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Oral Presentation Abstracts
Keynote Lecture Abstracts

Su-K-1

From the discovery of IL-6 to the development of anti-IL-6R anti body

Tadamitsu Kishimoto
Laboratory of Immune Regulation, Immunoology Frontier Research Center, Osaka University, Osaka, Japan

A series of our studies in IL-6 have revealed that it has a pleiotropic activity in various tissues and cells and its deregulated expression is responsible for several chronic inflammations and hemopoietic malignancies.

Humanized antibody against 80kd IL-6R (Tocilizumab) has shown significant therapeutic effect in RA, JIA, Castleman’s diseases and several other autoimmune inflammatory diseases, such as progressive sclerosis, giant cell arteritis, reactive arthritis, polymyalgia rheumatica and adult still’s disease. Cytokine storm induced by hyper activation of T cells has been shown to be controlled by Tocilizumab. Recently, TH17 is shown to be responsible for the pathogenesis of autoimmune diseases and IL-6 together with TGF-b are essential for the induction of TH17. We identified a new transcription factor called Arid5a which binds with the 3’-UTR of IL-6 mRNA and protects its degradation by competing with Regnase-1. Interestingly, this molecule is present in nuclei and inflammatory stimulation induced translocation of Arid5a from nuclei into cytoplasm and it competes with Regnase-1 for the protection of mRNA of IL-6.

Arid5a binds with the 3’-UTR of not only IL-6mRNA but also STAT3 mRNA in TH17 cells as well as T-bet mRNA in TH1 cells. Thus, Arid5a accelerates Th17cell differentiation in inflammation as well as exacerbation of IFN-γ-mediated septic shock.

All these results indicate that Arid5a is one of the key molecules for inflammation as well as the development of septic shock.

The results also suggest the therapeutic potential of anti-agonistic agents for Arid5a in the prevention of various incurable inflammatory diseases and septic shock.

Su-K-2

Anti-microbial action of inflammasomes at the mucosa

Richard A. Flavell
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Our bodies are in constant contact with a variety of microbes. Particularly in the intestine, the microbiome consists of an overwhelming number of organisms with which homeostasis must be regulated. If this cannot be achieved, bacterial and viral infection can lead to severe morbidity and death.

I will discuss the ways in which inflammasomes regulate these processes through a variety of mechanisms, including cell death and cytokine production.

Su-K-3

STAT3 is a master regulator of epithelial identity in KRAS driven tumorigenesis

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There is an apparent dichotomy that exists regarding the role of STAT3 in cancer. Studies in mice have demonstrated an intrinsic requirement for STAT3 in the early development of lymphoid, colon, liver, pancreatic, skin and stomach cancer. However, other studies demonstrated that STAT3 suppresses tumors in brain, breast, colorectal, lung, prostate and thyroid cancer. These functional and genetic studies demonstrated either an intrinsic promoting role for JAK/STAT3 signaling, or a suppressive role, dependent on cancer cell type. We sought to examine mechanisms that underlie the differential roles of STAT3 in cancer using a model of endogenous expression of oncogenic KRAS G12D in mouse lung and pancreatic epithelial cells. Our studies define a fundamental and previously unrecognized function of STAT3 in the maintenance of epithelial cell identity and differentiation. Loss of STAT3 preferentially associates with the acquisition of mesenchymal-like phenotypes, while persistent STAT3 tyrosine phosphorylation (pTyr705) confers a differentiated epithelial morphology. Our results imply a mechanism in which increased levels of pTyr705 STAT3
promote epithelial differentiation during tumor development. In contrast, loss of STAT3 augments tumor development with loss of epithelial identity. Our data point to the possibility that chronic activation of STAT3 limits progression of these cancers, and that the STAT3 signaling node may benefit intervention.

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Mo-K4-1

**Krebs Cycle repurposed for cytokines**

*Luke A.J. O’Neill*

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Metabolic reprogramming during the activation of immune cells has become a fascinating area of immunology. These events are not only important for energy production and biosynthesis but are also critically important for determining the phenotype of the cell. Macrophages in particular show marked metabolic differences based on their function with inflammatory macrophages being highly glycolytic whilst anti-inflammatory macrophages have more oxidative metabolism. Krebs cycle in particular shows differences. In LPS-activated macrophages succinate accumulates and is oxidised by succinate dehydrogenase, generating Reactive oxygen species via reverse electron transport at the node. What is emerging is therefore a critical role for Krebs cycle intermediates in governing cytokines profiles. These findings may point to new therapeutic options for inflammatory diseases.

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Tu-K5-1

**Tissue-Tregs and their nurturing cells**

*Diane Mathis*

*Harvard Medical School, Boston, United States*

Foxp3+ CD4+ regulatory T cells (Tregs) control most types of immune responses. Largely due to the ease with which we can access them, our view of Treg generation, phenotype and function is heavily colored by observations on cells found in lymphoid organs, in particular the spleen and lymph nodes. Our lab has been studying Tregs that infiltrate diverse tissues, eg the pancreas, adipose tissue, skeletal muscle and the large intestine. These “tissue-Treg” populations have distinct properties, adapted to their particular microenvironment and job. Interestingly, tissue-Tregs not only impact immune responses taking place in the vicinity, but can also influence non-immune processes. This presentation will focus on the diversification of tissue-Tregs and their sustenance by IL-33-producing mesenchymal stromal cells.

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We-K6-1

**Microbiota Control of Gut Immune Homeostasis**

*Dan Littman*

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The vertebrate intestinal tract is colonized by hundreds of species of bacteria that outnumber the total cells in the host, yet must be compartmentalized and tolerated to prevent invasive growth and harmful inflammatory responses. A key function of commensal microbes is to contribute to the adaptive immune repertoire and to diverse lymphocyte effector functions. T cell responses against non-invasive commensals contribute to shaping the repertoire of effector/memory and regulatory T cells. How T cells elicited by commensal bacteria can influence autoimmunity, resistance to pathogenic microbes, and anti-tumor responses are central questions that remain unsolved. We are studying the antigenic specificity of microbiota-induced T cells and the mechanisms by which their functions are acquired upon interaction with distinct commensal species. We find that Th17 cells, which are central to mucosal barrier defense but also participate in autoimmune disease, are induced by specific constituents of the microbiota, and acquire effector function only after additional exposure to endogenous adjuvants, such as the serum amyloid A proteins. Whereas some Th17 cells contribute to barrier defense and protection from potentially invasive enteropathogenic microbes, others can be highly inflammatory, but are normally restrained by commensal bacteria-induced regulatory T cells. Our studies in mice are not only relevant for human autoimmune diseases, many of which have Th17 cell involvement, but may also provide insights into how commensal microbe-specific T cell responses could be harnessed for mucosal vaccination and cancer immunotherapy.
Immune responses are accompanied by dynamic changes in gene expression. Gene expression is controlled at multiple points, including signal transduction, transcription and mRNA stability. So far, transcriptional regulation has been extensively studied. Many transcription factors including NF-kB and AP-1 are involved in induction of genes involved in inflammatory and immune responses. However, recent studies have revealed that control of gene expression at the mRNA level is as important as transcriptional control in the immune response. Gene expression profiles obtained from human Jurkat T cells stimulated with PMA plus ionomycin revealed that regulation of mRNA stability may account for as much as 50% of all measurements of changes in total cellular polyA mRNA. We have shown that Regnase-1 encoded by the Zc3h12a gene is an endoribonuclease involved in destabilization of a variety of mRNAs including IL-6, IL-12, and Regnase-1 itself mRNAs via the stem loop structure present in the 3'UTR of these genes.

Although originally identified as LPS-inducible gene, Regnase-1 is present in unstimulated cells, and disappears in response to Toll-like receptor ligands via an IKK-dependent proteasome degradation pathway or in response to T cell receptor stimulation through the cleavage by Malt-1. Thus, Regnase-1 acts as a brake in unstimulated cells as well as a negative feedback regulator after cellular activation. I would like to discuss the role of Regnase-1 in the immune response.

Mo-S1-3

MelLec: A new player in antifungal immunity

Gordon D. Brown
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The last few decades has seen a tremendous increase in our understanding of the mechanisms underlying the development of protective anti-microbial immunity. Key among these discoveries is the identification of pattern recognition receptors (or PRRs) expressed by immune cells which recognise conserved microbial components, such as beta-glucans. Recognition of these structures by PRRs, particularly by members of the C-type lectin receptor (CLR) family, triggers intracellular signalling cascades that initiate a variety of cellular and inflammatory responses, and induce the development of pathogen specific adaptive immunity. We now understand that innate recognition by CLRs is essential for the development of protective antimicrobial immunity. In this presentation, I will cover our discovery of a novel CLR that is providing new insights into the function and roles of these receptors.

Mo-S1-4

Gain of Function Mutation of RIG-I-Like Receptor Causes Autoimmune Symptoms

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MDA5 is an essential double-stranded RNA (dsRNA) sensor to elicit antiviral interferon (IFN) upon viral infection. Recently, MDA5 has been implicated in autoimmune diseases, however how MDA5 triggers the onset of autoimmune disorders remains unclear.

We obtained a mouse line that spontaneously developed autoimmune symptoms such as lupus-like nephritis and found that this mouse has a missense mutation (G821S) in Ifih1 gene encoding MDA5. The onset of all symptoms was totally dependent on the signaling from MDA5 G821S via an essential signaling adaptor, MAVS. While IFNAR deficiency in MDA5 G821S mutant mice partially ameliorated the phenotypes, suggesting the involvement of NF-kB-dependent inflammatory cytokines in the pathogenesis. MDA5 G821S failed to respond to dsRNA, however it constitutively activated IFN promoter. In humans, missense mutations in IFIH1 gene encoding MDA5 were reported in patients of systemic lupus erythematosus, Aicardi-Goutieres syndrome and Singleton-Merten syndrome (SMS). In one SMS patient, mutation in signaling has been shown to be associated with lethal autoinflammatory disease such as Aicardi-Goutieres Syndrome (AGS) and severe systemic lupus erythematosus (SLE). The activity of STING therefore requires tight control to prevent the sustained production of cytokines which are responsible for harmful autoimmune disease. Here, we will discuss the importance of STING-dependent innate immune signaling in controlling infectious disease, inflammation and cancer as well as review the importance of generating therapeutics that may control this pathway, for the prevention of a wide variety of disease.
DDX58 gene encoding another viral RNA sensor, RIG-I was reported. To examine the involvement of these mutations in autoimmune symptoms, we generated mutant mice expressing patient-related mutant of human MDA5 or RIG-I. We found that these mutant mice spontaneously developed various types of autoimmune symptoms, suggesting usefulness of these mice as human autoimmune disease models.

The involvement of signal transductions in autoimmune disease onset and possible preventions of the onset and treatments by inhibition of the signal transduction will be discussed.

Mo-S1-5
Metabolic regulation of innate immune function at barrier surfaces
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Employing models of allergen exposure, pathogen infection, and chronic inflammation, research in the Monticelli lab is examining how the innate immune system responds to tissue-specific cues to modulate cellular metabolism in order to coordinately regulate resolution of inflammation and promotion of tissue homeostasis at the body’s barrier surfaces. Our recent findings indicate that subsets of innate lymphoid cells (ILCs) utilize distinct metabolic pathways to fuel their proliferation and activation, and that this bioenergetic re-wiring influences their pro-inflammatory and tissue protective functions. Ongoing studies are examining how particular cell-extrinsic signals (such as cytokines) and cell-intrinsic factors (such as enzymes and solute carriers) control this metabolic reprogramming.

For example, we discovered that the amino acid enzyme Arginase 1 (Arg1) was a constitutive hallmark of the ILC2 lineage and that deletion of Arg1 limited ILC2 proliferation by inhibiting polyamine synthesis and reducing glycolytic capacity, thereby preventing development of airway inflammation. However, whether this enzyme has roles outside the ILC2 lineage is unknown. Unexpectedly, we’ve recently found that Arg1 expression is remarkably heterogeneous across ILC subsets and is dynamically regulated at a tissue-specific level in response to mucosal barrier damage. Expression of Arg1 delineated unique populations within the ILC1 and ILC3 lineages that were transcriptionally and functionally distinct from their Arg1 wild-type counterparts, suggesting that Arg1 enzymatic activity and downstream metabolites may control the ability of distinct ILC subsets to serve either pathologic or tissue-protective roles. Consistent with this, selective genetic deletion of ILC Arg1 in murine models of mucosal injury and infection resulted in reduced pro-inflammatory capacity while leaving the host-beneficial properties of ILCs intact, suggesting that this enzyme is a global instructor of the ILC family that governs pathologic versus tissue-protective roles during tissue damage and inflammation.

Mo-S1-6
Recognition of intracellular metabolites through C-type lectin receptors
Sho Yamasaki
Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Sensing and reacting to tissue damage is a fundamental function of immune systems. We previously found that Macrophage inducible C-type lectin (Mincle) is an activating C-type lectin receptor that senses damaged cells. We also found that Mincle also recognizes glycolipid ligands on pathogens, such as mycobacteria. To screen endogenous glycolipids ligands derived from damaged cells, we fractionated supernatants from damaged cells and identified a lipophilic component that activates reporter cells expressing Mincle. Mass spectrometry and NMR spectroscopy identified the component structure as β-glucosylceramide (GlcCer), a ubiquitous intracellular metabolite. Synthetic β-GlcCer activated myeloid cells and induced production of inflammatory cytokines; this production was abrogated in Mincle-deficient cells. Sterile inflammation induced by excessive cell death was exacerbated by hematopoietic-specific deletion of degrading enzyme of β-GlcCer (β-glucosylceramidase, GBA1). However, this enhanced inflammation was ameliorated in a Mincle-deficient background. GBA1–/– dendritic cells (DCs) in which β-GlcCer accumulates triggered antigen-specific T-cell responses more efficiently than WT DCs, whereas this enhancement was eliminated in DCs from GBA1–/– × Mincle–/– mice. These results suggest that β-GlcCer is an endogenous ligand for Mincle and possesses immunostimulatory activity.

Tu-S2-1
Interleukin 2 signal transduction and control of T cell biology: more than STATS
Doreen Cantrell
Department of Cell Signalling Immunology, School of Life Sciences, University of Dundee, Dundee, United Kingdom

Interleukin 2 (IL-2) and Janus kinase (JAK) regulate T cell biology by the co-ordinated phosphorylation of transcription factors, regulators of chromatin, mRNA translation, GTPases, vesicle trafficking and the actin and microtubule cytoskeleton. A key role for IL-2 is to control the transcriptional programs in T cells but IL-2/JAK signaling also regulates mRNA translation and protein synthesis to control T cell growth. To explore IL-2 and JAK control T cell function we have used high resolution mass spectrometry to quantify how IL-2 and Jak kinase inhibitors configure the proteomes of CD4+ TH1 cells and CD8+ cytotoxic T cells. We show that IL-2/JAK signaling regulates the expression of critical transcription factors, nutrient transporters, ribosomal proteins and metabolic enzymes to sustain the integrity of core metabolic processes essential for cellular fitness. One novel insight is that IL-2/JAK signaling regulates expression of the transcription factor NFIL3 (E4BP4) via control of the HIF1α transcription factor complex. These data reveal a fundamental IL-2 controlled signalling mechanism linking oxygen sensing to transcriptional control of T cell differentiation. These data inform how IL-2 controls effector T cell responses to hypoxia and provide a molecular understanding of how inhibitors of JAK kinases limit T cell function.

Tu-S2-2
IL-17 family cytokines in inflammation and cancer
Chen Dong
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Cytokines play important regulatory roles in immunity and inflammation. IL-17 family cytokines have emerged as critical players in inflammatory diseases. IL-17, also called IL-17A, is produced by specialized T cells, Th17 and other lymphocytes. Th17 cells are important in tissue inflammation and autoimmunity. On the other hand, IL-25, produced by epithelial cells, regulate type II immunity. In the workshop, I will discuss on our recent data on the function of IL-17 cytokines in inflammation and cancer. These cytokines may be targeted.
Tu-S2-3

Overlapping and distinct activities of IL-36 and IL-1 cytokines in inflammatory and infectious diseases

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The IL-1/IL-R and IL-36/IL-36R pathways share many features besides sequence and structural similarities unique that define the family of IL-1 and IL-1R cytokines. The IL-1R and IL-36R bind more than one agonist (i.e. IL-1α and IL-1β and IL-36α, IL-36β, and IL-36γ) as well as an antagonist, which is essential to balance the responses. Cytoplasmic proforms of the ligands are typically processed into mature forms with high activity. Moreover, they share a receptor accessory protein (IL-1RACp) and the adaptor MyD88 which are critical for signaling. However, while IL-1α/IL-1β are highly pleiotropic cytokines and their dysregulation is associated with many disease, the activity of IL-36 cytokines seem to be much more limited. Outstanding to date is their involvement in skin inflammation. In my presentation, I will highlight a few examples of inflammatory and infectious diseases, where my group has been addressing the roles of IL-1 and IL-36 cytokines.

Tu-S2-4

Cytokines networks in the induction and regulation of Th17 Cells

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Recently a subset of interleukin (IL)-17-producing T cells (TH17), which are distinct from TH1 or TH2 cells, was described and shown to have a crucial role in the induction of autoimmune tissue injury. TH17 differentiation is accomplished by three overlapping steps: Induction, Amplification and Stabilization mediated by distinct cytokines. Whereas TGF-b + IL-6 or IL-1 + IL-6 induces them, IL-21 amplifies TH17 cells, IL-23 stabilizes the phenotype of TH17 cells. Loss of any of the cytokines (TGF-β, IL-1, IL-6, IL-21 or IL-23) in the pathway results in a defect in generation of TH17. However not all TH17 cells are pathogenic and induce autoimmunity, IL-23 is a key cytokine that induces pathogenicity in TH17 cells (Lee et al., 2012). Using expression profiling at very high temporal resolution, novel computational algorithms and innovative nano-wire based “knock-down” approaches, we have developed a regulatory network that governs the development of TH17 cells. In addition to high-density temporal microarray analysis, we have performed single-cell RNA-seq of TH17 cells in order to characterize cellular heterogeneity, identify subpopulations, functional states and learn how gene expression variation affects TH17 effector functions. We have identified novel regulators of TH17 cells both in vivo and in vitro that do not affect TH17 differentiation but affects pathogenic vs. non-pathogenic functional states of TH17 cells. One of regulators CD5L (CD5like), produced in a soluble form by nonpathogenic TH17 cells, makes homo and heterodimers. Soluble forms of CD5L regulate differentiation of TH17 cells and inhibit development of autoimmunity and tissue inflammation.

Tu-S2-6

Osteoimmunology and autoimmunity

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Bone cells and immune cells share the same microenvironments in the bone marrow, communicating through various cytokines. Osteoblasts, osteoclasts and osteocytes are not only degrading or forming bone but have distinct roles in the immune regulation. Thus, much attention has been paid to the interdisciplinary filed, osteoimmunology, studying the interaction and shared molecules between bone and immune systems (Nat Rev Rheumatol 5, 667-76, 2009). Here I summarize the recent advance in osteoimmunology and its relevance in the studies on autoimmune diseases such as rheumatoid arthritis.

Self-tolerance is primarily established by positive selection followed by negative selection of self-reactive T cells in the thymus. We found that the transcription factor Fzfl2 plays a critical role in central tolerance by directly regulating tissue-restricted antigen expression in mTECs independently of Aire (Cell 163, 975-87, 2015). We also reported that the human variations of thymoproteasome subunit b5 impact CD8 T cell selection, indicating that genetic variations in antigen-peptide processing machinery influence T cell repertoire selection and susceptibility to autoimmune disease (Science Immunology 02 Jun 2017: 2, eaan5165).

We-S3-1

Modulation of the immune system by the gut microbiota

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The mammalian alimentary tract harbors hundreds of species of commensal microbes that critically influence a multitude of host physiological functions. Unfavorable alterations of the gut microbiota composition are called ‘dysbiosis’, and often correlate with negative health outcomes. Thus, the amelioration of microbiota dysbiosis is a promising route for future therapeutics for several diseases. To explore the role of individual components of the gut microbiota and to understand mechanisms distinguishing homeostatic from pathogenic microbiota-host interactions, we have adopted culture techniques for anaerobes, methods for generation and maintenance of gnotobiotic animals, together with the high-throughput sequencing technique. Combining these methods, we have been aiming to understand the functions, particularly immunological attributes, of the microbiota, and trying to identify responsible bacterial species and factors for shaping the immune system. We have succeeded in isolation of human and mouse gut-associated commensal bacterial strains that specifically affect the development and function of TH17 cells, Treg cells, Th1 cells or CD8 T cells. Our findings would allow for designing bacterial consortia that activate or suppress specific adaptive immune programs, potentially resulting in development of better therapeutics for numerous diseases involving the immune system, including infectious disease, autoimmunity, allergy, and cancer.
We-S3-3

Mucosal Multi-ecosystem of Epithelial Cells, Innate Lymphoid Cells and Commensal Microbiota for the Control of Symbiosis and Diseases

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Digestive tract is continuously exposed to infinite beneficial and harmful antigens including commensal and pathogenic microbe via the large mucosal epithelium. The intestinal mucosal surface is thus equipped with multi-complexed but harmonized biological components including epithelial-mesenchymal cells, mucosal immunocompetent cells and commensal microbiota, which form “Mucosal Multi-ecosystem” for the establishment of beneficial symbiosis condition as well as cooperative defense force. As an example, our study identified that commensal bacteria, Alcaligenes species can create “intra-tissue co-habitation niche” in Peyer’s patches (PPs), an example of commanding tissue for the induction and regulation of a balanced mucosal immunity. Innate lymphoid cells (ILCs) type3 have been shown to play critical role by the cooperative interaction with epithelial cells for the creation of intra-tissue co-habitation. Further, ILC3s have been shown to regulate epithelial cell glycosylation [e.g., fucosyltransferase 2 (Fut2) mediated fucosylation] for the creation of healthy gut microbiota and providing protective barrier against gut pathogens. The other form of innate immune-associated cells, mast cells (MCs) expressing P2X7 purinoceptor are also involved in the maintenance or disruption of healthy gut environment via the extracellular ATP and P2X7 cascade.

Further, our most recent study has suggested that Paneth cells located in the crypt region of epithelium contain Fut2-dependent population (Fut2 + Paneth cells). The development and function of Fut2 + Paneth cells are regulated by commensal bacteria dependent ILC3. These results suggested that the mucosal multi-ecosystem is a key element of creation and regulation of healthy environment of the intestinal tract for the balancing act between elimination and symbiosis.

We-S3-5

Gut reactions: Immune pathways in the intestine in health and disease

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The gastrointestinal (GI) tract is home to a large number and vast array of bacteria that play an important role in nutrition, immune system development and host defense. In inflammatory bowel disease (IBD) there is a breakdown in this mutualistic relationship resulting in aberrant inflammatory responses to intestinal bacteria. Our recent work has highlighted an important role for immune system tissue cell responses in the development and perpetuation of colitis and cancer. In this presentation I will discuss new therapeutic approaches that target this axis.

We-S3-6

Regulation of intestinal inflammation by epithelial barriers

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Intestine is a unique tissue, where many commensal bacteria, called microbiota, inhabit. Therefore, intestinal mucosa is protected from microbiota as well as pathogenic bacteria by several types of barriers. One of these barriers is constructed by mucus layers, composed of the inner firm mucus layer and outer loose mucus layer in the large intestine. Microbiota is present in the outer mucus layer, whereas there is no microbiota in the inner mucus layer. Separation of microbiota from the intestinal epithelial cells contributes to prevention of intestinal inflammation. Indeed, invasion of microbiota into the epithelial surface of the large intestine was shown in several mouse models of intestinal inflammation. However, the precise mechanisms by which the inner mucus layer is free of microbiota in the large intestine remain unknown.

Ly6/PLAUR domain-containing protein 8 (Lypd8) was found to be selectively expressed on the uppermost layer of colonic glands. Lypd8 was a highly glycosylated GPI-anchored protein, and cleaved and secreted into the colonic lumen, particularly the inner mucus layer. Depletion of these bacteria by antibiotics restored the bacterial free space in the inner mucus layer and ameliorated the intestinal inflammation of the mutant mice. Lypd8 bound to bacterial flagella and suppressed motile activity of flagellated bacteria. These findings demonstrated that Lypd8 mediates segregation of microbiota from the intestinal epithelial layer in the large intestine, and thereby contributes to the maintenance of gut homeostasis.

I will also discuss the sensitivity of Lypd8-deficient mice to intestinal inflammation in a dysbiotic condition.

We-S3-4

Sensing and reacting to pathogens via cytokine signaling at the skin barrier

Gabriel Nunez
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Staphylococcus aureus, a Gram-positive bacterium, is a leading cause of human infection capable of invading most tissues of the human body. The superficial skin is a major infection site for S. aureus, which normally resides in 10-20% of healthy individuals. S. aureus produces virulence factors to transform from a skin commensal to a pathogen. However, it remains unclear how the host senses virulent but not commensal S. aureus to trigger skin inflammation. Using a model of epicutaneous S. aureus infection in which virulence genes are induced upon epidermal colonization, we have identified S. aureus virulence factors that are critical for the detection of the virulent pathogen and the induction of skin inflammation. Furthermore, we have identified host molecules in keratinocytes and signaling pathways that are essential for triggering skin inflammation in response to virulent S. aureus.
ACROSS CLINICAL TRIALS, THE EXPANSION AND PERSISTENCE OF ADOPTIVELY TRANSFERRED T CELLS HAS CORRELATED WITH SUPERIOR PATIENT OUTCOMES. THIS, TRANSLATABLE STRATEGIES WHICH CAN ENGINEER ENHANCED SURVIVABILITY AND DURABILITY INTO TRANSFERRED T CELLS REMAINS A CRITICAL GOAL. USING THE TCGA DATABASE, WE FOUND THAT A SIGNIFICANT FRACTION OF BOTH HEMATOLOGIC AND SOLID TUMORS OVEREXPRESS FASLG, THE GENE ENCODING THE CANONICAL APOPTOSIS-INDUCING LIGAND FASLG. FURTHER, WE FOUND THAT FAS, THE RECEPTOR FOR FASLG, WAS HIGHLY EXPRESSED BY T-CELL SUBSETS ISOLATED FROM PATIENTS WITH MELANOMA AND DIFFUSE LARGE B-CELL LYMPHOMA PRIOR TO ENGINEERING FOR ADAPTIVE IMMUNOTHERAPY. WE THEREFORE HYPOTHESIZED THAT A COGNATE INTERACTION BETWEEN FASL AND FAS ON ADOPTIVELY TRANSFERRED T CELLS MIGHT LIMIT T CELL EXPANSION, PERSISTENCE AND ANTITUMOR EFFICACY. WE GENERATED A SERIES OF RETROVIRALLY-ENCODED FAS CONSTRUCTS WITH THE AIM OF ‘INSULATING’ ENGINEERED T CELLS FROM THE DEATHINDUCING FUNCTIONS OF FASLG. THESE CONSTRUCTS INCLUDED A FAS VARIANT BEARING A POINT MUTATION (FaslΔ264N) LIMITING FADD BINDING AND A TRUNCATION VARIANT MISSING THE MAJORITY OF THE DEATH DOMAIN (FaslΔDD). INTRODUCTION OF EITHER FaslΔ264N OR FaslΔDD INTO FAS-PERMISSIVE PRIMARY MOUSE OR HUMAN T CELLS PREVENTED Fasl-induced apoptosis. WHEREAS T CELLS ENGINEERED WITH AN EMPTY CONSTRUCT UNDERWENT APOPTOSIS WHEN CO-CULTURED WITH TUMOR CELLS, THIS PROCESS WAS PREVENTED IN T CELLS ENGINEERED WITH EITHER FaslΔ264N OR FaslΔDD. RELATIVE TO EMPTY VECTOR CONTROLS, T CELLS ENGINEERED WITH EITHER FaslΔ264N OR FaslΔDD EXHIBITED ENHANCED PERSISTENCE AND ENGRAFTMENT WITHIN THE TUMOR FOLLOWING TRANSFER IN VIVO. THIS ENHANCED SURVIVAL WAS IN TURN CORRELATED WITH SUPERIOR TUMOR REGRESSION IN A B16 MELANOMA MODEL. IN LONG TERM ENGRAFTMENT EXPERIMENTS, NEITHER THE FaslΔ264N NOR FaslΔDD-ENGINEERED T CELLS CLONALLY EXPANDED OR CAUSED AN ALPS SYNDROME. THUS, ‘INSULATING’ T CELLS FROM THE NEGATIVE INFLUENCE OF FASL IS A TRANSLATABLE STRATEGY RESULTING IN SUPERIOR T CELL PERSISTENCE AND ENHANCED ANTITUMOR EFFICACY FOLLOWING ADOPTIVE T CELL TRANSFER.

MACROPHAGE, MONOCYTE AND DENDRITIC CELL BIOLOGY: FROM DEVELOPMENT TO FUNCTIONS

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Dendritic cells (DCs), monocytes and macrophages play crucial and distinct roles in tissue homeostasis and immunity, but also contribute to a broad spectrum of pathologies and are thus attractive therapeutic targets. Potential intervention strategies aiming at manipulation of these cells will require in-depth insights of their origins and the mechanisms that govern their homeostasis. DCs and monocytes arise from common bone marrow (BM) precursor named macrophage-dendritic cell precursors (MDP), branching into exclusively DC- or monocyte-committed progenitors named common dendritic cell progenitors (CDPs) or common monocyte progenitor (cMoPs) respectively. CDPs give rise to plasmacytoid DC and migratory DC precursors termed pre-DCs. Pre-DCs seed tissues where they differentiate into the two major functionally specialized DC lineages, CD8α+/CD103+ DC1 and CD11b+ DC2. Recent evidence from our laboratory and others has showed that monocytes do not substantially contribute to all tissue macrophage populations in steady state and inflammatory conditions. Rather certain tissue macrophages in mice are derived from embryonic precursors, are seeded before birth and maintain themselves in adults by self-renewal. In addition, we now provide evidence that commitment to DC1 and DC2 subsets is imprinted early in the BM. Combining single cell sequencing with conventional transcriptomic analysis, we identified for the first time DC subset-specific precursors in the BM as well as previously unknown molecular checkpoints for DC lineage commitment as early as the CDP stage. Using again single cell sequencing and CyTOF, we also identified homologous DC progenitors in humans and redefined the human DC lineage from the BM to the tissues. These new insights into the origins of DCs, monocytes and macrophages should aid the rational design of therapies aimed at harnessing the functions of these cells in homeostasis and inflammation and will allow efficient targeting and manipulation during health and disease.
Immune-checkpoint blockade and T-cell based adoptive cell therapy have recently shown durable clinical effects on the patients with various advanced cancers. However, response rates are around 10-30%. Thus, the identification of biomarkers to select appropriate patients and immunotherapies as well as the improvement of immunotherapy efficacy possibly through combination strategy (including converting non-responders to responders for the immune-checkpoint inhibitors) are needed.

Pretreatment immune status of tumor microenvironments varies among cancer patients, and it correlates with prognosis and responses to various cancer therapies including surgery, chemotherapy, radiation, and immunotherapies. The immune status may be defined by cancer cell’s genetic characteristics (e.g. immunogenic passenger mutations, immunosuppressive driver mutations), patients’ immune-reactivity (e.g. SNPs), and environmental factors (e.g. smoking, microbiota). Because the immune status is different among cancer types, subtypes and individual patients, appropriate immune-interventions may need to be applied for each patient.

For combined immunotherapies, the following immunological issues may be considered; 1) identification of appropriate tumor antigens expressed in cancer initiating cells, 2) in situ tumor destruction to induce immunogenic cancer cell death, 3) enhancement of antigen presenting cells’ function, 4) in vivo activation of anti-tumor T-cells, 5) Reversal of cancer-associated immunosuppression (cancer induced primary and anti-tumor T cell induced adaptive immune resistances).

We have been screening various chemical compounds and antibodies capable of improving immunological conditions in tumor microenvironments, and found that some of them are able to augment anti-tumor effects particularly in combination with PD-1/PD-L1 blockade through various mechanisms in in vitro human and in vivo murine tumor models. Altogether, personalized combination therapy based on the evaluation of patients’ immune status may be exploited for further improvement of current cancer immunotherapies.

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**Th-S4-5**

**Escape from tumor immunity by soluble CD155**

Kazuko Shibuya  
Department of Immunology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan

Although CD155 is ubiquitously expressed on the cell surface of both hematopoietic and non-hematopoietic cells, tumor cells significantly upregulate its expression. The interaction of DNAM-1 immunoreceptor on CD8+ T cells and NK cells with CD155 on tumor cells plays an important role in tumor immunity in mice. However, unlike mouse, human CD155 contains two splicing isoforms that encode soluble form of CD155 (sCD155). We showed that the serum level of sCD155 was higher in patients with a variety of cancers than that in healthy individuals, and was correlated with the stage of stomach cancer. These results suggest a hypothesis that sCD155 is involved in the tumor escape. To address this hypothesis, we generated a B16/BL6 tumor transfectant expressing sCD155 (sCD155/BL6) and a control transfectant (mock/BL6). These transfectants were subcutaneously inoculated into mice, the growth was faster in sCD155/BL6 than in mock/BL6. We also found that lung metastasis was remarkably promoted in sCD155/BL6 compared with mock/BL6 after intravenous injection. These results suggest that sCD155 inhibits tumor immune response in primary and metastatic regions. Because DNAM-1 and TIGIT, which is known as an inhibitory receptor, shares the ligand CD155, we next intravenously injected either sCD155/BL6 or mock/BL6 into DNAM-1 KO and TIGIT KO mice for the identification of the ligand for sCD155 in tumor microenvironment. Whereas tumor metastasis of sCD155/BL6 was greater than that of mock/BL6 in TIGIT KO mice, those of sCD155/BL6 and mock/BL6 were comparable in DNAM-1 KO mice. Taken together, these results suggest that sCD155 suppressed DNAM-1-mediated tumor immunity.

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**Lunch Session Invited Speaker Abstracts**

**Mo-L1-1**

**Immune checkpoint blockade therapy in cancer and beyond**

Nagahiro Minato  
Graduate School of Medicine, Kyoto University, Kyoto, Japan

Since the conceptual proposal of cancer immunosurveillance by Burnet and Smith more than a half-century ago, a recent success of immune checkpoint blockade therapy in human cancers has provided a breakthrough in cancer immunotherapy. PD-1, originally discovered by Dr. Honjo’s group at Kyoto University in 1992, is a TCR-coinhibitory receptor and plays a crucial role in the checkpoint of peripheral T-cell self-tolerance, and in 2002 we reported that the PD-1 checkpoint also takes an important part in restraining endogenous anti-tumor immunity, providing a basis for current immune checkpoint blockade therapy. Current major research is directed to improved efficacy of the therapy as well as the search for genetic and other biomarkers predictive of the effectiveness. The clinical studies have reinforced the crucial importance of general accessibility of host immune effector system to cancer cells in tumor microenvironment. Another important aspect in cancer immunity is the host immune status. Considering the increased risk of cancer emergence with age, age-dependent alteration of immune capacity, or immunosenescence, may have a potential importance in cancer immunity. A recent report demonstrating that selective elimination of senescent cells in tissues lead to prolonged lifespan with decreased spontaneous tumor development may supports the notion. We recently succeeded in identifying senescent-associated (SA-) T cells, which play a central role in the immune senescence phenotype. While defective in proliferation capacity, SA-T cells are metabolically highly active and are involved in a variety of age-related disorders. In this talk, I shall briefly summarize the history and recent advancement in immune checkpoint blockade therapy and also introduce our recent studies on immunosenesence, which may potentially affect cancer immunosurveillance mechanism in hosts.

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**Mo-L2-1**

**Phase-orientated disease control by cytokines- lessons from rheumatoid arthritis**

Georg Schett  
University of Erlangen, Nuremberg, Germany

Autoimmune inflammatory diseases such as rheumatoid arthritis are based on (i) an initial transition of clinically silent autoimmunity to clinically apparent inflammation, (ii) the building-up of a robust systemic inflammatory process and (iii) the maintenance of this process by blocking naturally occurring resolution mechanisms. These different aspects of chronic inflammatory diseases are not necessarily controlled by the same cytokines. Recent data for instance suggest that the transition from autoimmunity to inflammation is essentially governed by IL-23, which controls the pathogenicity of autoantibodies and their cytokine producing potential. Essential effector cytokines in this process are IL-21 and IL-22, which influence the sialylation and thereby the effector function of antibodies. While the processes controlling
The Role of IL-17A in Psoriasis Pathogenesis and Treatment

James G. Krueger
The Rockefeller University, New York, United States

The pathogenic model of psoriasis has recently changed to focus on the induction of disease by a central cytokine pathway in which over-expression of IL-23 in psoriasis lesions drives expansion and activation of T-cells that over-produce IL-17. T-cells that produce IL-17 in psoriasis lesions are mainly conventional CD4+ and CD8+ T-cells that are designated as Th17 and Tc17, respectively. However, less abundant lesions are mainly conventional CD4+ and CD8+ T-cells that are involved in the induction of disease by a central cytokine pathway in which over-production of IL-17 production in psoriasis lesions, so the entire set of IL-17 producing lymphocytes is now called Type 17 (T17) cells. IL-17 acts as pathogenic cytokine in psoriasis principally through inducing transcription of many other cytokines and inflammatory mediators in keratinocytes. Some of the induced products, e.g., S100A7 are molecules that typify psoriasis, while others create “feed-forward” inflammatory circuits that regulate epidermal hyperplasia, influx of many different leukocyte types, and control expression of newly discovered psoriasis auto-antigens. Thus psoriasis becomes a self-perpetuating inflammatory reaction in focal skin areas due to chronic T-cell activation by autoantigens that are produced at high levels in skin lesions. This chronic immune process can be significantly reduced or eliminated through therapeutic targeting of either IL-23 or IL-17A with monoclonal antibodies. Targeting psoriasis with IL-17A monoclonal antibodies (secukinumab or ixekizumab) leads not only to impressive clinical improvement in the majority of treated patients, but it largely reverses epidermal changes that are IL-17 induced and it dramatically reduces immune cell infiltrates and molecular changes that are associated with psoriasis. Systemic inflammation, as measured by over production of IL-17 induced genes in leukocytes can be rapidly attenuated by IL-17 antibodies, but maximal benefit to the disease phenotype only occurs with long-term treatment, e.g., recent study show more disease improvement after 1 year of secukinumab treatment, compared to results obtained from 12-16 weeks of treatment.

Single-cell gene expression in tissues, tumors, and cell lines

Shinichi Hashimoto
Graduate School of Medical Sciences, Kanazawa University, Ishikawa, Japan

The cell populations, such as differentiated states or tissues and organs, are thought to be heterogeneous. Any expression profile based on a tissue sample will blend the true expression profiles of its constituent cells. To overcome this problem, single-cell methods have been developed for both microarrays and RNA-seq. Single-cell gene-expression profiling allows identification and characterization of different cell types and cell subtypes. Furthermore, phenotypic heterogeneity can be studied within the same cell type. Although several studies for single cell gene expression analysis have been reported, number of the observed cells is very limited. Therefore, we and other groups have developed novel strategy of single-cell transcriptome analysis for thousands of single cells. In these approaches, single cells are put into a high-density microwell plate or droplet and lysed in situ. mRNA is then captured on barcoding microbeads and reverse transcribed. In this study, we applied single cell transcriptome analysis to characterize complex heterogeneous samples in the human cancer tissue. These results showed the distinction of the cancer cell state with tumorigenicity, the EMT, stem like cell and infiltrated leukocytes. Finally, single cell transcriptome analysis is a powerful approach for characterizing and understanding cellular diversity in cancer tissues.

Lung Fibrosis: Future Directions in Research

Toshibiro Ito
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Fibrosis, which is caused by chronic inflammation, results in impaired tissue architecture and functions in many organs including the lung. Idiopathic pulmonary fibrosis (IPF), the most common lung fibrosis, is a chronic and progressive lung interstitial disease accompanying by the pathological diagnosis of usual interstitial pneumonia (UIP). The pathogenesis of IPF is complicated, but a number of factors, such as myofibroblasts, the extracellular matrix (ECM), inflammation, and genetic and environmental factors, are thought to be involved in the onset and progression of IPF. Despite the accumulated knowledge of IPF through basic and clinical research over the last decades, an effective medical therapy for IPF remains to be established. It leads to a median survival of 3 years, which is much worse than many types of cancer. One of the reasons why basic research has failed to yield an effective therapy for IPF in the clinical stage is that animal models of IPF remain to be established. This study showed the benefit of IPF research.

We and our collaborators have investigated humanized mice models of lung fibrosis induced by the infusion of human IPF fibroblasts. Using the humanized models, we have examined both the pre-existing drug and novel therapeutic candidates for IPF. One of the new therapeutic candidates for IPF is adipose-derived mesenchymal stem cell (ADSC). Compared with bone marrow-derived mesenchymal stem cells (BDMCs), ADSCs have several advantages such as easy accessibility and minimal morbidity on harvest. Moreover, in our studies, ADSCs produce many kinds of effective cytokines against fibrosis and reduce collagen expression in IPF fibroblasts, which indicates that ADSCs would be a promising therapeutic source for IPF.

Lung Fibrosis: Future Directions in Research

Tu-L5-1

Single-cell gene expression in tissues, tumors, and cell lines

Shinichi Hashimoto
Graduate School of Medical Sciences, Kanazawa University, Ishikawa, Japan

The cell populations, such as differentiated states or tissues and organs, are thought to be heterogeneous. Any expression profile based on a tissue sample will blend the true expression profiles of its constituent cells. To overcome this problem, single-cell methods have been developed for both microarrays and RNA-seq. Single-cell gene-expression profiling allows identification and characterization of different cell types and cell subtypes. Furthermore, phenotypic heterogeneity can be studied within the same cell type. Although several studies for single cell gene expression analysis have been reported, number of the observed cells is very limited. Therefore, we and other groups have developed novel strategy of single-cell transcriptome analysis for thousands of single cells. In these approaches, single cells are put into a high-density microwell plate or droplet and lysed in situ. mRNA is then captured on barcoding microbeads and reverse transcribed. In this study, we applied single cell transcriptome analysis to characterize complex heterogeneous samples in the human cancer tissue. These results showed the distinction of the cancer cell state with tumorigenicity, the EMT, stem like cell and infiltrated leukocytes. Finally, single cell transcriptome analysis is a powerful approach for characterizing and understanding cellular diversity in cancer tissues.

Tu-L4-1

Single-cell gene expression in tissues, tumors, and cell lines

Shinichi Hashimoto
Graduate School of Medical Sciences, Kanazawa University, Ishikawa, Japan

The cell populations, such as differentiated states or tissues and organs, are thought to be heterogeneous. Any expression profile based on a tissue sample will blend the true expression profiles of its constituent cells. To overcome this problem, single-cell methods have been developed for both microarrays and RNA-seq. Single-cell gene-expression profiling allows identification and characterization of different cell types and cell subtypes. Furthermore, phenotypic heterogeneity can be studied within the same cell type. Although several studies for single cell gene expression analysis have been reported, number of the observed cells is very limited. Therefore, we and other groups have developed novel strategy of single-cell transcriptome analysis for thousands of single cells. In these approaches, single cells are put into a high-density microwell plate or droplet and lysed in situ. mRNA is then captured on barcoding microbeads and reverse transcribed. In this study, we applied single cell transcriptome analysis to characterize complex heterogeneous samples in the human cancer tissue. These results showed the distinction of the cancer cell state with tumorigenicity, the EMT, stem like cell and infiltrated leukocytes. Finally, single cell transcriptome analysis is a powerful approach for characterizing and understanding cellular diversity in cancer tissues.
promising therapeutic candidate for IPF.

In this talk, we will discuss factors controlling lung fibrosis, future directions of IPF research to bring an effective therapy to the clinical stages, and the potential of ADSCs to treat IPF.

Tu-L6-1
Development and functions of resident macrophages
Frederick Geissmann
Memorial Sloan Kettering Cancer Center, New York, United States

A scientific literature that covers 150 years of research indicates that a cell type found in most tissues, conserved across metazoans, and commonly designed as ‘macrophages’ in vertebrates participates to the niches that support specialized tissues cells, acting as sentinel for the uptake of dying cells and pathogens, regulator of morphogenesis and tissue remodeling, homeostasis and metabolism, and are involved in inflammatory processes, degenerative diseases and tumor growth. The overarching hypothesis that drives our work is that understanding of the development, specification, growth, and organization of macrophages within tissues is essential to the molecular characterization of their functions at the tissue and organismal level and for the identification of genetic events, including germ-line polymorphisms and somatic mosaicism that cause macrophage dysfunction and disease pathogenesis.

We-L7-1
Strategic development of combination cancer immunotherapy
Kouji Matsushima
Department of Molecular Preventive Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

The recent development of immune-checkpoint Abs has revolutionized our concept of cancer therapy. We have clinically developed a humanized anti-CCR4 Ab with potent ADCC activity against Adult T-cell leukemia lymphoma (ATLL). Due to selective expression of CCR4 on Tregs, this Ab is also expected to overcome cancer-associated immune-suppression through the depletion of Tregs. We are also developing a humanized anti-CD4 Ab with potent ADCC activity, IT1208. In murine subcutaneous tumor models (B16 melanoma, Colon 26, and LLC), administration of the anti-CD4 Ab alone had strong antitumor effects that were superior to those elicited by CD 25+ Treg depletion or by any immune checkpoint Abs.

We-L8-1
Involvement of semaphorins in pathogenesis of autoimmune and inflammatory diseases
Atsushi Kumanogo
Department of Respiratory Medicine and Clinical Immunology, Osaka University Graduate School of Medicine, Osaka, Japan

Semaphorins were originally identified as neural guidance factors. It has been demonstrated that these proteins play pleiotropic functions in immune regulation, angiogenesis, tumor metastasis, and bone metabolism. We here present the pathological implications of Semaphorin 4D (SEMA4D) in anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and show the novel regulatory mechanisms of neutrophils through neutrophil-endothelium interactions to maintain immunological homeostasis, providing basic and clinical implications.

Inappropriate activation of neutrophils plays a pathological role in AAV. Serum levels of soluble SEMA4D were elevated in AAV patients and correlated with disease activity scores. Cell surface expression of SEMA4D was downregulated in neutrophils from AAV patients, a consequence of proteolytic cleavage of membrane SEMA4D. Membranous SEMA4D on neutrophils bound to plexin B2 on endothelial cells, and this interaction decreased NET formation. Recombinant plexin B2 suppressed neutrophil Rac1 activation through SEMA4D’s intracellular domain, and inhibited pathogen- or ANCA-induced oxidative burst and NET formation.

Collectively, these findings indicate that neutrophil surface SEMA4D functions as a negative regulator of neutrophil activation (an immune check point for neutrophils) and imply that SEMA4D is a promising biomarker and potential therapeutic target for AAV.

We-L8-2
The role of cytokine in the pathogenesis of age-related macular degeneration
Koh-Hei Sonoda
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Age-related macular degeneration (AMD) is the most common disease leading to acquired blindness in developed countries. Choroidal neovascularization (CNV) is a characteristic of AMD and is induced by regional inflammation as a result of age-related conformational changes of the chorioretinal interface. Genome-wide association studies have provided evidence that the immune system is involved in the pathogenesis of AMD; however, the role of inflammatory cytokines in CNV has not been well established. We have demonstrated IL-27, a member of the IL-6/IL-12 cytokine family, has an angiostatic effect and regulates the development of laser-induced experimental CNV in mice. We also demonstrated IL-17 had a strong potential for promoting neovascularization in a vascular endothelial growth factor-independent manner in laser-induced experimental CNV in mice. Infiltrated γ/δT cells and Thy-1(+) innate lymphoid cells were the main sources of IL-17 in injured eyes. In addition, subretinal fibrosis is another problem of AMD. It is an end stage of AMD, characterized by fibrous membrane formation after choroidal neovascularization. An initial step of the pathogenesis is an epithelial-mesenchymal transition (EMT) of retinal pigment epithelium cells. We have demonstrated several cytokines, including IL-6, have involved in this later phase of AMD. Collectively, cytokines plays an important role in the AMD pathogenesis, and it may be a new target to regulate regional cytokines in the eye.
ICIS-BioLegend William E. Paul Award Lecture Abstract

We-L9-1
Learning cytokine function from the host-pathogen encounter

Alan Sher
NIH / NIAID, Bethesda, United States

The discovery of essential roles for cytokines in host defense against pathogens has been one of the major drivers of research in the general field of cytokine biology. In addition to generating new therapeutic interventions in infectious disease, this work has led to important insights into the organization of the immune system. Indeed, the production of specific cytokines mediating control of defined phylogenetic classes of microbial invaders, has become a major functional criterion in the definition of both T lymphocyte and myeloid subsets. At the same time the elucidation of the regulatory activities of cytokines in protecting against infection driven immunopathology has contributed to our understanding of the functions of the same mediators in tissue repair and homeostasis. While identifying a plethora of potential targets for disease intervention, research on the host-pathogen interaction has at the same time uncovered a complex web in which cytokines can serve as either “friend” or “foe” depending on the context of their activities. Deciphering this web and its underlying principles has become a major challenge in the development of new cytokine-based therapies. In my talk, I will discuss some of the contributions of our laboratory in the use of parasite and mycobacterial infection models to understanding cytokine function and highlight fundamental questions and debates that my colleagues and I have encountered during our three decade journey in the field.

This work was supported by the Intramural Research Program of the National Institutes of Health, NIH.

Workshop Session Chair & Oral Presentation Abstracts

Mo-WS1-1
Roles of cytokines in the anti-fungal immunity

Shinobu Saijo
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Candida albicans (C. albicans) is a opportunistic fungus which causes a wide spectrum of infections from mild to severe and often lethal disease in the setting of immunosuppression. Defense against C. albicans is largely depends on C-type lectin receptors (CLRs) as well as IL-17 family cytokines. Here, we developed epicanthic candidiasis (ECC) mouse model, in which C. albicans was inoculated occlusively on shaved skin. In this model, skin inflammation reached a peak on day 2 with neutrophil infiltration and subsided by day 7 in WT, IL17a−/− and IL17f−/− mice, while IL17a−/−IL17f−/− mice showed more severe inflammation even on day 7 with the high fungal burden, indicating the importance of IL-17 for ECC defense. However, fungal recognition receptors TLR2, Dectin-1, Dectin-2, and the adaptor signaling molecules MyD88, FcRγ, and Card9 were dispensable for clearance of C. albicans in the skin. In addition, we detected lower mRNA expression of antimicrobial peptides (AMPs) and less number of neutrophils in the infected skin from IL17a−/−IL17f−/− mice than in that of WT mice. Neutrophils isolated from IL17a−/−IL17f−/− mice had less killing activity against C. albicans than those from WT mice in vitro. These findings indicate that IL-17 plays a pivotal role in cutaneous host defense by recruiting and activating neutrophils to eradicate C. albicans in the epidermis.

Mo-WS1-2
Pathogenic fungus, Trichophyton mentagrophytes negatively regulates host immune responses via Siglec receptors

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2 Chiba University, Medical Mycology Research Center, Chiba, Japan
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4 Kyushu University, Medical Institute of Bioregulation, Fukuoka, Japan

Innate immune cells such as macrophages and dendritic cells sense invading pathogens through pattern recognition receptors (PRRs), and control host immune responses. Among these PRRs, Siglec is a sialic acid-binding lectin and negatively regulates host immune responses through cytoplasmic ITIM sequence. To find Siglec-binding pathogens, we generated Siglec reporter cells that can detect ligand recognition by GFP expression, and found Trichophyton mentagrophytes. Trichophyton mentagrophytes is a pathogenic fungus that causes dermatomycosis. Although immunocompromised patients often suffer from dermatomycosis, Trichophyton mentagrophytes also infects healthy individuals and causes ringworm and athlete’s foot. Taken together, we speculated that Trichophyton mentagrophytes escapes from host immunity through the interaction with Siglec receptor. Trichophyton mentagrophytes potently bound to Siglec-5 and Siglec-9, and moderately interacted with Siglec-3, all of which are expressed on macrophages and monocytes. Upon ligation of Siglec with Trichophyton mentagrophytes in human monocytic U937 cells, SHP-1 but not SHP-2 was recruited to ITIM sequence in the cytoplasmic region, resulting in inhibition of immune responses including the production of TNFα. Sialidase treatment of Trichophyton mentagrophytes had no impact on the recognition of Siglec, suggesting that the ligand is not a sialic acid. We are now investigating the nature of this unique ligand. In conclusion, Trichophyton mentagrophytes negatively regulates host immune responses through immunosuppressive receptor, Siglec. This interaction should be a potential target for new anti-fungal drugs.

Mo-WS1-3
Two distinct ITAM-coupled receptors recognize mycobacterial mycolic acid-containing lipids and differently regulate immune responses

Eiichiro Iizasa1, Takayuki Uematsu1, Yasushi Chuma2, Hideyasu Kiyohara1, Mio Kutoba1, Masayuki Umemura3, Goro Matsuzaki1, Sho Yamakasi1,2,3,4, Hiromitsu Harag5
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6 Department of Molecular Immunology, Division of Host Defense, Research Institute for Microbial Disease, Osaka University, Osaka, Japan

Mycobacterium tuberculosis remains an important cause of morbidity and mortality worldwide. Macrophages (Mφs) represent first line
to defense against pathogens. Pattern recognition receptors (PRRs) expressed on Mps detect pathogen-associated molecular patterns (PAMPs) of pathogens to activate immune responses. Recent reports have highlighted the importance of ITAM-coupled receptors (ITAMRs) including C-type lectin receptors in the recognition of PAMPs to regulate immune responses. Mycobacteria paradoxically live in macrophages. Thus the regulation of immune responses are important for mycobacterial infection. In this study, we found that one of ITAMRs TREM2 directly recognized mycobacterial PAMPs, mycolic acid (MA) and MA-containing lipids. Its recognition of non-glycosylated MAs, such MA itself and glycerol monomycolate (GroMM), induced the production of MCP-1 but not TNF-α and any other inflammatory cytokines, as far as we examined. In contrast, Mincle, which is the receptor for Torehalose-di-mycolate (TDM), also recognized another glycosylated MA, GMM but not non-glycosylated MAs. Mincle recognition of these glycosylated MAs induced the production of various cytokines including MCP-1 and TNF-α. The glycosylated but not non-glycosylated MAs also induced nitric oxide (NO) production. Interestingly, TREM2 is found to also recognize these glycosylated MAs and inhibited these cytokine and NO productions. BCG infection of Trem2-deficient mice accelerated clearance of bacteria comparing to that of WT. These results shows the glycosylated MAs and non-glycosylated MAs differently regulate host immune responses through Mincle and TREM2. Although Mincle recruits macrophages for killing bacteria, TREM2 harness the host immunity to produce only MCP-1 to recruit macrophages lacking bactericidal activity for their propagation.

Mo-WS1-5
cGAS-STING signaling is required for host defense from WNV neuropathology

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West Nile Virus (WNV), an emerging and re-emerging RNA virus, is the leading source of arboviral encephalitic morbidity and mortality in the United States. WNV infection is largely controlled by the immune response in the periphery but in some cases WNV bypasses this response and invades the central nervous system (CNS), causing encephalitis and encephalomyelitis. Recent studies indicate that DNA sensor cyclic GMP-AMP (cGAMP) synthase (cGAS) and its downstream adaptor molecule STImulator of iNterferon Gene (STING) are required for host defense against neurotropic RNA viruses; however the mechanism of the cGAS-STING pathway on viral control is unknown. For this study, we evaluated the role of cGAS-STING signaling in host defense through virologic control and immune defense against WNV in the CNS using a murine model of infection. When infected with WNV, both cGAS and STING knock out (-/-) mice displayed increased morbidity and mortality compared to wild type (WT) mice. STING-/- mice exhibited increased and prolonged neurological symptoms compared to WT mice. Pathological examination revealed increased lesions, mononuclear cellular infiltration and neuronal death in the CNS of STING-/- mice, particularly in survivors. To determine if innate immune actions against WNV required cGAS-STING signaling, we infected primary bone-marrow derived macrophages (BMDM) and determined the virologic replication in BMDM. In contrast, in vivo analyses revealed that cGAS-/- and STING-/- mice have defective CD8 T cell responses that associate with increased CNS viral load pathology from WNV infection. Our findings demonstrate that cGAS-STING signaling plays an important role in CNS immune control against WNV infection, and reveal immune mechanisms of control against neuroinvasive virus infection.

Mo-WS1-6
Dengue virus degrades cGAS to prevent mitochondrial DNA sensing during infection

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This study put forward the immune-modulating capacity of the plant dsRNA and its potential physiological applications. The dsRNA demonstrated strong antiviral, tumor-suppressive capacity and the potential to suppress autoimmune diseases in the CNS.
Dengue virus (DENV) is the most prevalent mosquito borne virus, causing near to 400 million infections a year in more than 100 countries. In order to make a productive infection in humans, DENV actively inhibits the type I IFN system. Our group has reported that the virus encoded protease complex (NS2B3) cleave STING to avoid type I IFN production. To explore the sensors that collaborate with STING in DENV detection, we performed a systematic analysis of different pattern recognition receptors (PRRs) during infection. We report that the DNA sensor cGAS is involved in early detection of DENV infection, and interacts with cGAS during infection and promotes its degradation in an autophagy-lysosome dependent mechanism. This degradation results in an inhibition of the cGAS/cGAMP/STING cytosolic DNA sensing pathway in primary human dendritic cells. Using biochemical and immunofluorescence techniques, we demonstrate a novel mechanism by which the DNA sensor cGAS detects cellular collateral damage during DENV infection.

Mo-WS1-8
Targeting of viral replication complexes by LC3-guided interferon-inducible GTPases
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Background: A hallmark of viruses with positive-sense RNA genomes is the need to be overcome by viruses trying to invade a human host. In mice, that innate restriction factors represent escape structures was unknown. The replication of murine norovirus (MNV) and the localization of LC3 in cell culture systems. MNV is targeting the host immune system counteracts these structures was unknown.

Methods: The replication of murine norovirus (MNV) and the targeting of viral replication complexes by LC3-guided interferon-inducible GTPases (immunity-related GTPases [IRGs] and/or guanylate-binding proteins [GBPs]) with regard to MNV RC were investigated in bone marrow derived macrophages and mouse embryonic fibroblasts, derived from mice with various genetic deletions, with or without activation of the cells with interferon-gamma (IFNG). The survival of mice infected with MNV and the replication of MNV in the infected tissues were monitored using the mice with the defective IRG or GBP system. The replication of MNV in the localization of LC3 and GBPs on the MNV RC were also monitored in human cell lines with various genetic deletions after IFNG activation.

Results: The replication of murine norovirus (MNV) and the replication of MNV was inhibited by IFNG; such inhibitory effect of IFNG was dependent on targeting of IFN-inducible GTPases (IRGs and GBPs) to the RC of MNV via the LC3 conjugation system of autophagy (e.g. ATG5). However, the canonical autophagy pathway (e.g. ATG14) was not required for this antiviral activity of IFNG. Consistently, the mice with defective IRG or GBP system were more susceptible to MNV infection; in human cells, the LC3 conjugation system was also required for targeting of GBPs to the RC of MNV and both systems were required to control MNV replication.

Conclusions: IFN-inducible GTPases are known to destroy the membrane of vacuoles containing bacteria, protists, or fungi. Thus, our data suggest that viral RCs can be antagonized by a universal immune defense mechanism against the membranous shelters of pathogens.
Mo-WS1-9
Gate-16 is required for LC3-independent antimicrobial host defense through cytosolic distribution of interferon-inducible GTPases

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Although functions of autophagy-related (Atg) proteins in canonical autophagy are well understood, recent studies reveal the roles of various Atg proteins in other biological processes. While many of the Atg genes are highly conserved throughout evolution, Atg8 gene has undergone expansion in the genome of higher eukaryotes. Mammalian Atg8 homologues consist of LC3 and Gabarap subfamilies, all of which are involved in canonical autophagy. In contrast, the role of each Atg8 homologue members in non-canonical processes is not fully understood. Here we show a unique role of Gate-16 in an interferon-γ (IFN-γ)-mediated antimicrobial response. Cells lacking Gate-16, but not those lacking Gabarap and Gabarap1, or LC3a and LC3b, are defective in IFN-γ-induced clearance of vacuolar pathogens such as Toxoplasma. Gate-16, but not LC3b, specifically associates with Arf1 to mediate uniform distribution of IFN-inducible GTPases. Gate-16-deficiency reduces Arf1 activation, leading to formation of IFN-inducible GTPase-containing aggregates, hampering their function. Furthermore, mice lacking Gate-16 alone, either systemically or specifically in the myeloid compartment, are susceptible to Toxoplasma. Thus, Gate-16 is uniquely required for antimicrobial host defense through cytosolic distribution of IFN-inducible GTPases.

Mo-WS3-1
Itch and cytokines

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Itch is a noxious sensation that disturbs work and play while awake and prevents rest and rejuvenation during sleep. Itch accompanies many skin diseases from psoriasis to urticaria to various types of eczema and to ectoparasitosis such as scabies. Although the pathogenesis of itch is not well understood, it has neurophysiological similarities to and differences from pain. Recent investigations have defined both indirect and direct roles for cytokines in itch. Indirectly, cytokines activate cells of the immune system and resident cells in skin to release mediators that communicate with sensory nerves regulating neuromonal inflammation, pain, and/or itch. Certain cells, for example mast cells and eosinophils, communicate with sensory nerves during inflammation via mediators, including histamine, tryptase, and prostanoids, that induce pruritus. Information about how other immune cells, particularly T cells, communicate with nerves to influence neuromonal inflammation, pain, and pruritus is limited. Therapies directed at cytokines that are involved in proinflammatory immune responses, for example, IL-4, IL-13 (dupilumab in atopic dermatitis), IL-17 and IL-22 (secukinumab, ixekizumab, brodalumab in psoriasis), reduce the symptoms of itch. Emerging evidence indicates cytokines, including chemokines, involved in inflammation, for example, IL-1, IL-8, IL-10, monocye chemoattractant protein (MCP), CCL2, CCL4, CCL5, macrophage inflammatory protein 1 alpha (MIP1α), directly communicate with sensory nerves via activation of high-affinity receptors. Recent studies have shown that two members of the IL-6 cytokine family, IL-31 and oncostatin M (OSM), are important in T-cell-induced neuronal communication. IL-31 receptors are constitutively expressed on the surface of keratinocytes, eosinophils, and small diameter neurons. Nemolizumab (CIM331), a humanized monoclonal antibody against IL-31 receptor A, inhibits IL-31 signaling with improvement in pruritus when administered to patients with atopic dermatitis supporting the role of IL-31 in the pathogenesis of pruritus in this disease.

Mo-WS3-2
Critical role of CCR7 in peripheral tolerance to CD4+ T cells specific for desmoglein 3 (Dsg3), an autoantigen in pemphigus vulgaris

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A dogma of immunological tolerance has been established with transgenic mice using keratin promoters, which express neoantigens, not only in thymic epithelial cells, but also in epidermal cells in the periphery. In this study we focused on the peripheral tolerance to desmoglein 3 (Dsg3), the autoantigen in pemphigus vulgaris, and investigated the fate of Dsg3-specific T cells in two different settings. One is a thymus-transplanted chimeric model, in which Dsg3-/- thymus was transplanted to nude mice to create a unique condition where Dsg3 is expressed not in thymus but in the periphery. Then bone-marrow cells were transferred from Dsg3-specific TCR transgenic (Dsg3H1-Rag2-/-) mice to the chimeric mice. Dsg3-specific CD4+ T cells were transferred from Dr3H1T cells fully developed in the Dsg3-/- thymus, whereas the number of Dsg3H1T cells was significantly reduced in the spleen and lymph nodes of the chimeric mice when compared with Dsg3-/- recipient mice. The other is an adoptive transfer model of peripheral T cells from Dsg3H1-Dsg3-/- mice, in which Dsg3H1T cells exist in the periphery, to wild type mice. CSFE-labeled Dsg3H1T cells first proliferated on day 3, but sequentially disappeared on day 14. These models indicate the deletion of Dsg3-specific T cells in the peripheral lymphoid organs. Considering importance of antigen-presenting cells (APCs) in the deletion mechanism, we used CCR7-/- mice as recipients for further analysis, where CCR7-dependent migration of APCs is disturbed. In CCR7-/- recipients, Dsg3H1T cells showed only limited proliferation on day 3 and remained on day 14, indicating that CCR7 is critically involved in the process. To summarize these results, the skin as a peripheral tissue harbors a mechanism regulated by CCR7 to prevent harmful response of autimmune T cells. This experimental model provides a useful tool to dissect the detailed mechanism of peripheral tolerance.

Mo-WS3-3
Pathogenesis of autoreactive Th17 cells is driven by homeostatic cytokines stimulated by commensal microbiota

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Auto-reactive T cells are kept tolerant in the body by multiple mechanisms. How they are activated and precipitate tissue specific autoimmunity; especially the cytokine(s) that support their survival,
expansion and attack on self-tissues is not fully understood. To explore this question, we established a simplified model system. A transfer of fully differentiated, pure T-helper17 (Th17) cells with skin-reactive TCR (Th17 DSG3 H1 TCR Tg) into sub-lethally irradiated recipients, reproducibly caused severe autoimmune dermatitis. Intriguingly, the expansion of transferred auto-reactive Th17 and the penetrance of dermatitis in this model was abrogated by treating the recipients by cocktail of antibiotics which is known to deplete wide spectrum of commensal microbiota. To understand how microbiota helps expansion of self-reactive Th17, we compared homeostatic cytokines important for T cell homeostasis (IL-7, TSLP, IL-15, TGFβ) from various organs before and after antibiotics treatment. Our data indicated that production of these cytokines from organs are stable at homeostatic condition, but drastically change after antibiotic treatment, which influences expansion and survival of the fully differentiated autoreactive Th17. Thus, commensal microbiota interacts with, and stimulates cytokine expression from organs that support systemic T cells response, which can result in autoimmune symptoms.

Mo-WS3-4

IL-17E activates M2 macrophages to produce IL-8 and favors the recruitment of neutrophils in psoriatic skin

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Psoriasis vulgaris is a common chronic recurrent immune-mediated skin disease. We have recently found that IL-17E (i.e IL-25), a member of the IL-17 cytokine family, is over-expressed in lesional psoriatic skin when compared to non lesional and healthy donors. Within the psoriatic plaque, macrophages having a mixed M1/M2 phenotype internalize IL-17E in a receptor induced clathrin-mediated mechanism. In this study we investigated the biological effects of IL-17E in psoriasis. In vitro M2-polarized macrophages, but not M1, responded to IL-17E by producing inflammatory cytokines (e.g. TNF and IL-6 (p < 0.03)) and chemokines (e.g. IL-8 and MCP-1 (p < 0.03)) typical expressed by M1 cells. Nuclear factor-kappa B (NF-κB), p38 and STAT3 were required for generating the IL-17E-dependent effects. Of note, IL-17E did not stimulate the production of cytokines/chemokines involved in T cell polarization and recruitment. Supernatants of IL-17E-stimulated M2 macrophages contained high levels of IL-8 and favored the attraction of neutrophils to a higher extent compared to supernatants of resting macrophages. Chemical p38 inhibition impaired IL-8 production and neutrophil chemotaxis in vitro (p < 0.05). In vivo, intra-dermal injection of rml-IL17E in BALB/c mice induced severe dermal inflammation as assessed by immunohistological and FACS analysis, with increased neutrophil/eosinophil infiltration and reduced T-cell recruitment, compared to saline control group (p < 0.02). Consistent with the in vivo and in vitro data, the number of IL-17E+ cells in lesional skin correlated with the number of neutrophils (p = 0.05), while being inversely proportional to the number of infiltrating T cells (p = 0.01). Together, our data show that IL-17E favors the preferential recruitment of neutrophils via macrophage activation and define a novel pro-inflammatory role of IL-17E in psoriasis.

Mo-WS3-5

IL-10 derived from regulatory T cells in the skin limits immune responses in percutaneous sensitization

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The skin contains a substantial number of Foxp3-expressing regulatory T cells (Tregs) even in a steady state in both mice and human. Previous studies collectively suggest that Tregs activated in response to skin-related antigens in the skin-draining lymph nodes (dLNs) accumulate and reside in the skin, thereby preventing the spontaneous development of skin inflammation. However, it remains unclear whether and how Tregs distributed in the steady-state skin regulate immune responses to percutaneous sensitization with foreign antigens.

To address this issue, we established an experimental procedure for selectively eliminating cutaneous Tregs. Intradermal injection with low-dose diphtheria toxin (DT) into the ear skin of Foxp3DTR mice, in which Foxp3+ Tregs specifically express the diphtheria toxin receptor, significantly reduced Tregs only in the treated skin. Sensitization with hapten through Treg-deficient skin resulted in increased lymph node migration and enhanced T-cell stimulatory capacity of antigen-bearing dendritic cells (DCs) as well as enhanced development of IFN-gamma-producing CD8+ T cells in the dLNs. Cutaneous Treg depletion at the sensitization site also enhanced immune responses to NP-OVA including differentiation of follicular helper T cells and NP-specific germinal center B cells and plasma cells. We found that about half of Treg cells expressed IL10 in the skin under homeostatic condition while few Foxp3 conventional T cells did. Thus, next we investigated the role for IL-10 in cutaneous Treg-mediated suppression by generating mixed bone marrow chimeras in which only skin-resident Tregs are deficient in IL-10. Those chimeras showed increased DC migration into the dLN after sensitization with hapten, indicating that IL-10 derived from cutaneous Tregs was essential for suppression of DC maturation.

In conclusion, Tregs-residing in the skin are essential for preventing excessive immune responses to percutaneous sensitization by suppressing dermal DC migration and maturation via IL-10.

Mo-WS3-6

Interleukin-31 Modulates Cutaneous Th2 Inflammation

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T-helper 2 (Th2) cells are important mediators of atopic inflammation. In addition to canonical Th2 cytokines (IL-4, -5, and -13), Th2 cells recruited to skin produce IL-31, a lesser-known cytokine associated with itch. IL-31 is detectable in skin biopsy specimens from patients with ichthyoskinflammatory skin diseases including atopic dermatitis (AD). IL-31-transgenic animals develop spontaneous scratching behaviors and eczematous skin lesions. We generated IL31-deficient animals to better define the contribution of IL-31 to Th2-driven skin inflammation. Can IL-31 regulate pro-inflammatory cytokines in addition to its direct actions on cutaneous nerves? We hypothesized that IL31-deficient mice would display reduced susceptibility to Th2-mediated inflammation in models of AD-like dermatitis. We find that exuberant CD45 + infiltrates are recruited to WT and IL31-deficient skin in response to both epithelial alarmin release and antigen-specific challenge. Surprisingly, alterations in CD4 T cell sub-populations differ by challenge type. We conclude that IL-31 plays a dynamic, non-redundant role in feedback regulation of Th2 inflammation in skin.
Cytokines that exert their biological effect by activating the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway play a central role in inflammatory processes demonstrated by the clinical efficacy of JAK inhibitors in several chronic inflammatory diseases, including psoriasis.

With the purpose to support the development of a panJAK inhibitor for topical treatment of inflammatory skin diseases, we correlated the response of a proximal target engagement biomarker to distal inflammatory endpoints in in vivo pharmacology studies using the disease-relevant psoriasis xenograft mouse model. Briefly, skin keratome biopsies were obtained from active lesions of untreated patients with psoriasis vulgaris and grafted onto immune compromised mice. Target (JAK) activity and inflammatory endpoints were assessed in the psoriasis skin by the level of STAT3 phosphorylation and epidermal thickness and expression of keratin-16, respectively.

We used this model to test the efficacy of selective panJAK inhibitors derived from different chemical series. Engraffed skin was topically treated with test compounds twice daily for either 2.5 days (“mechanistic model”) or 28 days (“efficacy model”) to investigate treatment effect on target activity and downstream inflammatory processes, respectively. Interestingly, compared to vehicle-treated controls, significant inhibition of target activity was observed for some but not all tested compounds, which were largely equipotent, indicating that other factors, such as skin penetration, were affecting target engagement. Further, only the compounds with significant effect on target activity were found to have significant anti-inflammatory effect in the chronic inflammatory efficacy model. The results clearly indicate a direct translation between the short mechanistic model and the longer efficacy model in addressing drug responses.

In summary, we have established a short and predictive mechanistic mouse model to address modulation of JAK activity. This model has enabled us to efficiently identify and select which JAK inhibitors to progress for further development.

Mo-WS3-7

Establishment of a short and predictive mechanistic mouse model to support the development of topical JAK inhibitors

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Mo-WS5-1

Genetics of familial inflammatory disorders

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Inflammatory disorders are caused by interplay between genetic and environmental factors. The genetic studies of familial diseases caused by single gene mutation could provide crucial information about molecular basis of human disorders. In that sense, we have tried to identify the causative genes for familial inflammatory disorders. We have identified NLRC4 as a causative gene for familial cold-induced autoinflammatory disorders. The mutation in NLRC4 facilitated the oligomer formation of NLRC4, which increased IL-1b production and pyroptosis. The transgenic mice in which mutant Nlrc4 is expressed under invariant chain promoter developed severe inflammatory responses. The inflammatory response were partly suppressed by deleting Il1b or Il18 gene. The prouction of IL-18 is largely dependant on IL-1b and IL-18 would be crucial for causing macrophages activating pyroptosis. The transgenic mice in which mutant Nlrc4 is expressed under invariant chain promoter developed severe inflammatory response were partly suppressed by deleting Il1b or Il18 gene. The production of IL-18 is largely dependant on IL-1b and IL-18 would be crucial for causing macrophages activating pyroptosis.

Mo-WS3-2

Mutation of arginine 285 in IRF3 to glutamine selectively impairs activation of IRF3 by STING and TRIF dependent pathways

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The detection of viral infections is initiated by pattern recognition receptors (PRRs) that signal through the downstream adaptors MAVS, TRIF and STING to activate interferon regulatory factor 3 (IRF3). IRF3 is a positive regulator of both Type I and Type III interferons (IFNs) as well as a series of antiviral genes. IRF3 is activated when a positively charged surface on IRF3 is bound by a phosphorylated adaptor. Several recent studies have focused on the functional significance of the positively charged surface on IRF3, which is also the focus of this project. We originally identified the R285Q mutation in a patient suffering from herpes simplex encephalitis (HSE) who exhibited a markedly reduced response to infection with herpes virus but not to the RNA viruses we tested (Andersen, LL, et al., J Exp Med, 2015). As this observation is contradictory to the standard perception of IRF3 function, we decided to investigate the mechanism by which IRF3 is activated by the different adaptor proteins in more details. Basic surface residues within the region of IRF3 involved in docking to the adaptors were mutated and the effect of these mutations on the activation of IRF3 by each of the three adaptors was measured. The R211Q mutation led to an almost complete loss of IRF3 activity regardless of which pathway was used to activate IRF3. In contrast, the R285Q mutation resulted in a strong loss of activity when activated through both TRIF and STING but much less so when activated through MAVS. These observation are in agreement in what we found in the patient and suggest differences in how the three adaptors, MAVS, TRIF and STING, activate IRF3.

Mo-WS5-3

ADAR1 Deficiency Linked to Aicardi-Goutiéres Syndrome Causes Cell Death from RNase L Activation

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ADAR1 isoforms are adenosine deaminases that edit and destabilize double-stranded RNA reducing its immunostimulatory activities. Mutation of ADAR1 leads to a severe neurodevelopmental and inflammatory disease of children, Aicardi-Goutiéres Syndrome (AGS). In mice, Adar1 mutations are embryonic lethal but are rescued by mutation of the Mda5 or Mavs genes, which function in IFN induction. However, the specific IFN regulated proteins responsible for the pathogenic effects of ADAR1 mutation are unknown. We will show that the cell-lethal syndrome. I would like to discuss the molecular mechanism of NLRC4-mediated diseases and other hereditary inflammatory disorders.
Interestingly, IL-21 can also drive STAT3, which can result in the opposing actions when they activate different STAT proteins. Our result demonstrate that ablation of RNase L activity promotes survival of ADAR1 deficient cells even in the presence of MD5 and MAVS, suggesting that the RNase L system is the primary sensor pathway for endogenous dsRNA that leads to cell death. We will also show that a novel small molecule inhibitor of RNase L rescues ADAR1 deficient cells from cell death. These studies may therefore contribute to identifying therapeutic strategies for AGS patients with mutations in ADAR1.

Mo-WS5-4

γc Family Cytokines, Immunodeficiency, and the Fine-tuning of Cytokine Signaling

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The γc family of cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) collectively regulate normal development, growth, differentiation, and survival of lymphocytes. When dys-regulated signaling occurs, this can result in disease, including for example, severe combined immunodeficiency and autoimmunity. Like other type I cytokines, γc family cytokines activate STAT proteins and different cytokines can exhibit opposing actions when they activate different STAT proteins. Interestingly, IL-21 can also differentially use either STAT1 versus STAT3, which can result in the fine-tuning of signals induced by IL-21. Moreover, IL-2 can induce STAT5 dimerization versus tetramerization as another physiological mechanism for fine-tuning signaling. STAT5 tetramerization is critical for normal proliferation of T cells in vitro and in vivo and for the development of normal numbers of CD8 T cells and natural killer cells. In the absence of STAT5 tetramers, NK cells exhibit dys-regulated expression of genes involved in survival and CD8 T cells exhibit defective regulation of genes involved in cell cycle progression. In addition to these physiological mechanisms of fine-tuning, with Chris Garcia’s lab at Stanford, we have created and studied novel IL-2 partial agonists that can fine tune IL-2 signaling. These molecules can attenuate receptor heterodimerization and inhibit STAT5 activation and gene expression, with one of these molecules having the ability to augment survival of animals in a mouse model of graft versus host disease and to inhibit the proliferation ex vivo of leukemic cells from patients with the chronic-smoldering form of adult T cell leukemia. Thus, we provide both physiological and pharmacological ways of fine-tuning signaling of γc family cytokines to regulate the immune system.

Mo-WS5-6

Gain of function of MD5 in CD11c-expressing cells is sufficient to induce lupus-like nephritis

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MD5 is an essential cytoplasmic viral RNA sensor to induce type I interferon (IFN). Previously we reported that MD5 G821S mutant heterozygous (Ifhi1B+/-) mice spontaneously develop lupus-like nephritis in an IFN dependent manner. Considering that the gain of function mutation in IFHI encoding human MD5 protein has also been found in SLE patients, this Ifhi1B+/- mice has the potential to be a good tool to exploit clinical treatments for SLE patients with MD5 mutations. However, more precise mechanisms of the pathogenesis remain to be determined.

In order to elucidate the contribution of CD11c-positive dendritic cells (DCs), we generated conditional knock-in (cKI) mice expressing...
MDA5 G821S mutant protein in desired tissues. Although CAG-cKI mice with ubiquitous expression of Cre by CAG promoter activity exhibited immune complexes deposition and mesangial matrix proliferation as early as 4 weeks, they died before 6 weeks old. Interestingly, CD11c-cKI mice survived more than half a year with lupus-like nephritis, although the onset was delayed with less severe phenotype. Further analysis in CD11c-cKI mice revealed that splenic plasmacytoid DCs produced high amounts of IFN compared with other immune cells. Flowcytometric analysis revealed that the DCs are activated as judged by elevated expression of CD86. Furthermore, expression level of CD69 as a lymphocyte activation marker was increased in splenic T and B cells.

In accord with this, we observed autoantibody production in the serum.

Using conditional IL-4/IL-13-deficient mice and mixed bone marrow chimeras we investigated the contribution of different IL-4/IL-13-producing cell types for type 2 immune responses in vivo. We observed that expression of IL-4 by T cells outside the B cell follicle was sufficient to induce GC formation and efficient IgE and IgG1 switching in the context of a type 2 immune response. Further, T cell-derived IL-4/IL-13 was required for alum/OVA-induced accumulation of ILC2s and allergic inflammation of the lung but dispensable for elimination of gastro-intestinal helminths. Basophils played an important role for IgE-mediated allergic skin inflammation and protective immunity against secondary helminth infections which required IgE-elicted release of IL-4/IL-13 from basophils. To further investigate the IL-4/IL-13-elicted effector pathways in vivo, we generated mice which express a Cre-inducible and constitutively active version of STAT6. Crossing these mice to VillinCre mice resulted in spontaneous goblet cell hyperplasia and increased numbers of tuft cells in the intestinal epithelium. Expression of activated STAT6 in intestinal epithelial cells was sufficient for worm expulsion in the absence of CD4 T cells.

Mo-WS2-1

Regulation of type 2 immune responses by components of the innate and adaptive immune system

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Allergic inflammation and infection with helminths often elicit a strong type 2 immune response characterized by increased numbers of IL-4/IL-13 producing cells of the innate and adaptive immune system. Using conditional IL-4/IL-13-deficient mice and mixed bone marrow chimeras we investigated the contribution of different IL-4/IL-13-producing cell types for type 2 immune responses in vivo. We observed that expression of IL-4 by T cells outside the B cell follicle was sufficient to induce GC formation and efficient IgE and IgG1 switching in the context of a type 2 immune response. Further, T cell-derived IL-4/IL-13 was required for alum/OVA-induced accumulation of ILC2s and allergic inflammation of the lung but dispensable for elimination of gastro-intestinal helminths. Basophils played an important role for IgE-mediated allergic skin inflammation and protective immunity against secondary helminth infections which required IgE-elicted release of IL-4/IL-13 from basophils. To further investigate the IL-4/IL-13-elicted effector pathways in vivo, we generated mice which express a Cre-inducible and constitutively active version of STAT6. Crossing these mice to VillinCre mice resulted in spontaneous goblet cell hyperplasia and increased numbers of tuft cells in the intestinal epithelium. Expression of activated STAT6 in intestinal epithelial cells was sufficient for worm expulsion in the absence of CD4 T cells.

Mo-WS2-2

Inhibition of house dust mite-induced Th2 responses by Allergin-1 immunoreceptor on dendritic cells

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House dust mite (HDM) is a major allergen for allergic asthma mediated by T helper 2 (Th2) cells. However, the mechanism underlying HDM-induced Th2 responses remains incompletely understood.

Allergin-1 is an inhibitory immunoreceptor expressed on mast cells (MCs) and inhibited FcεRI-mediated signaling, resulting in suppression of anaphylaxis in mice (Hitomi et al, Nat Immunol., 2010). In this study, we demonstrate that Allergin-1 is also expressed on lung CD11b+ DCs and suppresses HDM-induced DC activation for Th2 responses. Allergin-1-deficient mice showed enhanced HDM-induced allergic airway inflammation and serum titer of IgE. CD11b+ DCs derived from the lung of HDM-administrated Allergin-1-deficient mice expressed higher mRNA levels of prostaglandin E synthase (Ptges) and cyclooxygenase 2 (Cox2), via the TLR4/MyD88 signaling pathway, which mediate PGE2 production. Addition of celecoxib, a selective COX2 inhibitor, in the coculture of HDM-administrated Allergin-1-
deficient lung CD11b<sup>+</sup> DC with naive CD4<sup>+</sup> T cells returned the increased IL-4 to the level in the coculture of WT DC with naive CD4<sup>+</sup> T cells. Moreover, transfer of PtgEs-knockdowned Allergin-1-deficient DCs into DC-depleted mice showed ameliorated HDM-induced airway inflammation compared with transfer of control shRNA-transduced Allergin-1-deficient DCs. Thus, Allergin-1 suppresses HDM-induced PGE2 production from lung CD11b<sup>+</sup> DCs and regulates Th2 responses.

Mo-WS2-3

**IL-22 induces Reg3γ production from lung epithelial cells and inhibits allergic airway inflammation in house dust mite-induced asthma models**

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**Background:** While the pathogenesis of asthma is mainly orchestrated by antigen-specific Th2 cells and their cytokines, recent findings indicate the involvement of other subsets of helper T cells and their cytokines. We and others have recently shown that IL-22 has regulatory roles in allergic airway inflammation; however, the underlying mechanisms remain to be determined.

**Methods:** Airway inflammation was provoked by intratracheal administration of house dust mite (HDM) extract in wild-type (WT) and IL-22-deficient (IL-22<sup>−/−</sup>) mice, and then evaluated by measuring the number of inflammatory cells in bronchoalveolar lavage fluid (BALF) and cytokine production from T cells. We also analyzed cells that produce IL-22 in the lung in HDM-induced allergic inflammation. In addition, we searched for molecules whose expression is regulated by IL-22 in the lung epithelial cells by RNA-seq analysis. Finally, we evaluated the roles of Reg3γ, one of IL-22-induced genes, in the regulation of HDM-induced allergic airway inflammation.

**Results:** IL-22<sup>−/−</sup> mice exhibited significantly enhanced eosinophil, CD4<sup>+</sup> T cell, and neutrophil recruitment into BALF, and IL-5, IL-13, and IL-17 production from draining lymph node lymphocytes as compared with WT mice. IL-22 was mainly produced by CD4<sup>+</sup> T cells in the lung. IL-22 induced Reg3γ production from lung epithelial cells and that neutralization of Reg3γ significantly exacerbated HDM-induced eosinophilic airway inflammation and Th2 cytokine production. Moreover, exostatin-like 3 (EXTL3), a functional Reg3γ-binding protein, is expressed in lung epithelial cells, and intratracheal administration of recombinant Reg3γ suppressed HDM-induced TSLP and IL-33 expression and accumulation of type 2 innate lymphoid cells in the lung.

**Conclusions:** IL-22 induces Reg3γ production from lung epithelial cells, and inhibits the development of HDM-induced allergic airway inflammation possibly by inhibiting TSLP and IL-33 production from lung epithelial cells.

Mo-WS2-4

**The transcriptional repressor Bach2 controls Th2-type immune response via interaction with Batf**

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**Introduction:** Bach2 is a transcriptional repressor that plays a critical role in regulating Th2-type immune response. However, the underlying molecular mechanisms remain unclear.

**Methods:** Bach2<sup>lox/lox</sup> mice and Batf<sup>lox/lox</sup> mice were crossed with CD4-Cre TG mice to generate T cell-specific gene manipulation mice (Batf<sup>KO</sup>: Bach2<sup>lox/lox</sup> × CD4-Cre TG, Batf<sup>KO</sup>: Batf<sup>lox/lox</sup> × CD4-Cre TG). Bach2 interaction protein(s) were identified by using an AlphaScreen with cell-free technology for protein synthesis.

**Results:** We screened Bach2 interaction protein(s), and Batf family transcription factors (Batf and Batf3) were identified by possible candidates. Binding of Bach2-Batf complex to the AP-1 consensus oligonucleotide was detected by an oligonucleotide precipitation assay. A ChIP-sequencing revealed that the major enriched motifs for Bach2-binding contain Batf and Irf4 binding motifs. The bindings of Batf and Irf4 to the Bach2 binding regions were increased in Bach2 KO effector CD T cells suggesting that Bach2-Batf complex inhibit the recruitment of the Batf-Irf4 complex. Furthermore, we found that Bach2 associates with the DNase 1 hypersensitive region (RHS6) at the control region of the Th2 cytokine gene loci. Increased bindings of Batf-Irf4 complex to the Bach2 binding regions and a rise of active enhancer mark of histone H3 (K27 acetylation level) were validated in Bach2 KO effector CD T cells. Moreover, the deletion of Batf gene improved the Th2-type lung inflammation in T cell-specific Bach2 KO mice.

**Conclusion:** Bach2-Batf interactions are required to prevent an excessive Th2 response and subsequent development of Th2-type lung inflammation.

Mo-WS2-5

**The 3D structure of the human IL-3 receptor complex and a novel mode of cytokine signalling**

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The human IL-3 receptor is a classic Type 1 cytokine receptor that signals in normal and malignant haemopoiesis. Composed of a specific alpha chain and a beta subunit which it shares with the GM-CSF and IL-5 receptors, the IL-3 receptor dimerises in the presence of IL-3 at the cell surface and triggers JAK-2 activation and other intracellular biochemical and signalling events which, once integrated, result in the...
stimulation of many cellular functions such as haemopoietic cell proliferation and differentiation⁴.

We and others have found that the IL-3 receptor signalling complex is dysregulated in malignant haemopoiesis. For example, the IL-3 receptor alpha chain (CD123) is overexpressed in acute myeloid leukaemia⁵ and chronic myeloid leukaemia progenitor cells⁶ providing a biological advantage⁷ whilst at the same time presenting a therapeutic opportunity to selectively target the leukaemic clones with monoclonal antibodies to CD123 such as CSL362⁸ and others. Similarly with JAK-2, a kinase that is physically associated with the IL-3 receptor beta chain⁹. The JAK-2 mutant V617F is constitutively active and accounts for some of the abnormalities described in myeloproliferative disorders, although the molecular events linking this mutation to biological outcomes remain to be elucidated.

We have now molecularly characterised human IL-3 receptor signalling by a combination of X-ray crystallography, proteomics, and a functional approach that selectively targets JAK-1 and JAK-2. Evidence will be presented that the full extracellular ternary IL-3 receptor complex assembles as a dodecamer, analogous, yet distinct, from the GM-CSF receptor dodecamer complex. Furthermore, intermediate forms of receptor assembly are observed that recruit a distinct set of the full IL-3 receptor signalling machinery. Finally, a sequential model of receptor activation emerges which reveals the mode of action of JAK-1 and JAK-2 in this signalling complex. These results have profound implications for the design and clinical management of human hematopoietic malignancies.

Mo-WS2-7

A unique DAMP with IL-33-inducing activity increases IL-33-expressing alveolar epithelial type II cells in lungs and induces primary cultured fibroblasts to produce IL-33 in vitro

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IL-33, a member of IL-1 family cytokine, is synthesized as a full-length active form, stored in the nucleus of epithelial cells, and released when the cells are damaged mechanically or become necrotic. IL-33 has the capacity to stimulate Th2 cells, mast cells, basophils, eosinophils and ILC2s to produce Th2 cytokines. Thus, IL-33 plays an important role in induction of allergic diseases. We previously reported that Strongyloides venezuelensis (S. venezuelensis) infection induces production of IL-33 in the lungs, which induces ILC2s to proliferate and to produce IL-5 and IL-13. Thus, Rag2−/− mice infected with S. venezuelensis develop eosinophilic inflammation and goblet cell hyperplasia in their lungs (Loeffler’s syndrome). Therefore, IL-33 production in the lungs is important for induction of allergic responses, while the mechanism for induction of IL-33 production is totally unknown. We noticed massive hemorrhagic areas in the lungs at day 5 after S. venezuelensis infection. Thus, we speculated that such macroscopic or microscopic injury might induce production of damage associated molecular pattern (DAMP), which in turn increases the production of IL-33 in the lungs. Here, we demonstrated lung tissue extracts contain DAMP, which induces IL-33 production in the lungs. We performed purification of DAMP with IL-33-inducing activity (IL33ia) from the lung tissues. Highly purified DAMP is 20kDa protein. Intranasal administration of the DAMP induces an increase in the number of IL-33-expressing alveolar type II cells (ATII) in the lungs. DAMP also stimulates primary fibroblasts to produce IL-33 in vitro. We prepared recombinant DAMP, which recapitulates IL33ia of natural DAMP. Many organs constitutively express this DAMP. We can induce DAMP with IL33ia in the peritoneal cavity by injection of alum. At day 7 after alum injection, we can harvest IL33ia and IL-33 in alum/cells aggregate. We will present our current data about new DAMP with IL33ia.

Mo-WS2-8

Roles of T-bet in ILC2-mediated eosinophilic airway inflammation

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Background: Innate lymphoid cells (ILC) are new subsets of immune cells that produce large amounts of cytokines upon cytokine and/or alarmin stimulation. Recent studies have shown that T-bet plays pivotal roles in the development of ILC3s and ILC1s; however, the roles of T-bet in lung ILC2s remain unknown.

Mo-WS2-6

Interleukin-33 activation of basophils promotes type 2 immunity to house dust mite challenge

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Allergic asthma is an inflammatory disease characterized by lung eosinophilia and airway obstruction controlled by type 2 cytokines. Recently, the importance of epithelial barrier cells and epithelial derived cytokines, like IL-33 and GM-CSF, in the initiation of allergic disease have been shown by administration of protease allergens as house dust mite (HDM) and papain. The importance of epithelial derived cytokines has further been demonstrated using IL-33−/− mice, in which IL-33 was found to be essential for T-cell-independent protease allergen induced airway inflammation. Meanwhile, innate sources of IL-4, IL-5 and IL-13, like basophils and type 2 innate lymphoid cells (ILC2s) have been shown to harbour the potential to induce asthma-like airway inflammation. Recently, it was found that in response to the protease allergen papain there is cooperation between IL-33-activated basophils and ILC2s, in which basophil-derived IL-4 boosts the terminal differentiation and activation of ILC2s. These data clearly demonstrate that basophils are implicated in allergy and asthma; however, how exactly basophils mediate HDM-induced eosinophilic exacerbation remains elusive. Using different depletion strategies, we found that conditional depletion of basophils, solely during the effector phase of the HDM-induced allergic response, resolved airway eosinophilia. Surprisingly, we could not observe a prominent role for ILC2’s in our model. These results demonstrate a previously unrecognized role for IL-33-activated basophils, independently of ILC2’s, in driving the effector phase of HDM-induced allergic airway inflammation.
**Purpose:** Purpose of this study is to determine the role of T-bet in ILC2-mediated airway inflammation.

**Methods:** The expression of T-bet in lung ILCs (defined as Thy1.2+Lin- cells) was examined. The roles of T-bet in the development of lung ILCs and airway inflammation induced by IL-33 administration were examined by using T-bet-deficient (T-bet-/-) mice. Gene expression profiles of T-bet-/- lung ILCs were analyzed by RNA sequencing. Roles of IL-9 produced by ILC2s were examined by neutralization of IL-9.

**Results:** T-bet was expressed in lung ILC2s (defined as Thy1.2+Lin- cells expressing ST2 or CD25) and IFN-g enhanced its expression. While the development of lung ILC2s at steady-state conditions was normal in T-bet-/- mice, IL-33-induced accumulation of lung ILC2s and eosinophilic airway inflammation were exacerbated in T-bet-/- mice. The exacerbated accumulation of ILC2s and eosinophilic airway inflammation by the absence of T-bet was evident even in a RAG2-/- background, suggesting that T-bet expressed in non-T/non-B population is involved in the suppression of IL-33-induced eosinophilic airway inflammation. Transcriptome analysis revealed that IL-9 expression was up-regulated in IL-33-stimulated lung ILCs in T-bet-/- mice compared with that in wild-type mice. Importantly, neutralization of IL-9 markedly attenuated IL-33-induced accumulation of lung ILC2s and eosinophilic inflammation in T-bet-/- mice.

**Conclusion:** T-bet suppresses IL-9 production from lung ILC2s and thereby inhibits IL-33-induced eosinophilic airway inflammation.

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**Mo-WS4-1**

**Posttranscriptional control of pro-inflammatory cytokine expression by Regnase-1 and Roquin**

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Proinflammatory cytokines are produced by innate immune cells upon sensing of pathogens by Toll-like receptors (TLRs). The expression of proinflammatory cytokines is controlled at the transcriptional and posttranscriptional levels. Posttranscriptional control of cytokine mRNA is critical for preventing excess and persistent production of cytokines by degrading them via a set of RNA binding proteins (RBPs) recognizing cis-elements present in the mRNA 3'-untranslated region. Among RBPs, Roquin recognizes stem-loop structures present in mRNAs encoding inflammatory proteins and degrades them by recruiting a CCR4-NOT deadenylase complex to its target mRNAs. Roquin-mutant mice spontaneously develop autoimmunity, possibly through enhanced GC formation in murine autoimmune diseases as compared to male mice, while the levels of IgG reactive autoantigens were not significantly different in males vs. females. Our previous gene expression data showed that interferon signaling was significantly upregulated in female ARE-Del-/- mice, while the levels of IgM reactive autoantigens were not significantly different between male and female ARE-Del-/- mice. Therefore, the interplay of type I and type II interferons is critical for the sex-biased autoimmune response, possibly through enhanced GC formation in murine autoimmune cholangitis.

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**Mo-WS4-2**

**The Dark Side of Interferon-gamma**

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The sex-bias in major autoimmune diseases is well known but the mechanisms are not well understood. We recently reported that chronic IFN-gamma (IFNg) expression via deletion of the IFNg 3' UTR AU-rich element (ARE-Del) mimics human primary biliary cholangitis (PBC) with a female predominance. There are female-biased immune functions including an elevated CD4/CD8 T cell ratio in the liver and spleen compared to male mice, and transfer of CD4+ T cells from ARE-Del-/- mice to female wild type mice induced moderate portal inflammation. Furthermore autoantigens, identified using peptide arrays, indicates that female ARE-Del-/- mice have a high induction of IgM reactive autoantigens associated with autoimmune diseases as compared to male mice, while the levels of IgG reactive autoantigens were not significantly different between male and female ARE-Del-/- mice. Therefore, the interplay of type I and type II interferons is critical for the sex-biased autoimmune response, possibly through enhanced GC formation in murine autoimmune cholangitis.

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**Mo-WS4-3**

**The importance of CGAMP horizontal transfer in DNA damage-driven inflammation**

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DNA damage is a potent inducer of inflammation. While this is well accepted, with the example of UV-induced DNA damage, a detailed understanding of the machineries at play in this inflammatory effect remains elusive. We have recently discovered that DNA damage
promoted by enzymatic recombination and DNA intercalation resulted in induction of type-I interferon (IFN) responses in various cell types through activation of the cytosolic cyclic GMP-AMP synthase (cGAS) pathway (Pepin et al., Nucleic Acids Research 2016, and Pepin et al., Nucleic Acids Research 2017). In the present work, we identify a novel role for viral oncopgenes in the facilitation of cGAS sensing and IFN production following DNA damage promoted by genotoxic agents and UV exposure. We also demonstrate that UV-driven DNA damage can propagate inflammation horizontally to adjacent cells through gap junction transfer of cyclic GMP-AMP (cGAMP). Our on-going studies collectively suggest a critical role for the cGAS pathway in the transcriptional regulation of IFN responses to DNA damage, potentially at play in UV-driven skin inflammation and systemic flares seen in systemic lupus erythematosus. Because drugs approved for use in humans can inhibit this horizontal amplification of inflammation, our work has the potential to help address select diseases where DNA damage is at the root of aberrant inflammation.

Mo-WS4-4

Malonylation as a novel inflammatory signal in macrophages

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Malonylation is a recently uncovered protein post-translational modification. It involves the attachment of a malonyl-CoA-derived malonyl group to lysines, resulting in an extra 86 Da mass, as well as a change in charge comparable to that of phosphorylation. Given the increasing number of links between the metabolic status and the function of immune cells, it led us to investigate whether protein malonylation might be a new mechanism by which the cell’s metabolism can control the immune response.

We have been able to show that protein malonylation can be induced in macrophages following activation. We have also been able to identify via mass spectrometry a wide array of proteins undergoing this post-translational modification, one of which is glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Although it is most widely known for its role as a glycolytic enzyme, GAPDH has blossomed in recent years from its mundane use as an endogenous control into an increasingly interesting multi-functional protein. Through the use of a GAPDH enzymatic activity inhibitor, and siRNA knockdown, we have shown that GAPDH activity is needed for the production of cytokines in macrophages, including TNFa, IL6 and IL1β. However, especially in the case of TNFa, the requirement for GAPDH goes beyond its glycolytic activity. GAPDH can bind TNFa mRNA, as well as other pro-inflammatory mRNAs, and block their translation. Following activation of macrophages with lipopolysaccharide (LPS), GAPDH can release these mRNAs and enable their translation.

Our data would suggest that there is a specific lysine within GAPDH catalytic domain that undergoes LPS-induced malonylation. By generating a malonylation-mimic mutant of this lysine, GAPDH activity is boosted while its binding to TNFa mRNA is inhibited. Overall, our results suggest that malonylation has important signalling functions in macrophages and can act as a key regulator of cytokine production and inflammation via GAPDH.

Mo-WS4-5

The protein kinase RIOK3 suppressed MDA5-dependent innate immune response

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Innate immune response is the first line of host defense against infection. MDA5 is one of the RIG-I-like receptors (RLRs), which are cytoplasmic viral RNA sensors and triggers the rapid innate immune response after viral infection. MDA5 recognizes relatively long dsRNA, leading to the multimer formation of MDA5 along the dsRNA required for activating the MAVS adaptor. In response to viral infection, MDA5 is activated by protein phosphatase 1α / γ-mediated dephosphorylation of the MDA5 CARDs.

Thus, the preceding phosphorylation of de novo MDA5 prevents excessive activation of MDA5. We previously identified RIO kinase 3 (RIOK3) as a kinase targeting MDA5 using yeast two-hybrid screening. Our studies revealed that RIOK3 phosphorylates Ser-828 in MDA5 C-terminal region. Moreover, we found that RIOK3-mediated phosphorylation interferes with MDA5 multimer formation and suppresses its signaling. These findings suggest that switching between phosphorylation and dephosphorylation in both N-terminal and C-terminal region is a key factor in MDA5 functional regulation.

To examine the role of RIOK3 in vivo, we recently established RIOK3 knockout mice with CRISPR-Cas9 systems. In mouse embolic fibroblast (MEF) and bone marrow-derived macrophage (BMM) but not bone marrow-derived dendritic cell (BMDC), knockout of RIOK3 increased type I IFN expression in response to EMCV infection or poly(I:C) stimulation, which are recognized by MDA5. On the other hand, the innate immune response against VSV infection or LPS, dsDNA, 2’-3’-cGAMP stimulation was comparable between wild and RIOK3-/- MEFs. Taken together, these data suggest that RIOK3 works as a negative regulator of MDA5-mediated signaling in cell type specific manner, but barely affects RIG-I, TLR4, or STING-mediated signaling. Hereafter, we are going to investigate the function of RIOK3 in viral infection in vivo using an EMCV infection model.

Mo-WS4-6

Functional diversity of zinc-finger antiviral protein isoforms during viral infection

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The host response to viral infections initiates with expression of IFNs and antiviral factors that combat infection. However, return to homeostasis is important, as unrestrained IFN production results in pathology. Post-transcriptional regulation of immune genes has been implicated in resolution of IFN responses. IFN-λ is an important mediator of antiviral protection in HCV infection. We have previously shown that expression of IFNL1 is regulated post-transcriptionally through a functional SNP (rs4803217) within the 3’UTR of IFNL3, which affects mRNA stability and correlates with HCV clearance or persistence depending on the rs4803217 genotype in infected patients.
This is mediated by miRNAs and AU-rich elements. Here, proteomic screens and RNA immunoprecipitation revealed that the short isoform of zinc-finger antiviral protein (ZAP, ZC3HAV1) interacts with instability motifs in the IFNL3 3'UTR. Since ZAP is expressed as a long and a short isoform, it raises the possibility that ZAP isoforms have distinct cellular functions. The long isoform of ZAP, ZAP-L, has been characterized previously as an antiviral restriction factor. ZAP-L protein is expressed at basal levels, while ZAP-S is induced upon IFN stimulation through alternative splicing. ZC3HAV1−/− hepatocytes show elevated expression of IFNs and ISGs upon sensing of viral nucleic acids and flaviviral infection. At the same time, ZC3HAV1−/− hepatocytes present increased flaviviral replication despite higher IFN levels. Subcellular fractionation and confocal laser scanning microscopy reveals distinct localization of ZAP-S and ZAP-L within the cell. While ZAP-S is diffusely cytoplasmic, ZAP-L forms perinuclear foci and co-localizes with early and late endosomes, which is mediated by its C-terminal prenylation motif. Overall, our data suggest that the two ZAP isoforms have differential specificity to host and viral RNAs based on their intracellular localization. Through this, ZAP-L restricts viral replication early during infection, while ZAP-S curbs excessive IFN mediated inflammatory responses once the infection is controlled.

Mo-WS4-8

Intratumoral IRF5 regulates programs an anti-breast tumor immunity resulting in microenvironment that suppresses the suppression of breast tumor growth and metastasis

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Transcription factor interferon regulatory factor 5 (IRF5) is a critical mediator of the host immune response to pathogens and cellular response to DNA damage. It has recently been shown to play a role in macrophage polarization, with high levels expressed in M1 (pro-inflammatory) macrophages and low levels in M2 (anti-inflammatory). We previously found that expression IRF5 was down-regulated in the progression of human breast cancer, with loss of IRF5 expression correlating with metastasis. We also found that restoring IRF5 expression in the metastatic human mammary epithelial cell line MDA-MB-231 led to recruitment of CXCR5(+) B and T cells to the tumor. These findings support a role for IRF5 in regulating tumor immunity. In order to directly address the function of IRF5 in the breast cancer microenvironment, we generated syngeneic models of mammary tumorigenesis by orthotopically injecting Irf5+/+ and Irf5−/− 4T1 cells into mammary fat pads of BALB/c mice. Kinetics of tumor formation, growth and metastasis were monitored by bioluminescence imaging and metastasis confirmed by counting metastatic lung nodules. Immune response to Irf5+/+ and Irf5−/− 4T1 tumors was studied by comparing tumor-infiltrating leukocyte populations using flow cytometry. Results from this study show that restoring IRF5 expression in 4T1 tumor that normally lacks IRF5 reduces the growth of primary tumor and inhibits metastasis. First, Irf5−/− tumors were significantly smaller than Irf5+/+ tumors. Second, mice baring Irf5+/+ tumors had significantly reduced metastasis than mice with Irf5−/− tumors. Third, the analysis on tumor infiltrating leukocytes showed a significantly larger population of type 2 macrophage and smaller population of CD8+ cytotoxic T cells compared to Irf5−/− tumors. Thus, we have identified IRF5 as a critical mediator controlling interaction between tumor and the immune system. Our findings point to strategies that enable reprogramming of the tumor-immune microenvironment to enhance anti-tumor immunity and inhibit breast cancer metastasis.

Mo-WS6-1

An eye commensal tunes the immune response at the ocular surface by eliciting IL-17 from mucosal γδ T cells

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The surface of the eye is a mucosal site exposed to the environment, but existence of a resident ocular microbiome has been highly contentious. We used a mouse model of ocular surface disease to reveal that the surface of the eye indeed harbors resident microbes that tune the local immune response, affording protection from pathogenic organisms. We isolated and identified Corynebacterium mastitidis as a bona fide ocular surface commensal that elicits a commensal-specific interleukin 17 response from γδ T cells in the ocular mucosa. This causes recruitment of neutrophils and secretion of antimicrobials into...
the tears, and provides resistance to infection with C. albicans and P. aeruginosa. As a microorganism that confers a protective phenotype, C. mastitidis satisfies all 4 Koch’s postulates for a causative agent, and constitutes an important proof of concept that a resident commensal flora indeed exists on the surface of the eye and contributes to ocular mucosal homeostasis.

Mo-WS6-2

The role of Dectin-1-IL-17F axis in the homeostasis of the intestinal immune system

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Dectin-1 (gene symbol: Clec7a), one of C-type lectin family members, is the receptor for 1, 3-b-glucans and plays an important role for the host defense against fungal infection by inducing ROS and Th17-inducing cytokines. Because various foods, such as mushrooms and yeasts, contain b-glucans and Dectin-1 is expressed in intestinal myeloid cells, we examined the effects of Dectin-1 deficiency on the intestinal immune system. We found that Clec7a+/− mice are resistant against Dextran Sodium Sulfate (DSS)-induced colitis due to over expansion of Treg cells. Treg cell expansion was caused by Lactobacillus murinus, whose population was expanded in Clec7a+/− mouse colon due to a decrease of a group of antimicrobial proteins that specifically suppress L. murinus growth. IL-17F, but not IL-17A, was induced in the down-regulation of a group of antimicrobial proteins.

Mo-WS6-3

Pulmonary Regnase-1 functions as a posttranscriptional switch in anti-bacterial immunity

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The pulmonary antimicrobial immune defense is mediated by the cooperation of multiple cell types including airway epithelial cells (AECs) and professional immune cells. However, mechanisms underlying pulmonary immune regulation are not fully uncovered. Here we show that Regnase-1, an RNase critical for suppressing activation of immune cells by degrading inflammatory mRNAs, is expressed in AECs and plays pivotal roles in orchestrating pulmonary surface barrier function and immune responses against Pseudomonas aeruginosa. Regnase-1 in the lung is rapidly degraded upon exposure with P. aeruginosa or the stimulation of TLR. To investigate the influence of Regnase-1 disappearance from the lung, we analyzed the lung of Regnase-1+/− mice, and we found the overt accumulation of neutrophils and 15-fold increase of bronchoalveolar lavage fluid IgA concentration in Regnase-1+/− mice compared with wild-type mice. These phenotypes were largely attenuated in mice lacking Regnase-1 only in radiosensitive cells, suggesting the vital role of Regnase-1 in the AECs. RNA-sequencing revealed that the loss of Regnase-1 in AECs enhanced the expression of newly identified Regnase-1 target genes involved in direct exclusion of pathogens (Muc5b, Stipi, Lf), neutrophil recruitment (Cxcl1, Cxcl5), Th17 recruitment (Cxl120), and transportation of IgA in AECs (Pigr), as well as the attraction of IgA producing plasma cells (Ccl28). Indeed, mice lacking Regnase-1 specifically in AECs showed the enhancement of natural and adaptive immunity through the induction of neutrophils, Th17 cells, and IgA producing plasma cells. Concordantly, these mice showed the reinforcement of neutrophil inflammation and antigen-specific IgA secretion in the course of P. aeruginosa airway infection in vivo, confirming the resistance through the accelerated elimination of the pathogen. These results demonstrate that Regnase-1 degradation in pathogen-sensing AECs is the key mechanism for the activation of anti-bacterial immunity.

Mo-WS6-4

Lfç controls the formation of neutrophil extracellular traps against Candida albicans infection

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Neutrophils are the first line of host defense against fungal infection that sense microbe size and selectively release neutrophil extracellular traps (NETs) in response to hyphal Candida albicans but phagocytose small yeast. It is well known that NET formation depends on microtubule and actin networks. However, the molecular mechanisms by which dynamic cytoskeleton networks regulate NET formation are still unclear. Here we showed that a guanine nucleotide exchange factor Lfc, which is crucial in coupling microtubule dynamics to RhoA GTPase activation, is critical for NET formation. Wild-type neutrophils incubated with phorbol 12-myristate 13-acetate (PMA) formed NETs in spread form. In contrast, the majority of Lfc-deficient neutrophils exhibited diffused nuclear phenotype associated with neutrophil elastase translocation and histone citrullination under the same treatment. Interestingly, ROS production was comparable between PMA-incubated wild-type and Lfc-deficient neutrophils further indicating there were profound defects in the late stage of spread NET formation in Lfc-deficient neutrophils. Moreover, Lfc-deficient neutrophils were less able to control Candida albicans infection due to their incapability in spread NET formation. Overall, our results suggested Lfc is a critical regulator for spread NET formation in response to PMA or Candida albicans incubation.

Mo-WS6-5

Polarized interferon-mediated immune response against enteric pathogens reveal novel mechanisms of immune tolerance in the human gut

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Intestinal epithelial cells (IECs) lining the surface of our gastrointestinal tract have their apical sides in constant contact with the luminal commensal flora. As such, IECs are faced with a major challenge as they must tolerate the presence of the microbiota while maintaining full responsiveness against enteric pathogens. How IECs achieve such tailored immune response remains unclear but it is known that an inappropriate response against the microbiota participates in inflammatory bowel diseases. Here we identify and characterize the IECs-specific molecular mechanisms that govern this unique immune response and ultimately lead to gut immune homeostasis.

Using human mini-gut organoids and human intestinal epithelial cell (hIECs) lines we found that primary non-transformed hIECs assemble a polarized immune response against enteric pathogens (bacterial and viral). Not only do hIECs secrete cytokines and interferons (IFNs) in a polarized manner (apical vs. basolateral secretion), hIECs can mount a distinct immune response as a function of infection side. Enteric pathogen infection of hIECs from their apical side (luminal side) leads to an acute production of both type I and III IFNs which is quickly downregulated. On the contrary, infection from their basolateral sides (lamina propia side) triggers a stronger innate immune response characterized by prolonged production of type III IFNs. We demonstrated that this polarized immune response is dependent on the polarized nature of hIECs. We identified the clathrin/AP1-dependent polarity program as a specific regulator of TLR3 signaling allowing hIECs to mount a moderate immune response against apical challenges and a strong immune response against basolateral stimulation. Interestingly, mice lacking AP1 display colitis due to an over-reaction to their microbiota. This polarized response would represent a strategy to maintain gut immune homeostasis by avoiding excessive response against microbes located in the luminal side while maintaining full responsiveness against invasive pathogens that have passed the epithelium barrier.

Mo-WS6-6
Myd88 deficiency results in dysbiosis favoring generation of spontaneous lymphomas and carcinogen-induced colonic tumors

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Recognition of microbial products occurs via Myd88 (Myeloid differentiation factor 88) a downstream signal transducer for IL1R, IL18R and the majority of TLRs (Toll-like receptors). Previously we reported that IL18R/Myd88 exerts a protective role in colon carcinogenesis induced by AOM-DSS (Azoxymethane-Dextran Sodium Sulfate) treatment. Here we identify myeloid Myd88 and IL18R as the sources of this protection, in that animals with either IL18R or Myd88 deficiency in Cd11b+ cells resemble complete IL18R or Myd88 deficiency. Microbiome sequencing indicated an increase in the abundance of colitogenic species including Escherichia coli serotype H707, Blautia sp and Enterococcus faecalis, all of which correlated with polyb multiplicity. Concordantly, expression of Beta-Glucoronidase, PKS (polyketide synthase) and Invasin in these bacterial species account for the pro-carcinogenic effects. Notably, this phenotype was transmissible to wild type animals as detected post- cohousing with mice bearing targeted deletion of Myd88 in myeloid cells and by fecal transplantation. Thus, IL18R/Myd88 signaling in myeloid cells was required for the regulation of microbial composition of the colonic lumen.

Additionally, Myd88 deficient animals harbor a large quantity of viral infectious particles in their colonic mucosa corresponding to EMV2, an ecotropic endogenous retrovirus with low or undetectable levels of expression in wild type C57BL/6 animals. Up to 20% of Myd88 knockouts spontaneously developed T cell lymphomas containing abnormalities in chromosome 17 and 15, with high expression of Mela antigen, and viral particles. Consistently, exposure of the colonic mucosa to DSS increased the frequency of thymic lymphoma 2-fold. DSS treatment also resulted in the induction of other tumor types including liver, intestinal and kidney tumors although to a lower frequency.

Further studies are underway to investigate the contribution of EMV2 in the progression of colonic tumors as well as the effect of the dysbiotic microbiota and their products on spontaneous tumor formation elicited by this endogenous retrovirus.

Tu-WS7-1
Myd88/IRAK2-dependent interplay between myeloid and adipocytes in the initiation and progression of obesity-associated inflammatory diseases

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Low-grade systemic inflammation is often associated with metabolic syndrome, which plays a critical role in the development of the obesity-associated inflammatory diseases, including insulin resistance and atherosclerosis. Here, we investigate how Toll-like receptor–MyD88 signaling in myeloid and adipocytes coordinately participates in the initiation and progression of high fat diet–induced systemic inflammation and metabolic inflammatory diseases. MyD88 deficiency in myeloid cells inhibits macrophage recruitment to adipose tissue and their switch to an M1-like phenotype. This is accompanied by substantially reduced diet-induced systemic inflammation, insulin resistance, and atherosclerosis. MyD88 deficiency in adipocytes ameliorated diet-induced inflammatory diseases. Collectively, these results implicate a critical MyD88/IRAK2-dependent interplay between myeloid cells and adipocytes in the initiation and progression of obesity-associated inflammatory diseases.

Tu-WS7-2
Induction of regulatory T cells from Th1 cells through metabolic reprogramming

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In response to antigen stimulation, naïve CD4 T cells differentiate into several subsets including effector helper T (Th1, Th2 and Th17) and inducible regulatory T (iTreg) cells. Although these differentiated CD4 T cell subsets are more plastic than previously thought, Th1 cells are one of the most stable subsets, and conversion of Th1 to iTreg cells

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has been shown to rarely happen. The methods of converting effector Th1 cells into iTreg cells are urged to be established for the treatment of Th1-mediated inflammatory and autoimmune diseases.

Here we report that Foxp3+ cells can be induced in vitro from differentiated Th1 cells by simply culturing Th1 cells in the absence of TCR stimulation for 4 days (resting procedure), then culturing with TGF-β. We named these Th1-derived Foxp3+ cells Th1Reg cells. Th1Reg cells showed both Th1 and iTreg-like phenotypes and possessed suppressive ability in vitro. Vitamin C facilitated stable Foxp3 expression in Th1Reg cells both in vitro and in vivo. Th1Reg cells could also be induced from in vivo-derived Th1 cells that are prepared from mice with graft versus host disease. Surprisingly, the resting process did not induce apparent changes in epigenetic modifications at the Foxp3 enhancer and promoter regions, but down-regulated the mTOR activity of Th1 cells. The resting procedure suppressed glycolysis, which is downstream of the mTOR pathway, but enhanced oxidative phosphorylation. Pharmacologic and gene expression analyses suggested that metabolic shift from glycolysis to oxidative phosphorylation is important for the induction of Th1Reg cells. In addition, both expression of Smad3 and its TGF-β-induced phosphorylation, which is reported to be suppressed by mTOR, were reduced in Th1 cells, and recovered after the resting. These data indicate that the metabolic reprogramming is important for the conversion from Th1 cells into iTreg-like cells.

Tu-WS7-3

A critical role of mitochondrial oxidation in the production of type I interferon by human plasmacytoid dendritic cells

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Introduction: Plasmacytoid dendritic cells (pDC) play a crucial role in innate viral immunity as the most potent producers of type I interferons (IFN-I) in the human body. However, the metabolic regulation of IFN-I production in such vast quantity remains poorly understood, with conflicting evidence in the literature. Methods: To investigate the role of metabolic pathways in IFN-α production, we employed flow cytometric methods and extracellular flux analysis (EFA) to measure changes in metabolic activity downstream of TLR7 and -9 ligation. We also assessed the effect of metabolic inhibitors on IFN-α expression.

Results: Basal oxygen consumption and respiratory capacity both significantly increased upon 6 h HSV or flu stimulation, indicating an increase in the rate of and capacity for aerobic metabolism. Small-scale, statistically significant changes in extracellular acidification were also observed. Likewise, mitochondrial membrane potential significantly increased under the same conditions, while small but significant increases were also apparent in 2-NBDG uptake, a fluorescent glucose analog taken up through glucose transporters with similar kinetics. IFN-α production correlated with these changes in metabolic activity: we showed significant IFN-α inhibition with separate treatments of the inhibitors etomoxir (carnitine palmitoyltransferase 1), UK5099 (mitochondrial pyruvate translocase), and 2-deoxyglucose (hexokinase). In addition, IFN-α production was completely ablated 6 h post-flu stimulation with treatment with the inhibitors oligomycin (ATP synthase), rotenone (complex I), and antimycin A (complex III). Cell viability was unaffected by all treatments, suggesting a necessity of mitochondrial oxidation in the production of IFN-α. Conclusion: We have demonstrated evidence for the necessity of mitochondrial oxidation in the innate antiviral response of pDC, the inference being that the increase in ATP production provides energy for the translation of large amounts of IFN. Our data also suggest a role for glycolysis, which likely relates to the resultant pyruvate as a major substrate of the TCA cycle.

Tu-WS7-4

Stress-induced dynamic regulation of mitochondrial STAT3 and its association with cyclophilin D reduce mitochondrial ROS production

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Signal Transducer and Activator of Transcription 3 (STAT3) has been tied to various physiological and pathological functions, mainly as a transcription factor that translocates to the nucleus upon tyrosine phosphorylation induced by cytokine stimulation. In addition, a small pool of STAT3 resides in the mitochondria where it serves as a sensor for various metabolic stressors including reactive oxygen species (ROS). Mitochondrially-localized STAT3 largely exerts its effects through direct or indirect regulation of the activity of the electron transport chain (ETC). It has been assumed that STAT3 amounts in the mitochondria are static. We showed that various stimuli, including oxidative stress and cytokines, triggered a signaling cascade that resulted in a rapid loss of mitochondrially-localized STAT3. The loss of STAT3 from the mitochondria is due to its proteolysis. Recovery of the mitochondrial pool of STAT3 over time depended upon phosphorylation of Ser727 in STAT3 and new protein synthesis. Under these conditions, mitochondrially-localizedSTAT3 becomes competent to bind to cyclophilin D (CypD). Binding of STAT3 to CypD was mediated by the N-terminus of STAT3, which was also important for reducing mitochondrial ROS production after oxidative stress. These results outline a role for mitochondrially-localized STAT3 in sensing and responding to external stimuli.

Tu-WS7-5

Insights into the tumor suppression mechanisms of Suppressors of Cytokine Signaling 1 (SOCS1) and SOCS3 in hepatocellular carcinoma

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SOCS1 and SOCS3 are considered tumor suppressors in the liver. Mice lacking either SOCS1 or SOCS3 show increased susceptibility to hepatocellular carcinoma (HCC). Previously we have shown that SOCS1 attenuates the oncogenic potential of p21CIP1/WAF1 (p21) via ubiquitination and proteasomal degradation. Contrary to SOCS1, SOCS3 has been reported to promote p21 expression. However, both SOCS1 and SOCS3 can activate p53, a transcriptional activator of Cdkn1a that encodes p21. p21 promotes oncogenesis via several mechanisms including activation of NRF2, a transcriptional factor that induces cytoprotective genes against oxidative stress. The goal of this study is to understand the disparate regulation of p21 by SOCS1 and SOCS3, and to elucidate their role in p21-dependent oncogenic processes in HCC.
We generated mice lacking SOCS1, SOCS3 or both in the liver, and evaluated their susceptibility to HCC induction by diethylnitrosamine (DEN). Hepatocyte-specific SOCS1- or SOCS3-deficient mice showed increased susceptibility to DEN-induced HCC, but the loss of both SOCS proteins attenuated HCC progression despite increased STAT3 activation caused by SOCS3 deficiency. We show that elevated p21 expression and increased p53 phosphorylation in SOCS1-deficient liver are mediated by SOCS3. Besides Cdkn1a, certain other p53 target genes are induced in the SOCS1-deficient liver in a SOCS3-dependent manner. Loss of p21 in SOCS1-deficient livers attenuated the induction of Nfe2l2 coding for NRF2 and its target genes, and diminished HCC susceptibility. Overall, our findings indicate that loss of SOCS1 leads to a compensatory increase in SOCS3 expression presumably to attenuate cytokine signaling, but SOCS3 appears to exacerbate the paradoxical oncogenic functions of p21 by promoting the NRF2-mediated antioxidant functions, thereby contributing to HCC pathogenesis.

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Tu-WS7-6

IL-1β induced cell death under glucose deprivation is dependent on SIRT6- Hexokinase 2 cross talk

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Introduction: Emerging evidences indicate the involvement of a dynamic network connecting cellular bioenergetics, inflammatory response and redox homeostasis in tumor progression. Since there is a considerable overlap of signaling mediators involved in these interactions, the role of pro-inflammatory IL-1β in inducing these events in Glioblastoma multiforme (GBM) was investigated.

Methods: Nucleosomal scanning assay (NuSA) was performed in glioma cells to determine the effect of SIRT6 (Sirtuin 6) on positional stability of nucleosomes on HK2 promoter in the presence IL-1β. The role of SIRT6-HK2 axis in IL-1β treated glucose deprived cells was determined by Western blot analysis, co-immunoprecipitation and ChIP (chromatin immune-precipitation) upon knock-down and over-expression of SIRT6 or HK2.

Results: IL-1β induced expression of Hexokinase 2 (HK2) - the enzyme that catalyzes the first committed step of glycolysis, was accompanied by heightened SIRT6 levels. SIRT6 served as negative regulator of HK2, and IL-1β-induced HIF-1α (Hypoxia inducible factor 1α) prevented SIRT6 abundance and consequently its availability for regulating HK2 transcription. While treatment with IL-1β alone had no effect on glioma cell viability, massive cell death was observed in IL-1β treated cells under glucose deprivation in a ROS dependent manner. This was accompanied by increase in HK2, Nrf2 and Xanthine oxidoreductase (XOR) levels. SIRT6 not only promoted IL-1β induced death under glucose deprivation by regulating ROS levels, but also affected Nrf2 expression and its interaction with HK2. Importantly, SIRT6 mediated HK2-Nrf2 interaction was crucial for regulation of XOR promoter activity. Interestingly, XOR inhibition further elevated IL-1β and HIF-1α levels under glucose deprived inflammatory conditions. Taken together, IL-1β induced SIRT6 not only regulates HK2 transcription but is also involved in maintaining cellular redox homeostasis.

Conclusion: Relative abundance of SIRT6 determines tumor cell fate by modulating metabolic and redox status of cells.

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Tu-WS7-7

T-bet suppresses the IFN-gamma-mediated induction of a T cell intrinsic type I IFN signature during T helper 1 responses

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Type I and type II interferons (IFN) have critical roles in host defense and immunoregulation, with some aspects of their actions overlapping and others being quite distinct. In this study, we compared the actions of type I (IFN-beta) and type II (IFN-gamma) IFN on CD4 T cells, and found that T-bet has a profound impact on the distinct transcriptomes and metabolic profiles triggered by these cytokines. T-bet has been well described as a lineage defining transcription factor (LDTF) for T helper 1 (Th1) cells that regulates IFN-gamma production. However, little is known about its role beyond determining lineage. Here, we found that in the absence of T-bet, IFN-gamma aberrantly enhanced STAT2 activity and a type 1 IFN transcriptomic program, while reducing glycolysis. Moreover, during the Th1 response induced in vivo by Toxoplasma infection, type I IFN signature genes were enhanced in T-bet deficient CD4 T cells compared with wild type cells. We found that T-bet restrained the type 1 IFN signature otherwise triggered by IFN-gamma by inhibiting autocrine production of type I IFNs as well as constraining STAT1, STAT2 and IRF7 expression. Accordingly, blocking the type 1 IFN receptor (IFNAR) during IFN-gamma treatment inhibited the type 1 IFN signature and restored glycolytic capacity in T-bet deficient cells. This inhibitory activity of T-bet on multiple components of the type 1 IFN circuitry ensures the development of a polarized type II IFN response and appropriate effector cell expansion in Th1-dominated infections.

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Tu-WS7-8

Involvement of the MAP kinase pathway in PKR inhibition by Theliers virus

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Theliers’s Murine encephalomyelitis virus (TMEV) belongs to the Theilovirus species within the Cardiovirus genus. The leader (L) protein encoded at the N terminus of viral polyprotein is multifunctional and was shown to antagonize the innate immune response. We reported that wild type but not mutant L protein can inhibit stress granules (SG) assembly in infected cells.

Recent results suggested that this inhibition was consequent to L-mediated inhibition of the double-stranded RNA (dsRNA)-activated protein kinase R (PKR). Indeed, wild type but not L-mutant viruses inhibited PKR phosphorylation. However, we found no evidence for interaction between L protein and PKR or viral dsRNA suggesting that PKR inhibition by L might be indirect. Recently, we identified MAP kinases of the RSK family as binding partners of the L protein. To analyze the involvement of RSK kinases on SG assembly, elf3 (SG marker) immunolabeling was performed on cells where RSK1 and/or RSK2 expression was knocked down using shRNAs or knocked out by CRISPR-Cas9. Interestingly, wild type virus failed to inhibit PKR phosphorylation and stress granule assembly in cells.
that lacked BSK1/2 expression, showing the involvement of RSK kinases in PKR inhibition.

In conclusion, RSK kinases are required for TMEV L-mediated inhibition of PKR activity.

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**Tu-WS9-1**

**Overview of anti-cytokine therapy and differential use of biologics based on lymphocyte phenotype in inflammatory autoimmune diseases**

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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammation and joint damage that causes significant morbidity and mortality. Rapid and appropriate intervention by intensive treatments is prerequisite to halt joint damage and long-term functional disabilities. Recent progress in the treatment strategy has brought about paradigm shift for the management of RA, namely, the combined use of methotrexate, a synthetic disease-modifying antirheumatic drug (DMARD), and biologic DMARDs targeting TNF, IL-6 and T cells has revolutionized treatment of RA. Clinical remission is now realistic targets for the treatment, achieved by a large proportion of RA patients, which leads to structural remission without damage in bone and cartilage as well as functional remission. Furthermore, orally available small but strong molecules targeting signaling kinase are emerging. Oral administration of tofacitinib targeting the Janus kinase (JAK) is significantly effective than placebo in active patients with methotrexate-naïve, inadequately responsive to methotrexate or TNF-inhibitors. The efficacy was rapid and as strong as TNF-inhibitors. Multiple kinase inhibitors are currently developed. On the other hand, how differentially use multiple inhibitors remain unclear. Psoriatic arthritis (PsA) is a chronic and progressive inflammatory arthritis. Because PsA is a clinically heterogeneous disorder, we have tried to classify PsA by phenotypic differences of peripheral lymphocyte using 8-color flow cytometry and found that PsA can be divided to four types, activated Th17-dominant, Th1-dominant, both of them and neither of them. We currently try to treat patients with different bDMARDs based on the difference of lymphocyte phenotype, which may lead to precision medicine of PsA. Thus, differential use of biologics based on lymphocyte phenotype leads to a precision medicine for the treatment of inflammatory autoimmune diseases such as PsA and RA.

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**Tu-WS9-2**

**TNFR2⁺ Regulatory T CELLS (Tregs) Subpopulations Are Highly Suppressive And Are Increased On Anti-Tnf Treatment In Rheumatoid Arthritis (RA) Patients**

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**Introduction:** In RA, Tregs fail to control chronic inflammation. TNF-α is involved in inhibition of Tregs differentiation and activation but the respective roles of its two receptors is unclear.

We aimed to establish the role of TNFR2 on Tregs in control of inflammation by studying: 1) the action of TNF on Treg function in the presence and absence of TNF2 in vitro and 2) in a model of skin inflammation in TNFR2KO mice, 3) the evolution of TNFR2-expressing Tregs from RA patients during anti-TNF-treatment.

**Methods:** Mice deficient in the TNFR2 gene (TNFR2KO) and CRE-FoxP3-TNFFR2 lox/lox mice were used. Cell phenotype was evaluated by FACS. Tregs stability was evaluated by analyzing methylation status of 9 CpG motifs of the Foxp3-locus (bisulfite sequencing of CD4⁺ CD25⁺ purified cells). Skin inflammation was induced by an imiquimod-containing ointment. Peripheral blood Tregs were characterized before and after 3 months of anti–TNF treatment in 12 RA-patients and in 19 patients with axial spondyloarthritis (AxSpA).

**Results:** In vitro, TNF-α enhanced Foxp3 maintenance through TNFR2 signaling in cultured Tregs. In vivo, TNFR2-negative-Treg cells from both TNFR2KO and CRE-FoxP3-TNFFR2 lox/lox mice, had lower spontaneous suppressive capacities (lower inhibition of effector T cell proliferation and IFN-γ production). Foxp3 methylation was higher in Tregs from TNFR2KO mice than wt mice. This suggested that TNFR2 expression confers higher stability to Tregs.

**Conclusion:** TNFR2 signaling on Tregs may play a major role in controlling inflammation and can be activated both by TNF-α and anti-TNF treatment. Further studies to dissect TNFR2 dependent pathways on Tregs are warranted.

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**Tu-WS9-3**

**TRAIL suppresses joint inflammation and osteoclastogenesis through inhibiting activated T cell responses in inflammatory arthritis**

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Rheumatoid arthritis (RA) is a complex, heterogeneous systemic autoimmune disease involving a wide array of joint inflammation and bone erosion, eventually leading to serious disability. Targeting to key points of the pathogenesis of RA is the current mainstay of new therapeutics development. TRAIL belongs to TNF superfamily, and accumulated evidences suggest TRAIL may be implicated its actual biological function in immune-regulation in besides triggering apoptosis. In this study, we demonstrated that TRAIL significantly inhibited joint inflammation, restored bone erosion and suppressed systemic inflammatory cytokines in an inflammatory arthritis animal model. Interestingly, the suppression of joint inflammation was not due to the
Tu-WS9-4

Distinct single cell gene expression signatures of monocyte subsets differentiate between TNF-alpha inhibitor treatment response groups in Rheumatoid Arthritis

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Our group demonstrated that pre-treatment serum type I IFN-β/α ratio > 1.3 predicts non-response to anti-TNFα therapy (TNFi) in RA patients. However, mechanisms that underlie the IFN-β/α ratio that predicts response are unknown. Effects of IFN on unicom cell populations may be masked in whole blood or mixed cell populations. We used single cell analysis to investigate whether monocyte gene expression differs significantly between RA patients according to their pre-TNFi serum IFN-β/α ratio. Single classical (CL) and non-classical (NC) blood-derived monocytes were isolated from seropositive RA subjects prior to biologic therapy. Subjects were grouped by pre-TNFi serum IFN-β/α ratio: IFN-β/α > 1.3(n=6) and IFN-β/α < 1.3(n=9). 87 target genes were analyzed. Genes that varied significantly between groups by categorical analyses were tested in multivariate logistic regression models. JAK1, IL1A, TLR2, CD32A, CD36, CX3CR3, IL8, IRAK1, and TYK2 expression were retained in the mixed monocyte gene expression model for differentiating between groups. JAK1 and IL1A were also retained in the models from monocyte subsets. TLR9, STAT1, and FCER were retained in the CL model. STAT2 and IFR27 were retained in the NC model. Regression models from the monocyte subsets provided increased discriminatory potential in comparison to the mixed monocyte model. Within-cell co-expression patterns demonstrate biological differences in monocyte subsets of RA patients with an IFN-β/α ratio > 1.3, the ratio of type I IFNs which predicts non-response to TNFi. When monocyte subsets were analyzed separately, differentiation by gene expression was strongest and distinct expression signatures were identified, suggesting that further study of monocyte subsets will illuminate molecular differences that determine response to TNFi in RA. A better understanding of mechanisms that underlie the IFN-β/α ratio that determines treatment response should allow us to focus down to a more specific marker that would be easier to measure, and, may reveal other targets for therapy. This work will help to develop a more individualized approach to treatment in RA.

Tu-WS9-5

Anti-CX3CL1 monoclonal antibody therapy suppresses the development of bleomycin-induced and growth factors-induced skin fibrosis in mice

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Objective: Systemic sclerosis (SSc) is a connective tissue disease characterized by inflammation, fibrosis, and vascular injury of the skin and internal organs. We previously reported that expression of CX3CL1 (fractalkine) and its unique receptor, CX3CR1 was augmented in patients with SSc. In this study, we examined the effect of anti-mouse CX3CL1 monoclonal antibody (mAb) therapy for skin fibrosis in two different mouse models of SSc.

Methods: The first SSc model was established on C57BL/6J mice and CX3CR1-deficient mice by daily intradermal injections of bleomycin. In the second model, BALB/c newborn mice received subcutaneous injections of transforming growth factor-β followed by that of connective tissue growth factor. The anti-CX3CL1 mAb originally generated was injected in both models.

Results: The bleomycin injection quickly increased serum levels of CX3CL1. Administration of anti-CX3CL1 mAb or CX3CR1 deficiency significantly suppressed the skin thickness and dermal collagen accumulation induced by bleomycin. In addition, the dermal infiltration of CX3CR1+ cells, macrophages (especially CD11b+Ly6Chi and CD11b+CD206+ subsets), and CD3+ cells was reduced by anti-CX3CL1 mAb therapy or CX3CR1 deficiency. The mRNA expression levels of fibrogenic molecules such as osteopontin and thymic stromal lymphopoietin were markedly augmented in the skin by bleomycin injection. However, anti-CX3CL1 mAb therapy significantly suppressed them. Anti-CX3CL1 mAb administration resulted in a tendency of protection and/or reorganization of the injured microvasculature in bleomycin-injected skin. In growth factors-induced skin fibrosis model, the mRNA expression of CX3CL1, CX3CR1, and macrophage markers was increased during the fibrotic course. Pre-treatment of anti-CX3CL1 mAb significantly ameliorated the skin fibrosis.

Conclusions: Blockade of the CX3CL1/CX3CR1 pathway can efficiently ameliorate the skin fibrosis in two strategically different SSc mouse models. Anti-CX3CL1 mAb therapy is an ideal candidate for consideration in clinical trials of SSc.

Tu-WS9-6

A new GM-CSF-dependent pathway in inflammation

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Data from preclinical and clinical studies have demonstrated that granulocyte macrophage colony-stimulating factor (GM-CSF) can function as a key proinflammatory cytokine. However, therapies that directly target GM-CSF function could lead to undesirable side effects, creating a need to delineate downstream pathways and mediators. Evidence will be provided that GM-CSF drives CCL17 production by
Tu-WS11-1

The IL-1 family: new and old cytokines

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The IL-1 family of cytokines comprises 11 members, including 7 agonists (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36g), 2 established antagonists (IL-1Ra, IL-36Ra) and 2 additional cytokines (IL-37, IL-38) the biological functions of which are not fully established. I will show some of our findings on IL-36 and IL-18.

We have shown that IL-36 agonists stimulate dendritic cells and naive T cells to produce Th1 responses. To examine the role of IL-36 as a vaccine adjuvant to vaccines to induce Th1 responses, new-born mice were infected with tetanus toxoid-Alum with or without IL-36. We observed that the addition of IL-36 is associated with a high lethality due to a cytokine storm mediated by TNF-α. We have also examined the role of IL-36R signaling in a Th1 model of host defense. IL-36R-/-, IL-1R-/- and TNF-α-/- and wild-type (WT) mice were infected with M. tuberculosis. We observed that, in contrast to TNF-α and IL-1R, IL-36R signaling is dispensable to control of M. tuberculosis infection.

The biological effects of IL-18 are tightly regulated at the level of its production and maturation as well as by a soluble inhibitor, IL-18 binding protein (IL-18BP). We developed an assay to specifically measure free IL-18 in human and mouse and found that levels of free IL-18 are elevated in some autoinflammatory diseases, including adult onset Still’s disease (AOSD). We conducted a phase 2 clinical trial with recombinant IL-18BP in patients with AOSD and obtained positive results with 50% response in patients with refractory disease. By using IL-18BP-/- mice in a model of macrophage activation syndrome induced by repeated CpG injections, we showed that unopposed IL-18 signaling leads to severe manifestations.

In conclusion, IL-36 is dispensable in host defense against M. tuberculosis. IL-18 is an interesting target for future therapy in some autoinflammatory diseases.

Tu-WS11-2

Interleukin 27 controls pain sensitivity in pathophysiological conditions; to immunity and beyond!

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Numerous studies have shown that pain sensation is affected by various immune-related molecules such as cytokines in tissues constituting sensory pathway. Interleukin (IL)-27 has been reported to have an anti-inflammatory effect through regulation of T cell differentiation, especially Th17 cells, and induction of IL-10-producing Tr1 cells. Although IL-17 and IL-10 are reportedly involved in pain sensation, roles of IL-27 in pain sensation have yet to be determined. Here we show evidences that constitutive existence of IL-27 controls threshold of pain sensation in various pathophysiological conditions. Mice lacking IL-27 signaling possessed chronic pain-like hypersensitivity. Reconstitution with IL-27 in these mice quickly restored the hypersensitive threshold of aversive behavior, suggesting the mechanisms were independent of cytokine induction, such as IL-10. In addition, the mechanism by which IL-27 controlled pain-like behavior did not involve well-known pain-related molecules, such as prostaglandins and opioids. Our data shed new lights on the role of IL-27 in pain control and pain-related disorders, aside from its immunoregulatory roles.

Tu-WS11-3

Th22 cells as a new helper T cell subset involved in RA pathogenesis through their ability to promote osteoclast differentiation via IL-22 production

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Background: Although elevated levels of IL-22 in the synovial fluids of RA patients were reported, its pathological roles remain unclear. In this study, we examined the distribution of Th22 cells in synovial tissues in RA patients, and the influences of Th22 cells on osteoclast differentiation in order to elucidate the role of Th22 cells in RA pathogenesis.

Methods: CD4+ IL-22+ IL-17-IFN-γ-Th22 cells and chemokine receptor-ligands (CCL17, CCL20, CCL28) in synovial tissues in patients with RA and osteoarthritis (OA) were evaluated by immunohistochemistry. Human monocytes were cultured with IL-22, IL-17 or IFN-γ in the presence of M-CSF and RANKL. Th1 cells, Th17 cells or Th22 cells were sorted from peripheral blood and co-cultured with monocytes.

Results: Th22 cells were markedly infiltrated in synovial tissue in patients with active RA, but not in patients with OA. CCL17, CCL20 and CCL28 were abundantly expressed in RA synovial tissues compared to OA. Addition of IL-22 to the in vitro culture of monocytes markedly increased numbers of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts number, whereas IL-17 had marginal effects. The gene expression of NFATc1 and cathepsin K was significantly increased by addition of IL-22 to the culture in a dose dependent manner. Co-culture of Th22 cells with monocytes induced TRAP-positive osteoclasts formation more efficiently than that of either Th1 cells or Th17 cells. IL-22 neutralizing antibody inhibited osteoclast formation in co-culture of Th22 cells with monocytes.

Conclusion: Th22 cells possess strong potency of tissue migration and accumulate into inflamed synovial tissues where the ligands such as CCL28 are highly expressed. The results indicated that Th22 cells have the capacity to promote osteoclast differentiation through production of...
Tu-WS11-4

Interleukin-27 inhibits the generation of memory CD4+ T cells during malaria infection

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IL-27 is a heterodimeric cytokine of IL-12 family consisted of p28 and EB13 subunits, and have regulatory function for T cell immune responses. We previously reported that a subpopulation of malaria antigen-specific Foxp3 CD4+ T cells produce IL-27 and inhibit IL-2 production as well as proliferation of other CD4+ T cells during infection with malaria parasites.

Malaria antigen-specific Th1 cells are induced during the infection with Plasmodium berghei ANKA, and play critical roles for the protection against the infection. However, these Th1 cells disappeared quickly after the cure from the infection by anti-malaria drug treatment. We hypothesized that T cell-derived IL-27 may contribute to the loss of memory responses after the cure. To test this hypothesis, we compared the memory responses between C57BL/6 and IL27p28−/− mice after infection and cure with P. berghei ANKA. Memory responses of CD4+ T-cells were quickly lost in C57BL/6 mice after the cure, while they were maintained in IL-27p28−/− mice. Apoptosis of malaria-specific CD4+ T cells was accelerated in C57BL/6 mice when compared with IL27p28−/− mice during the treatment. In addition, parasite-specific antibody responses were higher in IL27p28−/− mice. After the re-challenge, IL-27p28−/− mice showed little parasitemia, and their CD4+ T cells exhibited enhanced IFN-γ response. These results suggest that IL-27 inhibits the generation of memory CD4+ T cells by accelerating apoptosis of parasite-specific CD4+ T cells during contraction phase of the immune response against P. berghei ANKA.

Tu-WS11-5

Structure of an engineered IFN-λ/IFN-λR1/IL-10Rβ complex provides insight into the functional dichotomy of type III versus type I IFNs

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Type III interferons (IFN-λs) are cytokines that share a common signaling pathway, downstream target genes, antiviral and antiproliferative activities with type I IFNs, but drive these processes with less potency and via different cell surface receptors. The intrinsically low-affinity of IL-10Rβ, one of the two cognate IFN-λ receptors, was believed to be a limiting factor to crystallizing the wild-type signaling complex. Here, we report the crystal structure of the IFN-λ receptor ternary complex enabled by the in vitro evolution of a high-affinity IFN-λ to stabilize the complex. Structural and sequence analyses provide insights on the mechanism of IL-10Rβ engagement with IFN-λ and other IL-10 cytokines. We harnessed the enhanced affinity of our engineered IFN-λ as means to enhance the potency of STAT signaling, gene induction, and antiviral and antiproliferative activities. More importantly, an in vivo study of hepatitis B virus infected human-liver chimeric mice shows enhanced antiviral activity by our high-affinity IFN-λ without increased toxicity relative to the wild-type IFN-λ, or untreated control mice. We provide evidence that IFN-λ antiviral activities can be improved through protein engineering, and suggest an avenue by which these promising molecules may be pursued for clinical use.

Tu-WS11-6

IL-33 potentiates the inflammatory response to Toxoplasma gondii

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Mice deficient in the IL-33 receptor ST2 are susceptible to infection with the protozoan parasite Toxoplasma gondii, but how IL-33 promotes resistance to infection remains elusive. Previous reports have attributed this susceptibility to aberrant skewing of the immune response away from type 2 immunity, with which ST2 has been classically associated, toward an excessive type 1 response and consequent lethal immune pathology in the central nervous system (CNS). Although expression of ST2 by tissue-resident Tregs supports a possible deficiency in immune regulation, ST2-knockout mice were also found to have higher parasite burdens in the CNS, suggesting a potential defect in the inflammatory response. In recent years, a direct role for IL-33/ST2 in enhancing type 1 responses has been appreciated, leading us to reconsider the function of IL-33 in the response to T. gondii. Using whole organ in vitro antigen recall assays of spleen, bone marrow, and brain tissues, we found that addition of exogenous IL-33 to Soluble Toxoplasma Antigen (STA)-restimulated cultures enhanced interferon gamma production during both acute and chronic infection. Further, we observed that IL-33 enhanced the proliferation and differentiation of restimulated parasite-specific CD4+ and CD8+ effector T cells in vitro. To confirm the physiological relevance of our system, we used IL-33 reporter mice to confirm in vivo expression of IL-33 in intact tissues relevant to T. gondii infection, including the meninges and cerebral lymph nodes. As IL-33 is expressed at high levels in many tissues, including the central nervous system, and is released during tissue damage caused by infections such as T. gondii, these results suggest that IL-33 enhances the inflammatory response to T. gondii and consequently control of this parasitic infection.
Tu-WS8-1
High-affinity monoclonal IgA derived from mouse Intestine as a modulator of the gut microbiota

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Background: Accumulating evidence suggests that dysbiosis plays a role in the pathogenesis of intestinal diseases including inflammatory bowel disease (IBD) as well as extra-intestinal disorders. Immunoglobulin A (IgA) is the main antibody isotype secreted into the intestinal lumen. IgA plays a critical role in the defense against pathogens and in the maintenance of intestinal homeostasis. However, how secreted IgA regulates gut microbiota is not completely understood.

Methods & Results: In this study, we isolated monoclonal IgA antibodies from small intestine of healthy mouse. As a candidate of efficient gut microbiota modulator, we selected a W27 IgA that binds to multiple bacteria but not beneficial ones such as Lactobacillus casei. Via specific recognition of an epitope in serine hydroxymethyltransferase (SHMT), a bacterial metabolic enzyme, W27 could bind to and suppress the cell growth of Escherichia coli but not Lactobacillus casei in vitro, indicating an ability to improve the intestinal environment. By modulating the gut microbiota in vivo, W27 oral treatment could have therapeutic effect on both lymphoproliferative disease and colitis models in mice.

Conclusion: W27 IgA oral treatment is a potential remedy for inflammatory bowel disease, acting through restoration of the host-microbial symbiosis.

Tu-WS8-2
Antibiotics disrupt intestinal macrophage homeostasis to induce long-lived inflammatory T-cell responses and defective protection against bacterial and parasitic infections

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Intestinal macrophages are highly adapted to recognize and respond to components of the gut microbiota without provoking an inflammatory response but are critical in host defense against invading intestinal pathogens. However, it is unknown how intestinal macrophages become conditioned to maintain this homeostatic balance. We show here that the intestinal microbiota shapes the regulatory functions of macrophages in the colon, mediating host responses to infections and inflammation. We provide evidence that broad-spectrum antibiotic use disrupts intestinal macrophage homeostasis causing macrophages to become hyper-responsive to bacterial stimulation. Thus, re-colonization of antibiotic-treated mice with conventional microbiota induced intestinal macrophages to produce excess amounts of pro-inflammatory cytokines (TNFα/IL-6/IL-12/IL-1β). This drives a subsequent, macrophage-dependent long-term increase in activated, IFNγ-producing Th1 cells in the colon along with sustained disruption of the microbiota. The consequences of this dysregulated macrophage activity for T-cell function were demonstrated by enhanced susceptibility of recolonized mice to bacterial infection requiring Th17 responses for clearance (Citrobacter rodentium) and, in addition, for helminth infection requiring Th2 responses for clearance (Trichuris muris). Indeed, colonic T-cell from recolonized mice were defective in the generation of Th17 responses during C. rodentium infection and were unable to generate IL-13-producing Th2 responses during T. muris infection. Administration in vivo of the short-chain fatty acid (SCFA) butyrate (usually generated from dietary fibre by the gut microbiota) partially restored the normal anergy of colonic macrophages to microbial stimulation and abolished the increased Th1 responses after re-colonization. Furthermore, butyrate enabled sufficient Th17 responses to be generated during C. rodentium infection for bacterial clearance. In summary, the gut microbiota is essential for maintaining macrophage-mediated intestinal immune homeostasis, mediated at least in part by a SCFA-dependent pathway. Disruption of the gut microbiota with antibiotics prevents this process, promoting inflammatory cytokine production and enhanced susceptibility to intestinal infections. These data highlight the potential impact of broad-spectrum antibiotic use in human health.

Tu-WS8-3
Osteoblasts mediate immunosuppression during sepsis by regulating lymphopoiesis

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Adult hematopoietic stem cells (HSCs) are maintained in bone marrow (BM) and give rise to all blood cell types. The maintenance and the differentiation of blood cells including immune cells are essential for host defense. Cells involved in bone homeostasis share microenvironments with HSCs in BM and contribute to hematopoiesis under physiological conditions, but bone cell function in acute immune reactions has been poorly understood. Sepsis is the acute host inflammatory response to severe infection associated with high mortality, which is often caused by the immunosuppression mediated by lymphocyte apoptosis. However, it is unknown how lymphopenia persists after the accelerated lymphocyte apoptosis. Here we show that sepsis rapidly suppressed osteoblastic bone formation and reduced the number of common lymphoid progenitors (CLPs) in the BM as well as the peripheral T and B cell numbers, suggesting the role of osteoblasts in the regulation of CLPs. To investigate the precise role of osteoblasts in the regulation of CLPs, we generated osteoblast-ablated mice by mating Osterix (Osx)-Cre mice with pAkT mice. In these mice, Cre-expressing cells can be ablated by ganciclovir administration. Similar to sepsis, transient ablation of osteoblasts led to a marked decrease in the CLP number. IL-7, which is important for maintaining lymphocytes, in BM was decreased during sepsis. Osteoblast-specific IL-7 conditional knock-out (cKO) mice were generated by crossing Il7fl/fl mice with Osx-Cre or osteocalcin-Cre. These cKO mice exhibited the lymphopenic phenotype together with a lower CLP number, indicating that osteoblast-
derived IL-7 supports CLPs in the BM. Activation of osteoblasts by intermittent parathyroid hormone treatment improves sepsis-induced lymphopenia. The survival rate for sepsis was decreased in osteoblast-ablated mice. This study demonstrated that IL-7 derived from osteoblasts regulates lymphopoeisis differentiation in the acute immune reaction, indicating that bone cells serve as a novel therapeutic target of life-threatening process in the immune reactions.

Tu-WS8-4

IL-17A controls autoimmune disease by inhibiting the expression of IL-17 lineage cytokines through a negative feedback loop involving IL-24

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Background: The Th17 response has been associated with autoimmune diseases in patients and in animal models. IL-17A is recognized as the Th17 signature cytokine and IL-17-producing T cells are pathogenic effectors in models of autoimmunity, including experimental autoimmune uveitis (EAU). Paradoxically, however, IL-17A treatment given to EAU-challenged mice was reported to ameliorate the disease (PMID: 19234216) and clinical trials targeting IL-17A in uveitis have been largely disappointing (PMID: 25648267).

Methods: We investigated susceptibility to EAU in interphotoreceptor retinoid binding (IRBP)-immunized mice on an IL-17A-/- background. Additionally, T cells from IL-17A-sufficient and deficient IRBP T cell receptor transgenic R161H mice were polarized under Th17 conditions and were adoptively transferred to WT recipients to examine their cytokine profile and their ability to induce EAU.

Results: Surprisingly, IL-17A-/- EAU mice developed essentially undiminished uveitis. IL-17A-/- R161H T cells, polarized to Th17 and infused into wild type recipients, induced similar disease to IL-17A-sufficient R161H T cells. These Th17-polarized IL-17A-/- R161H T cells produced elevated amounts of other Th17 lineage cytokines, namely, IL-17F, GM-CSF and IL-22, and this was reversed by supplementing the cultures with recombinant IL-17A. RNAseq analysis revealed that the IL-17A-/- T cells expressed lower levels of IL-24 compared to their IL-17A sufficient counterparts. Mechanistic studies in vitro indicated a negative feedback loop where IL-17A induces Th17 cells to produce IL-24, which subsequently suppresses production of Th17 lineage cytokines. Studies in vivo showed that injection of recombinant IL-24 ameliorated adoptive Th17-induced EAU, and conversely, silencing IL-24 expression in the adoptively transferred Th17 cells increased their pathogenicity and enhanced disease severity.

Conclusions: Our data suggest that IL-17A exerts a negative feedback on Th17 cells by inducing IL-24, which limits the expression of Th17-related cytokines and dampens Th17 effector pathogenicity.

Tu-WS8-5

Non-linear scaling of CD8+ T cell responses by bystander DCs

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Naive CD8+ T cells integrate signals from peptide antigen-MHC complex (pMHC; signal 1), costimulatory molecules (signal 2), and cytokines (signal 3) upon their interaction with antigen-presenting dendritic cells (DCs). Despite the vast knowledge on molecular regulators of effector CD8+ T cell responses, it remains elusive whether CD8+ T cells integrate all signals from a single interacting DC or whether additional DCs are required for exponential expansion of CD8+ T cells. To address this question, we combined conventional flow cytometry with intravitral two-photon microscopy (2PM) and light sheet fluorescence microscopy of lymph nodes (LNs) in a DC vaccination model. Subcutaneous injection of at least 20,000 peptide-pulsed DCs induced exponential expansion of a starting population of 15–25 antigen-specific CD8+ cells, which corresponded to a DC : T cell ratio of 8 or more in draining LNs. We then analyzed the effect of pMHC-negative “bystander” DCs as supplement for pMHC+ DCs, keeping the total number of injected DCs to 20,000. In the presence of bystander DCs, injection of as few as 1,250 pMHC-carrying DCs (corresponding to 20 pMHC+ DCs per popliteal LN) sufficed for exponential expansion and effector differentiation of CD8+ cells. 2PM imaging showed that the rescue of T cell expansion by bystander DCs was independent of direct interactions. In contrast, IL12a-/- bystander DCs did not potentiate expansion of CD8+ cells, indicating that bystander DCs augment antigen-specific CD8+ T cell responses via secretion of inflammatory cytokines. In sum, our results demonstrate that activated DCs that do not present cognate pMHC significantly lower the pMHC requirement for exponential expansion of responding CD8+ T cells. Furthermore, our data suggest that CD8+ T cell expansion follows a “coincidence detection” model in which cognate interaction with single DCs do not suffice without additional signal 3 from the surrounding immune microenvironment.

Tu-WS8-6

A Novel Role for Epstein-Barr Virus-Induced Gene 3 as An Intracellular Molecule That Enhances IL-23 Receptor Expression by Binding to Calnexin and IL-23 Receptor

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The IL-6/IL-12 family cytokines have unique characteristics in that they consist of two distinct subunits forming a heterodimer and each subunit shares with each other. Epstein-Barr virus-induced gene 3 (EBI3) is a common subunit shared by IL-27 and IL-35. Here, we have uncovered a novel role of EBI3 as an intracellular molecule independently of these cytokines. Initially, we found that EBI3 expression is
induced in naive CD4^+ T cells after stimulation with plate-coated anti-CD3 plus anti-CD28. To investigate the role of EB13 in naive CD4^+ T cells, we then utilized T cell-dependent colitis model, which is induced by transfer of naive CD4^+CD25^-CD62L^+ T cells into RAG2-deficient mice. EB13-deficient CD4^+ T cells failed to induce the colitis with reduced IFN-γ production in CD4^+ T cells of intestinal lamina propria. IFN-γ production was also decreased in EB13-deficient CD4^+ T cells differentiated under the pathogenic Th17 polarizing conditions with IL-23 in vitro, whereas this effect was not mediated by a soluble factor. In the EB13-deficient CD4^+ T cells, IL-23 receptor (R) expression was greatly reduced at protein level but not at mRNA level due to increased degradation of IL-23R at proteasome but not at lysosome. Moreover, forced expression of EB13 in HEK293T cells augmented IL-23R expression via binding to a molecular chaperone, calnexin, and IL-23R. Interestingly, genome-wide association studies and targeted re-sequencing studies recently revealed that the IL-23R variant G149R is linked to protection against the development of inflammatory bowel diseases in humans. Forced expression of EB13 failed to augment the IL-23R variant G149R expression, due to significantly reduced binding of EB13 to the variant. Taken together, the present study revealed a novel role for EB13 as an intracellular molecule that promotes the proper protein folding of IL-23R by binding to calnexin and IL-23R through augmenting its chaperone activity, resulting in development of colitis.

Tu-WS8-7

The role of BATF-3 dependent DC in the formation of fat associated lymphoid clusters

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The omentum is a visceral adipose tissue present in the peritoneal cavity that contains fat associated lymphoid clusters (FALCs). Despite the ability of the FALCs to form organized structures capable of T and B cell priming, little is understood about how inflammatory cells are recruited to these sites. Intraperitoneal immunization with a vaccine strain of Toxoplasma gondii results in a major reorganization of the FALCs that is dependent on Batf3-dependent cDC1s required for initial priming of a protective CD4 and CD8 Th1 type T cell response. The use Cre-expressing parasites shows that infected macrophages migrate from the peritoneum to the lymphoid clusters present in the omentum that results in rapid remodeling of these structures and T cell priming. Unexpectedly the cDC1s are a critical early source of IL-12 that induces IFN-γ production required to induce the migration of antigen-laden macrophages from the peritoneum to the omentum. In Batf3/- mice after the administration of recombinant IL-12p70 resulted in migration of peritoneal cells and recovery of CD4 T cell priming but was not sufficient for CD8 T cell priming. Together, these data identify a key role for cDC1 in co-ordinating the initial reorganization of FALCs required for their role in T cell priming.

Tu-WS10-1

Overview of WS10

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In WS10, we will focus on disease development caused by molecules and cells related to the NLRP3 inflammasome or to neuro-immune interactions. We selected two abstracts related to the NLRP3 inflammasome and eight abstracts related to neuro-immune interactions. I will introduce each abstract in the first several minutes of this workshop.

Tu-WS10-2

Selective blockade of NLRP3 inflammasome by TCM in lupus nephritis

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Lupus nephritis (LN) is one of the most common and severe complications in systemic lupus erythematosus patients. The current treatments for LN mainly rely on glucocorticoids and other immunosuppressants. These drugs are partially effective and yet have considerable side effects, especially for long-term administration. Thus, new therapeutic approaches are clinically warranted for disease control. It has been shown that renal NLRP3 inflammasome activation play a key role in the pathogenesis of LN. Because LN results from autoimmune conditions, windows of opportunity exist for improving therapy by targeting NLRP3 inflammasome and its related molecular pathways. Traditional Chinese medicine (TCM) has been successfully used worldwide in treating various diseases, including autoimmune and renal conditions, with tremendously low toxicity and side effects, although individual mechanisms of action responsible for each of them to render beneficial effects are mostly vague.

In this report, we demonstrated that TCM-derived pure compounds exerted reno-protective effects in LN through regulating NLRP3 inflammasome, using our recently established screening platform with cell models, covering both immune cells and intrinsic renal cells, and a spontaneous mouse model of LN. Most of these tested components have been proved as of extremely low of cytotoxicity at a cell level, although further in vivo toxicology study is required for drug development purposes in the near future.

Tu-WS10-3

NLRP3 and AIM2 inflammasome function in autoimmune NZB/W F1 mouse macrophages

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Inflammasomes are molecular complexes activated by infection and cell stress, responsible for activating caspase-1 that subsequently facilitates IL-1β processing and cell death. We have discovered inflammasome deficiencies in the autoimmune NZB mouse strain. NZB has a point mutation leading to loss of expression of inflammasome initiator NLRP3, and is also deficient in AIM2 inflammasome function. NZB mice develop anti-erythrocyte antibodies and haemolytic anaemia, and also anti-nuclear antibodies typical of lupus. We hypothesise that the inflammasome deficiencies in NZB alter the interaction of the host with both...
Microflora and pathogens, promoting prolonged production of cytokines, which contribute to loss of tolerance. A more aggressive model of lupus is the first cross progeny of NZB and NZW mice, which develop severe lupus nephritis. Here we have investigated AIM2 and NLRP3 inflammasome function in cells from NZB, NZW and NZB/W F1 mice. Outputs such as cell death, ASC speck formation and IL-1β processing were measured in response to AIM2 inflammasome stimuli, electroporated DNA and mouse cytomegalovirus (MCMV) infection and the NLRP3 inflammasome stimuli nigericin, ATP, alum and Candida albicans. The NZW parental strain differs from the F1 mice, and consequently function that was intermediate between NZB and NZW. Despite moderate deficiency in the F1 mice, both inflammasome systems were capable of giving substantial responses under conditions of high stimulus. The partial inflammasome deficiency in NZB/W F1 mice could contribute to the initiation of autoimmune. However, with abundant self danger molecules as triggering stimuli later in disease, NLRP3 inflammasome may exacerbate kidney damage. This work highlights the complex role that inflammasomes could play in autoimmune disease.

Tu-WS10-4

Interleukin-20 induces podocyte apoptosis and is upregulated in early diabetic nephropathy

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Interleukin (IL)-20, a proinflammatory cytokine of the IL-10 family, is involved in acute and chronic renal failure. The aim of this study was to elucidate the role of IL-20 during diabetic nephropathy development. We found that IL-20 and its receptor IL-20R1 were upregulated in the kidneys of mice and rats with STZ-induced diabetes. In vitro, IL-20 induced MMP-9, MCP-1, TGF-β1 and VEGF expression in podocytes. IL-20 was upregulated by hydrogen peroxide, high-dose glucose and TGF-β1. In addition, IL-20 induced apoptosis in podocytes by activating caspase-8. In STZ-induced early diabetic nephropathy, IL-20R1-deficient mice had lower blood glucose and serum BUN levels and a smaller glomerular area than did wild-type controls. Anti-IL-20 monoclonal antibody (7E) treatment reduced blood glucose and the glomerular area and improved renal functions in mice in the early stage of STZ-induced diabetic nephropathy. ELISA showed that the serum IL-20 level was higher in patients with diabetes mellitus than in healthy controls. The findings of this study suggest that IL-20 induces cell apoptosis of podocytes and plays a role in the pathogenesis of early diabetic nephropathy.

Tu-WS10-5

Aicardi-Goutières syndrome-like inflammation in mutant mice with constitutively activated MDA5

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MDA5 is a cytoplasmic double-stranded RNA sensor that triggers type I interferon (IFN-I) production. The gain-of-function mutations in MDA5 cause Aicardi-Goutières syndrome (AGS), a genetic disorder characterized by an infancy-onset inflammatory encephalopathy. AGS is also caused by mutations in six other genes affecting nucleotide metabolism: TREX1, RNASEH2A, RNASEH2B, RNASEH2C, ADAR1, and SAMHD1. However, mice deficient in these genes were reported not to develop obvious encephalitis.

Here we revealed spontaneous encephalitis in mutant mice with gain-of-function mutations of MDA5 generated by ENU mutagenesis, which were previously reported to develop lupus-like autoimmune symptoms. FDG-PET analysis showed a MAVS-dependent high accumulation in the kidney and brain, suggesting severe inflammation in the both organs. The mice displayed upregulation of IFN-I in the brain with no infiltration of lymphocytes and microglia were the major source. Through histological and flow cytometric analysis, enrichment of astrocytes and microglia, which is called astrocytosis and microgliosis respectively, was observed in the whole brain in both MAVS and IFN-I dependent manner. Although multiple autoantibodies were detected in the sera of the mice and also AGS patients, the mutant mice intercrossed with Rag2 deficient mice also exhibited upregulation of IFN-I, astrocytosis and microgliosis, indicating autoantibodies or lymphocytes did not contribute to development of the encephalitis. Furthermore, microglia in mutant mice were in an activated status with high CD45 expression, less production of neurotrophic factors and enhanced phagocytic capability. These data suggested that not only IFN-I but also microgliosis was involved in the pathogenesis of this encephalitis. Although there was no detectable calcification, IFN-I upregulation in the absence of lymphocyte infiltration is a common feature with human AGS. Taken together, these mice have the potential to be used for exploiting clinical treatments of AGS. Moreover, we would like to propose that microglia can be one of targets for AGS therapy.

Tu-WS10-6

Regulation of glial cells by Tregs in the chronic phase after stroke

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Foxp3+ regulatory T cells (Tregs) play essential roles in immune tolerance. In addition, Tregs have been shown to possess specialized functions in non-immune tissues such as visceral adipose tissue, skeletal muscle, and colonic lamina propria where Tregs contribute to tissue homeostasis. In this study, we discovered that Tregs accumulate in the brain one to two weeks after ischemic brain injury induced by a transient middle cerebral artery occlusion (MCAO). Analysis of gene expression profile revealed that the Tregs isolated from injured brain were different form other tissue Tregs. Tregs accumulated in the brain after ischemic injury expressed high levels of ST2 (IL-33 receptor) and Amphiregulin (Areg). Depletion of Tregs in DEREG mice resulted in the increase of the number of microglia as well as GFAP-positive reactive astrocytes (astrogliosis), and delayed neurological recovery. Rag-deficient mice also showed enhanced astrogliosis, and adoptive transfer of Tregs reduced astrogliosis. These data suggest that Tregs are neuroprotective through regulation of astrocytes. Areg may be involved in the regulation of astrogliosis, since administration of Areg reduced the number of activated astrocytes. FTY720 treatment reduced the number...
of Tregs in the brain, suggesting that Tregs were generated in the periphery and migrated into the brain. Brain Tregs expressed CCR6 and CCR8, and intraventricular administration of their ligands, CCL1 and CCL20 increased the infiltration of Tregs in the brain, and improved a neurological function. These results suggest that the accumulation of Tregs in the chronic phase after stroke is important for the neurological recovery and induction of brain-specific Tregs could be a useful therapy for alleviation of neurological symptoms.

Tu-WS10-8

The microbiome controls the development of CNS autoimmunity by regulating T cell activation and migration

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The commensal microbiome heavily influences the host immune system and plays an integral role in immunity to infection and cancer. Conversely, changes in the microbiota have been linked with the development of autoimmune diseases, such as multiple sclerosis (MS). Here, we have used a murine model of MS, experimental autoimmune encephalomyelitis (EAE), to examine the role of the microbiome in priming pathogenic CD4+ T cells and γδ T cells. Our findings suggest that intestinal dysbiosis can alter γδ T cell activity. Treatment of mice with oral antibiotics significantly attenuates the clinical course of EAE induced by active immunization with MOG and CFA. This was reflected in a significant reduction in VLA-4, CCR6 and LFA-1 expression on T cells and infiltration of IL-17+ IFN-γ+ and IFN-γ+IL-17+ CD4 T cells into the CNS. However, T cell cytokine production in the periphery remained unchanged. Furthermore, depletion of the microbiome in recipient mice impaired the ability to induce EAE by transfer of pathogenic T cells from mice with a normal gut flora, suggesting that the microbiome plays a critical role in the migratory and encephalitogenic activity of T cells. Innate IL-1 plays a key role with IL-23 in activating IL-17-secreting γδ T cells that are pathogenic in EAE. Il1r1−/− mice are significantly more resistant to EAE than wild-type mice. Transfer of Vγ4+ γδ T cells from naive mice increased the susceptibility of Il1r1−/− mice to induction of EAE. In contrast, Vγ4+ T cells from microbiome-depleted mice had limited ability to confer susceptibility to EAE in Il1r1−/− mice. Our findings demonstrate the microbiome is necessary to prime γδ T cells during induction of EAE and moreover, is required for the licensing of T cells to traffic from the periphery into the CNS.

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Tu-WS10-9

Photopic light intensity inhibits retinal inflammation via down-regulating local adrenergic system

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We previously reported that specific neural activations involving sensory and sympathetic neurons trigger neuro-inflammation in the CNS via blood-brain barrier breaching at specific vessels. We demonstrated that gravity-, electric stimulation- or pain-mediated specific sensory inputs activate specific sympathetic pathways to release norepinephrine, which in turn enhances expression of chemokines through activation of NF-κB in the specific blood vessels to open a gateway for immune cells toward the CNS. We termed the phenomena as the gateway reflex. Because light is a common neuronal stimulus to the retina, we examined the effect of light on local retinal inflammation. While the photoperiod length affects immune responses, whether light intensity can impact the course of autoimmune diseases remains unknown. Here, we showed that exposure to photopic light suppresses...
subsequent ocular inflammation, including chemokine and cytokine expressions, immune cell infiltration, and the clinical scores in a mouse model of autoimmune posterior uveitis. Mechanistic analysis showed that photopic light exposure increases norepinephrine concentration in eyes and down-regulates retinal expression of α1A-adrenoceptor (α1AAR), which is known to enhance NF-κB activity most likely via their desensitization. Consistently, blockade of α1AAR signaling under mesopic light condition recapitulated the protective effect of the photopic light stimulation. These results reveal that photopic light can inhibit ocular inflammation via regulating local noradrenergic system. Thus, we revealed a previously unrecognized role for light intensity in the control of ocular inflammation, which can be called “light-gateway reflex”. Our study also suggests that photopic light treatment or targeting of retinal α1AAR pathway might represent a novel therapeutic strategy for autoimmune uveitis.

Tu-WS10-10
Symmetrical inflammation is developed by the sensory neurons between joints in a rheumatoid arthritis model

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One criterion for the diagnosis and a fundamental characteristic of rheumatoid arthritis (RA) is symmetric inflammation, which results in wide spread and severe malformation and immobility in bilateral joints. Furthermore, symmetric clinical symptoms are observed in various inflammatory diseases including psoriasis, pulmonary fibrosis, glomerulonephritis, and sympathetic ophthalmitis. Several studies have suggested that a neurological mechanism is involved in the symmetric symptoms, however, the detailed molecular mechanism to link inflammatory and neurological pathways has not been demonstrated yet. Here, we show using cytokine-induced arthritis, a mouse model of RA that the asymmetrical inflammation in ankle joints is developed by the interactions via sensory-interneurons at the lower thoracic cords. Symmetrical ankle joint inflammation was significantly improved by surgical ablation or pharmacological inhibition of this neural pathway. We identified ATP as a key molecule to induce the symmetric inflammation and to activate the neural pathway. Thus, blockades of this regional neural pathway by suppressing ATP or attenuating the excessive activation of sensory-interneuron crosstalk may have therapeutic value for inflammatory diseases, particularly those with symmetric symptoms.

Tu-WS10-11
Brain micro-inflammation at specific vessels establishes a new neural circuit, which dysregulates the gastrointestinal homeostasis under stress conditions

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It is well known that chronic stresses exacerbate illness. However, the molecular mechanism remains poorly understood. Here, we show one of the underlying molecular mechanisms using an adoptive transfer system of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. In general, EAE mice develop tail and hind limb paralysis, but the symptom is not lethal. Under a chronic stress condition, however, EAE mice showed high mortality, associated with severe gastrointestinal failure. Interestingly, while donor pathogenic CD4+ T cells specific for a myelin antigen accumulated at the fifth lumbar spinal cord under a normal condition, the stress condition directed them to invade at the specific vessels of boundary area of the third ventricle, thalamus, and dentate gyrus to establish brain micro-inflammation. Importantly, instead of EAE induction, a direct cytokine injection to induce brain micro-inflammation at specific vessels was sufficient to establish severe gastrointestinal failure in mice with stress. Resulting brain micro-inflammation activated the specific neural pathway including the paraventricular nucleus (PVN), dorsomedial nucleus of hypothalamus (DMH), and vagal neurons to cause severe gastrointestinal failure. Suppression of the brain micro-inflammation or blockage of the neural pathway inhibited the gastrointestinal failure and improved mortality. These results showed a direct link between brain micro-inflammation and gastrointestinal homeostasis via a specific neural pathway under stress. We therefore suggest that brain micro-inflammation(s) could act as a switch to activate the new neural pathway(s) to regulate organ homeostasis.

Tu-WS12-1
Role of T follicular helper (TFH) and Th1 in flu specific humoral immunity

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TFH cells are a helper subset to localize in B cell follicle and germinal center (GC) of second lymphoid organ and to control B cell response that largely rely on TFH secreted cytokines, IL-4 and IL-21. We recently identified IFN-γ dependent antibody, IgG2 antibodies predominate in the response to vaccination with inactivated influenza A virus (IAV) and were responsible for protective immunity to lethal challenge with pathogenic H5N1 and pandemic H1N1 IAVs. The IgG2 antibody responsible for protective immunity was independent on germinal center (GC) and TFH based on the evidences that B or T cell-specific ablation of the Bcl6 did not have an impact on IgG2 responses specific for HA antigen. We identified that IL-21 and IFN-γ secreted from CXCR3+CXCR5+ Th1 cells accounted for the low affinity antibodies are not entirely necessary for protective IgG2 responses in systemic vaccination.

The mucosal IgA antibody specific for HA in the lung has a potential to cross-react with other strains of influenza virus (breath). We found that virus replication was needed to induce protective and cross-reactive IgA antibody. The production of neutralizing IgA antibodies associated with lung residential CD4 T cells that expressed CD103, CXCR3 and IFN-γ, and with IL-6 dependent B cell activation. These results indicated that flu replication promoted unique lung
environment to confer cross-reactive neutralization in IgA antibodies. Therefore, induction of IgG2 antibody in systemic vaccination and IgA response in the lung by live flu administration should be promising strategies to induce effective neutralizing antibodies to combat emerging pandemic influenza viruses.

Tu-WS12-2
Hypoleptinemia impairs T<sub>FH</sub> cell function and confers the risk of poor vaccine responses

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Despite being considered one of the greatest achievements of modern medicine, vaccination is often limited by poor responses in a significant fraction of the general population. For example, influenza vaccine is clinically effective in most young adults, but it is clinically ineffective in the majority of elderly individuals. Improved understanding of vaccine immunology and identification of new biomarkers for vaccine efficacy are required to guide the optimization of immunization strategies and the development of new vaccines. Here we report hypoleptinemia as an important risk factor for poor vaccine responses. We found that low leptin was strongly associated with low antibody responses to influenza and hepatitis B virus (HBV) vaccines in different age groups. Similarly, leptin receptor-deficient mice showed impaired antibody responses after influenza virus infection or immunization. In a more physiological setting, fasting mice failed to mount vaccination-mediated protection to influenza infection, but this was largely rescued by leptin replacement. Mechanistic studies revealed that leptin promotes both mouse and human follicular helper T (T<sub>FH</sub>) cell differentiation and IL-21 production in a Stat3-dependent manner. T<sub>FH</sub> cells are specialized CD4<sup>+</sup> T cell subset that is essential for supporting vaccine-generated OT-II TCR-transgenic mice with peripheral T cell-specific deletion of either E2A (E2A<sup>Δ<sub>C</sub></sup>) or HEB (HEB<sup>Δ<sub>C</sub></sup>). Naïve CD4<sup>+</sup> OT-II T cells from mutant or control mice were transferred into congenic C57BL/6 mice, and the recipients were immunized with OVA in alum. Seven days after immunization, E2A<sup>Δ<sub>C</sub></sup> donor cells differentiated into pre-T<sub>FH</sub> and germinal center (GC) T<sub>FH</sub> cells comparably to control cells, whereas HEB<sup>Δ<sub>C</sub></sup> donor cells underwent pre-T<sub>FH</sub> cell differentiation but failed to mature into GC T<sub>FH</sub> cells. HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells exhibited normal T<sub>FH</sub> signature gene expression but a substantial increase in the Gpr183 gene encoding Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2), which recognizes 7α,25-dihydroxycholesterol secreted by stromal cells in the outer T cell zone that affects T cell localization at the T/B border. EBI2 expression on HEB<sup>Δ<sub>C</sub></sup> donor cells was induced normally at day 2 and downregulated at day 4 post-immunization. However, at day 5 post-immunization and later, HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells re-expressed high levels of EBI2, and accumulated at the T/B border. The failure of HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells to enter B cell follicles and undergo subsequent GC T<sub>FH</sub> maturation was completely rescued by further conditional deletion of Eomes, which was highly expressed in HEB<sup>Δ<sub>C</sub></sup> donor cells during the first 2 days of priming. Our results suggest that HEB fine-tunes the localization of pre-T<sub>FH</sub> cells by preventing EBI2 re-expression through the suppression of Eomes, allowing for their entry to the follicles to promote subsequent maturation into GC T<sub>FH</sub> cells.

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Tu-WS12-4
Mechanisms underlying differentiation and function of adipose tissue resident regulatory T cells

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Inflammation mediated by adipose tissue (AT) resident immune cells is a major driver of insulin resistance and type 2 diabetes (T2D). Maintaining the balance between anti- and pro-inflammatory immune cells is crucial for the prevention of adipose tissue inflammation. This balance however is tipped in favour of pro-inflammatory cells under obese conditions. A specialised population of regulatory T (Treg) cells that reside in the adipose tissue counteracts inflammation and preserves insulin resistance. This Treg cell population declines during obesity, which leads to the exacerbation of inflammation in adipose tissue. We discovered that AT Treg cells specifically required the cytokine IL-33 for their differentiation and maintenance. IL-33 led to the expansion of Treg cells specifically in the AT and reverted insulin resistance when administered to obese mice. TCR signalling induced transcription factor IRF4 contributed to Treg cell IL-33 sensing by regulating the expression of IL-33 receptor ST2. Furthermore, I will discuss how novel physiological signals regulate IL-33 signalling to facilitate differentiation and maintenance of AT Treg cells.

Tu-WS12-5
T-bet<sup>+</sup> memory-phenotype CD4<sup>+</sup> T cells are spontaneously generated via tonic IL-12 in steady state and exert cytokine-dependent, innate-like effector function

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E-box binding proteins (E-proteins) have recently been demonstrated to regulate follicular helper T (T<sub>FH</sub>) cell differentiation, but the mechanisms underlying E-protein-mediated T<sub>FH</sub> differentiation remain elusive. To evaluate T cell-intrinsic effects of E-proteins, we generated OT-2 TCR-transgenic mice with peripheral T cell-specific deletion of either E2A (E2A<sup>Δ<sub>C</sub></sup>) or HEB (HEB<sup>Δ<sub>C</sub></sup>). Naïve CD4<sup>+</sup> OT-II T cells from mutant or control mice were transferred into congenic C57BL/6 mice, and the recipients were immunized with OVA in alum. Seven days after immunization, E2A<sup>Δ<sub>C</sub></sup> donor cells differentiated into pre-T<sub>FH</sub> and germinal center (GC) T<sub>FH</sub> cells comparably to control cells, whereas HEB<sup>Δ<sub>C</sub></sup> donor cells underwent pre-T<sub>FH</sub> cell differentiation but failed to mature into GC T<sub>FH</sub> cells. HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells exhibited normal T<sub>FH</sub> signature gene expression but a substantial increase in the Gpr183 gene encoding Epstein-Barr virus-induced G-protein coupled receptor 2 (EBI2), which recognizes 7α,25-dihydroxycholesterol secreted by stromal cells in the outer T cell zone that affects T cell localization at the T/B border. EBI2 expression on HEB<sup>Δ<sub>C</sub></sup> donor cells was induced normally at day 2 and downregulated at day 4 post-immunization. However, at day 5 post-immunization and later, HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells re-expressed high levels of EBI2, and accumulated at the T/B border. The failure of HEB<sup>Δ<sub>C</sub></sup> pre-T<sub>FH</sub> cells to enter B cell follicles and undergo subsequent GC T<sub>FH</sub> maturation was completely rescued by further conditional deletion of Eomes, which was highly expressed in HEB<sup>Δ<sub>C</sub></sup> donor cells during the first 2 days of priming. Our results suggest that HEB fine-tunes the localization of pre-T<sub>FH</sub> cells by preventing EBI2 re-expression through the suppression of Eomes, allowing for their entry to the follicles to promote subsequent maturation into GC T<sub>FH</sub> cells.

This work was supported by the Intramural Research Program of NIAID/NIH.
CD4+ T cells are composed of naïve, pathogen-specific memory, and pathogen-independent memory-phenotype (MP) cells. Naïve and pathogen-specific memory cells play key roles in adaptive immunity while the homeostatic mechanisms regulating the generation of MP cells and their biological functions are unclear. In this work we show that MP cells are autonomously generated from peripheral naïve cells in the absence of infectious stimulation in a TCR- and CD28-dependent manner. We further demonstrate that MP cells contain a T-bethi subpopulation and that this subpopulation requires tonic IL-12 derived from CD8α+ (type 1) dendritic cells for its generation. Importantly, these cells rapidly produce IFN-γ in response to IL-12 in the absence of pathogen recognition and provide antigen-nonspecific host resistance against Toxoplasma gondii and Mycobacterium tuberculosis infection that drives Th1-type immunity while enhancing the adaptive CD4+ T cell responses. Together, these findings reveal that T-bethi MP CD4+ T cells are spontaneously generated from naïve precursors by IL-12 and possess a novel innate-like effector function by which they produce an early, cytokine-driven protective response against Th1-inducing pathogens.

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Tu-WS12-6

Myosin light chain 9 and 12 are functional ligands for CD69 that regulate airway inflammation

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Recent decades have witnessed a rapid worldwide increase in chronic inflammatory disorders such as asthma. CD4+ T helper type 2 (Th2) cells play critical roles in the pathogenesis of allergic airway inflammation and CD69 expression on activated CD4 T cells is required to induce allergic inflammation in tissues. However, how CD69 mechanistically controls allergic inflammation remains poorly defined. In lymphoid tissues, CD69 regulates cellular retention via inhibition of S1P1 expression and requires no specific ligands to function. In contrast, we show herein that myosin light chain (Myl) 9 and 12 are new functional ligands for CD69. The blockade of the CD69-Myl9/12 interaction ameliorates allergic airway inflammation in OVA-induced and house dust mite (HDM)-induced mouse models of asthma. Within the inflamed mouse airways, we found that the expression of Myl9/12 was increased and platelet-derived Myl9/12 localized to the luminal surface of blood vessels and formed intravascular net-like structures. The analysis of nasal polyps of eosinophilic chronic rhinosinusitis (ECRS) patients revealed that Myl9/12 expression was increased in the inflammatory lesion, and was distributed within net-like structures in the intravascular space. In addition, we detected Myl9/12 in the perivascular spaces where many CD69+ cells were positioned within Myl9/12 structures. Thus, the Myl9/12-CD69 interaction is a key event in the recruitment of activated CD69+ T cells in inflamed tissues and could be a therapeutic target for intractable airway inflammatory diseases.

We-WS13-1

Identification of human common monocyte progenitors

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Monocytes give rise to macrophages and dendritic cells (DCs) under steady-state and inflammatory conditions, thereby contributing to host defense and tissue pathology. Inflammation can prompt tissue-infiltrating monocytes to differentiate into monocyte-derived macrophages and DCs, which are associated with both homeostatic host-defense reactions and inflammatory diseases. In mice, monocytes are divided into classical Ly6ch and non-classical Ly6csh-expressing subsets. Ly6ch monocytes are present only in the blood, whereas Ly6csh monocytes are found in both the blood and other tissues, where they differentiate into macrophages and DCs. In this context, most Ly6csh monocytes are derived from Ly6ci monocytes. In human, monocytes are comprised of major CD14CD16+ and other CD14CD16+ and CD14CD16- monocytes. A couple of years ago, a common monocyte progenitor (cMoP) that is restricted to the monocyte lineage has been identified in mice. Here, we introduce human cMoP, which was identified as a CLEC12A/CD64+ subpopulation of conventional granulocyte-monocyte progenitors (cGMP) in umbilical cord blood and in bone marrow. Human cMoP gave rise to monocyte subsets without showing any potential for differentiating into myeloid or lymphoid cells ex vivo. Within the cGMP population, we also identified revised GMP that completely lacked DC and lymphoid potential, and the revised GMP gave rise sequentially to cMoP, pre-monocytes, and monocytes. Collectively, our findings revise the current understanding of human myeloid cell differentiation pathways. Given that monocytes and monocyte-derived macrophages and osteoclasts cause a variety of inflammatory disorders, our studies shed light not only on human monocyte differentiation.

We-WS13-2

Repression of SMAD3 by STAT3 and c-SKI is essential for conventional dendritic cell differentiation

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Transforming growth factor-β (TGF-β) plays pleiotropic regulatory roles in dendritic cell (DC) development. However, the signalling mechanisms how TGF-β differentially regulates the development of DC subsets remain largely unknown. Here, we show that signalling downstreamregulation of one of the TGF-β receptor-regulated SMADs, SMAD3 by STAT3 and c-SKI is essential for conventional or classical DC (cDC) differentiation. We found that the expression of SMAD3 was decreased, whereas SMAD2 remained expressed in cDCs. SMAD3 deficiency facilitated cDC differentiation with the increase of CD135+CD115+Lin−cDC precursor cells and cDCs along with the decrease of CD117+CD135+CD115+macrophage-DC precursor (MDP) cells in bone marrow, whereas SMAD2 deficiency did not affect DC differentiation. We found that SMAD3 transcribed by SMAD2 and SMAD3 repressed the genes essential for cDC differentiation such as Flt3, IRF4 and STAT3 in the progenitor cells. Furthermore, STAT3 repressed SMAD3 for Flt3L or GM-CSF/IL-4-induced cDC differentiation in synergy with c-SKI, a negative regulator of SMAD-mediated TGF-β signalling. Our data reveal the cross-regulation between SMAD3 and STAT3 specific for cDC differentiation from MDP.

We-WS13-3
Mapping the human DC lineage through the integration of high-dimensional techniques

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Dendritic cells (DC) are professional antigen-presenting cells that orchestrate immune responses. The human DC population comprises two main functionally specialized lineages, whose origins and differentiation pathways remain incompletely defined. Here, we combine two high-dimensional technologies-single-cell messenger RNA sequencing (scmRNAseq) and cytometry by time-of-flight (CyTOF)-to identify human blood CD123+CD33+CD45RA−DC precursors (pre-DC). Pre-DC share surface markers with plasmacytoid DC (pDC) but have distinct functional properties that were previously attributed to pDC. Tracing the differentiation of DC from the bone marrow to the peripheral blood revealed that the pre-DC compartment contains distinct lineage-committed subpopulations, including one early uncommitted CD123high pre-DC subset and two CD45RA−CD123low lineage-committed subsets exhibiting functional differences. The discovery of multiple committed pre-DC populations opens promising new avenues for the therapeutic exploitation of DC subset-specific targeting.

We-WS13-4
SIRPa+ dendritic cells regulate homeostasis of fibroblastic reticular cells via TNF receptor ligands in the adult spleen

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Stromal cells contribute to the structural organization of secondary lymphoid organs (SLOs) by producing cytokines and chemokines, which regulate the positioning and segregation of incoming dendritic cells (DCs) and T and B cells. We have previously shown that signal regulatory protein α (SIRPa) and its ligand CD47 are important for the homeostasis of type 2 conventional DCs (cDC2) that abundantly expresses SIRPa, as well as that of stromal cells such as podoplanin (Pdpn)−positive fibroblastic reticular cells (FRCs) in the spleen. The cellular and molecular basis for such regulation by SIRPa and CD47 has remained largely unclear, however. Here we showed that DC-specific ablation of SIRPa or CD47 (SIRPaΔ Δ or Cd47ΔΔ) markedly reduced the number of cDC2 as well as of Pdpn+ FRCs and T cells in the spleen. Such ablation also reduced CCL19 and CCL21 production and impaired the survival of FRCs in the spleen. In addition, the reduction in the number of cDC2 caused by tamoxifen-inducible ablation of SIRPa was followed by the consecutive depletion of FRCs and T cells in the spleen. By contrast, Irf4Δ Δ and RbpjΔ Δ mice, both of which also impaired development and homeostasis of cDC2, did not manifest any reduction in the number of FRCs or T cells in the spleen. These results suggest that cDC2 maintain homeostasis of FRCs and SIRPa specifically regulates such function. Moreover, we found that the cDC2 prevented the proliferation or survival of stromal cells by producing TNFR ligands such as TNF-α. Indeed, the expression of Tnf mRNA was down-regulated in the CD4+ cDC2 subset sorted from SIRpaΔ Δ or Cd47ΔΔ mice. SIRPa− cDC2 thus regulate the steady state homeostasis of FRCs in the adult spleen via the production of TNFR ligands, with the CD47−SIRPa interaction in cDC2 likely being indispensable for such regulation.

We-WS13-5
Glibenclamide reduces monocyte functions against Mycobacterium tuberculosis infection

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Diabetes mellitus is an important risk factor for development of tuberculosis. Our previous study found that glibenclamide reduced neutrophil cytokine production, migration and killing in responses to bacterial infection. Glibenclamide is a widely used antidiabetic drug in low and middle-income countries where the incidence of tuberculosis is high. It is a broad-spectrum ATP-binding cassette transporter inhibitor and a K+ ATP channel blocker which inhibits cryopyrin/Nalp3 inflammasome activation and proteolytic maturation of IL-1β and IL-18 by caspase-1 in neutrophils and macrophages in response to Burholderia
pseudomallei (melioidosis). In mice infected with M. tuberculosis (Mtbc), the production of IL-1β is essential for host resistance through the enhancement of cyclooxygenase that limits excessive Type I interferon (IFN) production and fosters Mtbc containment. Since melioidosis and tuberculosis share many immunological characteristics, we hypothesize that glibenclamide may also interfere with monocyte-mediated immune responses against Mtbc and alter the balance between protective IL-1β and immunosuppressive Type I interferon-mediated immunity. Here we demonstrate that purified human monocytes from diabetic individuals who had been treated with glibenclamide showed reduction of IL-1β and IL-8 secretion but revealed enhancement of bacterial growth when exposed to Mtbc. Additionally, these responses also occurred when monocytes from non-diabetic control individuals were pre-treated with glibenclamide in vitro. Moreover, this pre-treatment enhanced IFN-α expression but not involved with prosta-glandin level in response to Mtbc infection. Taken together, our data show that glibenclamide might be responsible, at least in part, to the increased susceptibility of diabetic individuals to Mtbc infection by reducing IL-1β production and killing of monocytes.

We-WS13-6

MIP-1α deficiency prevents lipotoxicity-induced hepatic insulin resistance and nonalcoholic steatohepatitis

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We reported previously that loss of the C-C chemokine receptor CCR5 prevents obesity-related insulin resistance independently of MIP-1-CCR2. Moreover, we found that hepatic expression of macrophage inflammatory protein 1α (MIP-1α), a CCR5 ligand, increases markedly in obese mice. To investigate the role of MIP-1α in lipotoxicity-induced hepatic insulin resistance and nonalcoholic steatohepatitis (NASH), the metabolic phenotypes of MIP-1α+/− mice and their littermates were compared by feeding them a high-cholesterol/high-fat (CL) diet for 16 weeks. Expression of MIP-1α mRNA increased significantly in the liver of wild-type (WT) mice fed the CL diet compared with that in chow fed WT mice (P < 0.01), particularly in the macrophage/Kupffer cell fraction. An immunofluorescence analysis of the liver revealed that MIP-1α was expressed by F4/80+ macrophages in CL fed mice. The excess hepatic lipid accumulation and peroxidation induced by the CL diet decreased in MIP-1α+/− mice compared with those in MIP-1α+/+ mice (P < 0.01). CL diet-induced glucose intolerance and hyperinsulinemia improved in MIP-1α+/− mice compared with those in MIP-1α+/+ mice. In addition, phosphorylation of IRβ and Akt was enhanced in the liver of MIP-1α+/− mice, accompanied by attenuated NF-kB and MAPK signaling, and decreased plasma TNFα levels (P < 0.05). Furthermore, CL diet-induced stellate cells activation and fibrogenesis decreased in the liver of MIP-1α+/− mice. A flow cytometry analysis demonstrated that MIP-1α+/− mice fed the CL diet had 37% fewer CD11c+CD206+ (M1) macrophages but 59% more CD11c+CD206− (M2) macrophages than those in MIP-1α+/+ mice, resulting in a predominance of M2 over the M1 population. Importantly, chimeric mice lacking MIP-1α only in myeloid cells (MIP-1α+/− into MIP-1α+/− bone marrow transplant) were protected from CL diet-induced insulin resistance and NASH. Thus, MIP-1α deficiency prevented lipotoxicity-induced insulin resistance and NASH by polarizing M2 macrophages in the liver.

We-WS13-7

The innate immune receptor Dectin-2 mediates the phagocytosis of cancer cells by Kupffer cells for the suppression of liver metastasis

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Metastasis to distal organ is a fatal cause of cancer death and liver is one of the most common site of metastasis leading to poor prognosis. Although it has been known that innate immune cells play critical roles in the regulation of liver metastasis, how they control the tumor development is still largely unclear. Here, we found that Dectin-2, a C-type lectin receptor (CLR) essential for anti-fungal innate immunity, is required for the suppression of liver metastasis in vivo. Such antitumor system was not triggered in subcutaneous tumor growth and lung metastasis, indicating the liver-selective function of Dectin-2. Analysis for the mechanism revealed that Dectin-2-mediated tumor rejection required Kupffer cells, liver-residing macrophages, which express Dectin-2 dominantly in liver. Moreover, Dectin-2 enhanced the uptake and clearance of cancer cells by Kupffer cells. Interestingly, Kupffer cells are uniquely endowed with Dectin-2-dependent phagocytotic function, as neither bone marrow-derived macrophages nor alveolar macrophages show such phenotype. We further examined the involvement of other CLRs in Dectin-2-regulated anti-tumor responses and found that macrophage C-type lectin (MCL), a CLR known for forming complex with Dectin-2, also contributed to the uptake of cancer cells by Kupffer cells and the rejection of liver-metastasizing cells. Another CLR Dectin-1 suppressed liver metastasis as well, however, the engulfment of cancer cells by Kupffer cells was not regulated by Dectin-1. Instead, Dectin-1 promoted the anti-tumor cytotoxicity of liver non-parenchymal cells, which is mostly mediated by natural killer cells. Dectin-2 and MCL were not involved in such host killing machinery against tumor cells. Collectively, these results indicate that Dectin-2 selectively suppresses liver metastasis through the enhancement of cancer cell engulfment by Kupffer cell, in cooperation with MCL and distinctly from Dectin-1. This study provides the promising insights for the development of novel anti-cancer therapy targeting CLRs.

We-WS14-1

A safe way for insulting antigen with adjuvant without cytokine toxicity in vaccines

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Vaccines exert a tremendous effect on the prevention of infectious diseases. Infectious disease vaccines contain components of microorganisms, which make the vaccine consisting of an antigen (Ag) and an immunostimulatory substance (a pattern molecule, a ligand of Toll-like receptor (TLR), etc.). It is due to pattern molecules that side effects
(fever, fatigue, nausea, etc.) occur after vaccination. Synthesis or highly purified antigens lose pattern molecules, which increases safety but reduces vaccine activity as seen in the current influenza vaccine.

Pattern molecules exogenously added to purified antigens are called adjuvants. Such a vaccine can be designed to be noninfectious and is called a subcomponent vaccine. The advantage is that both antigens and pattern molecules can be structurally defined, and safety can be individually tested in advance.

Adjuvants are indispensable for activating innate immunity to induce cellular immunity but have been made indistinguishable from inflammation. Existing adjuvants such as Alum cause inflammatory reactions but do not directly activate TLR. Therefore, induction of cellular immunity is too weak to adapt for anticancer immunity.

Human antigen-presenting dendritic cells were found to express only TLR2 and TLR3. TLR2 recognizes bacterial lipoprotein and peptidoglycan. BCG-CWS is an agonist of TLR2. TLR3 recognizes poly I: C. It was known that antiviral antitumor activity is strong against viral double-stranded (ds) RNA (polyyl: C is its analog). Many clinical trials using polyyl: C were performed, but tumor regression was seen with effective dose, where serious adverse events developed and described as ‘untolerable’. If these are modified to develop non-inflammatory adjuvants, it will be possible to create highly safe memory vaccines. Adjuvants can also be applied to prophylactic vaccines against infectious diseases, and development of non-inflammatory adjuvants beyond Alum is expected.

We-WS14-2
Role of HMGB1 in inflammation and cancer

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High-mobility group box protein 1 (HMGB1) is nuclear protein conserved among almost all eukaryotic cells. HMGB1 is thought to be a danger signal mediator when it is released both actively from activated immune cells and passively from dead cells into extracellular milieu upon pathogen infections or sterile inflammations. Released HMGB1 act as an inflammatory cytokine and promote inflammatory disorders. However, in vivo function of HMGB1 is still elusive. Recently we have established Hmgb1 gene conditional knockout (KO) mice and examined the role of HMGB1 in innate immune responses, inflammation and cancer. Interestingly, we observed that the number of glanurocytic neutrophils are decreased when mice were inoculated HMGB1 KO tumor cells. We would like to show and discuss these data.

We-WS14-3
CD163 is involved in the protumour activation of macrophages in human and murine sarcoma

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Recent findings showed that the significance of CD163-positive macrophages in tumour progression. However, few studies related to the functions of CD163 in macrophages have been published. Therefore, we tried to uncover the involvement of CD163 in macrophage activation using CD163-deficient mice and human samples. At first we performed immunohistochemistry of CD163 using 45 samples of undifferentiated pleomorphic sarcoma (UPS). High density of CD163-positive macrophages was closely related to shortened progression free survival time and higher histological grade. Tumor development of sarcoma (MCA205 and LM8) cells were significantly abrogated in CD163-deficient mice as compared with WT mice. Co-culture study using peritoneal macrophages and MCA205 cells was performed, and we found that proliferation of MCA205 was significantly increased by co-culture with WT macrophages whereas this macrophage-induced proliferation of MCA205 was suppressed when CD163-deficient macrophages were used. IL-6 and CXCL2 production were found to be suppressed in CD163-deficient macrophages compared with WT macrophages. Silencing IL-6, but not CXCL2, abrogated the macrophage-induced proliferation of MCA205. By means of coculture studies using human monocyte-derived macrophages and human sarcoma cell lines, similar macrophage-induced tumour cell proliferation was observed in TYLMS-1 and NMFH-1 cell lines, and notably silencing of CD163 abrogated the macrophage-induced tumour cell proliferation. Taken together, CD163 is related to protumour activation of macrophages and considered to be closely involved in tumour development and progression in murine and human malignant tumours. This work is collaboration with Dep. of Pathology, Kyushu University.

We-WS14-4
Combining depletion of myeloid-derived suppressor cells with dexamethasone ameliorate tumor regression in melanoma-bearing mice

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Aggravated inflammation is often associated with metabolic disturbances that contribute to cancer initiation. Further, overt immunosuppression support cancer progression with accumulation of proinflammatory cytokines, and infiltration of immunosuppressive cells. Therefore, combinatorial targeting of both inflammation and immunosuppression pathways may improve cancer immunotherapy. Herein, we examined immune response, metabolic imbalance affecting fat and lean tissue in vivo in B16F10 tumor-bearing mice. Moreover, we investigated mono- or multimodal-immunotherapies that target both production of inflammatory cytokines and myeloid-derived suppressor cells (MDSCs) in order to augment tumor regression. To this end a group (n = 12) of either B16F10-bearing mouse receiving dexamethasone (DEX), or (n = 12) depleting monoclonal antibodies (mAbs) anti-Gr1 (RB6-8C5), both (n = 12) DEX and anti-Gr1, or (n = 12) vehicle were tested. First, we observed that polymorphonuclear (PMN)-MDSCs (Ly6G+) and monocytic (M)-MDSCs (Ly6C+) found within the spleen of B16F10-bearing mice express interleukin (IL)-10, whilst in melanoma-bearing mice. Moreover, we investigated mono- or multimodal-immunotherapies that target both production of inflammatory cytokines and myeloid-derived suppressor cells (MDSCs) in order to augment tumor regression. To this end a group (n = 12) of either B16F10-bearing mouse receiving dexamethasone (DEX). In both tumors (Ly6G+) and monocytic (M)-MDSCs (Ly6C+) found within the spleen of B16F10-bearing mice express interleukin (IL)-10, whilst in melanoma-bearing mice. Moreover, we investigated mono- or multimodal-immunotherapies that target both production of inflammatory cytokines and myeloid-derived suppressor cells (MDSCs) in order to augment tumor regression. To this end a group (n = 12) of either B16F10-bearing mouse receiving dexamethasone (DEX), or (n = 12) depleting monoclonal antibodies (mAbs) anti-Gr1 (RB6-8C5), both (n = 12) DEX and anti-Gr1, or (n = 12) vehicle were tested. First, we observed that polymorphonuclear (PMN)-MDSCs (Ly6G+) and monocytic (M)-MDSCs (Ly6C+) found within the spleen of B16F10-bearing mice express interleukin (IL)-10, whilst in melanoma-bearing mice. Moreover, we investigated mono- or multimodal-immunotherapies that target both production of inflammatory cytokines and myeloid-derived suppressor cells (MDSCs) in order to augment tumor regression. To this end a group (n = 12) of either B16F10-bearing mouse receiving dexamethasone (DEX), or (n = 12) depleting monoclonal antibodies (mAbs) anti-Gr1 (RB6-8C5), both (n = 12) DEX and anti-Gr1, or (n = 12) vehicle were tested. First, we observed that polymorphonuclear (PMN)-MDSCs (Ly6G+) and monocytic (M)-MDSCs (Ly6C+) found within the spleen of B16F10-bearing mice express interleukin (IL)-10, whilst in melanoma-bearing mice. Moreover, we investigated mono- or multimodal-immunotherapies that target both production of inflammatory cytokines and myeloid-derived suppressor cells (MDSCs) in order to augment tumor regression. To this end a group (n = 12) of either B16F10-bearing mouse receiving dexamethasone (DEX).
inflammation (DEX) in immunotherapy may facilitate tumor regression.

We-WS14-5

IL-34 as a prognostic biomarker and a therapeutic target in cancer

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Interleukin-34 (IL-34) is a hematopoietic cytokine that was reported for the first time in 2008 as a second ligand of CSF1R in addition to M-CSF. IL-34 and M-CSF show no sequence homology, but have similar functions, affecting the proliferation, survival and function of myeloid cell lineage. In contrast to M-CSF, IL-34 shows unique signaling and expression patterns. Physiologically, IL-34 expression is restricted to skin and brain, where it acts as a regulator of langerhans cells and microglia, respectively. However, IL-34 expression can be induced under various pathological conditions, and correlates with disease severity, chronicity and progression. In cancer, IL-34 also plays major roles in multiple aspects of the tumor microenvironment, including tumor growth, metastasis, angiogenesis and therapeutic residence. Clinical data from cancer patients strongly suggest a correlation between high expression of IL-34 with tumor aggression, poor prognosis and clinical outcome of cancer therapy. Accordingly, IL-34 may serve as a critical prognostic biomarker and a therapeutic target of great potential in cancer treatment.

We-WS14-6

Involvement of a chemokine, CCL3, in chemotherapeutic-induced tumor eradication by rapid recruitment of CD4-positive cytotoxic T cells into tumor sites

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Background: Evidence is accumulating to indicate the potential roles of CD4+ cytotoxic lymphocytes (CTLs), a distinct subset from other CD4+ helper T cell subsets, in tumor immunity because they can degranulate massively cytolytic proteins such as granzyme B and perforin upon activation. However, it remains elusive on their roles in chemotherapeutic-induced tumor eradication process. Hence, we delineated cyclophosphamide (CTX)-induced tumor eradication process in mice with a focus on CD4+ CTLs.

Methods and Results: We inoculated a murine hepatoma cell line, BNL 1ME A.7R.1 (BNL), subcutaneously into naïve Balb/c mice and gave a single intraperitoneal injection of 150 mg/kg CTX after a tumor formed. CTX injection eradicated tumors in wild-type mice (6 / 9 heads), whereas none of the tumors disappeared in nude mice (p<0.05). Moreover, WT mice whose tumor disappeared after CTX treatment rejected re-challenged BNL, but not an unrelated murine colorectal cancer cell line, CoL26. Furthermore, CD4+ but not CD8+ cell depletion abrogated CTX-induced tumor eradication. Consistently, CTX treatment increased intratumoral CD4+ CTLs, which expressed a cytolytic granule molecule, CD107a, and granzyme B. When congeneric CD45.1 splenocytes were transferred to tumor-bearing CD45.2 mice after CTX treatment, transferred CD4+ cells were recruited into tumors on day 3. Moreover, the recruited cells expressed CD107a without antigen presentation at draining lymph nodes and proliferation in tumor tissues. CTX administration enhanced mRNA expression of a CC chemokine, CCL3, in tumor tissues. CTX-mediated tumor regression was attenuated in mice deficient in CCR5, the receptor for CCL3. Consistently, CTX-induced accumulation of intratumoral CD107a-expressing CD4+ T cells was less in mice receiving CCR5-deficient mouse-derived splenocytes than those receiving WT mouse-derived splenocytes.

Conclusion: CTX induces intratumoral expression of CCL3, which recruits CD4+ CTLs into tumor sites, thereby eradicating tumors and subsequently inducing specific tumor immunity.

We-WS14-7

Time-scale analysis of interplay between immunogenic tumor and immune response

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Our knowledge of how immune checkpoints shape anti-tumour immunity is continuously growing, creating new possibilities for cancer immunotherapy. Nevertheless, some fields in this research area remained quite unexplored, such as the time course for transition from functional to dysfunctional state of anti-tumour immune response. We created bioluminescence imaging model that allowed us to precisely monitor the behaviour of immunogenic tumour (B16OVA-Luc2) under the different immunological conditions, which in turn provided us with valuable information of exact time frame when tumour escape from immune control occurs.

In our model, immunization with tumour antigen ovalbumin (OVA-mice) resulted in time-limited immune control of tumour growth that we discovered to be far more dynamic than can be observed with standard calibrator measurement. Anti-tumour immune response in OVA-mice was critically dependent on IFN-γ and CD8+ T cells, whereas in the absence of NK cells tumor growth suppression in OVA-mice was still conceivable, yet duration of immune control over tumour growth was significantly shorter. Although we found anti-PD1 mAb treatment to be partially effective in OVA-mice, PD-1 itself turned out not to be a reliable marker of exhausted OVA-specific CD8+ T cells: expression of PD-1 and its ligand PD-L1 on OVA-specific CD8+ T cells and B16OVA-Luc cells, respectively, were significantly higher in OVA-mice (in both wild type and IFN-γ−/− phenotype) compared to non-immunized mice, even during the time of evident immune control of tumour growth. In contrast, we found tumour escape to strongly correlate with both reduced clonality of OVA-specific CD8+ T cells and their ability to proliferate in tumour microenvironment. Ongoing studies are focused on further exploring time dependent changes in OVA-specific CD8+ T cells as well as in immunogenic tumour, in order to better define the events that precede tumour escape from immune control.
Adaptive T-cell immunotherapy provides a promising approach to cancer therapy. The persistence and resistance to exhaustion of transferred T cells are critical for improvement in patient outcomes. Stem cell memory T (TstemCM) cells have been proposed as a new class of memory T cells which have longevity and proliferative potential. It has been shown that mouse and human CD8+ TstemCM cells can be generated in vitro from naïve CD8+ T cells by the Wnt signaling, however, the methods for inducing TstemCM cells from activated or memory T cells remain to be established. Here, we established a new strategy of generating TstemCM-like cells in vitro (designated as “iTstemCM” cells) from activated CD4+ and CD8+ T cells in mice and humans by coculturing with stromal cells expressing a Notch ligand. These iTstemCM cells lost PD-1 expression proinflammatory receptor, to gain wide-ranging target recognition. The main players in the innate immune system are classiﬁed into 3 different groups; myeloid lineage cells including macrophages, dendritic cells and granulocytes, innate T cells including γδ T cells, NKT cells and MAIT (mucosal associated invariant T) cells, and innate lymphoid cells (ILCs). ILCs have been divided into 3 groups based on their cytokine production; group 1 ILC including NK cells and ILC1 produce IFNγ, group 2 ILC (ILC2) produce type 2 cytokines such as IL-4, IL-5, IL-6 and IL-13, and group 3 ILC, including lymphoid tissue inducer (LTI) cells and ILC3s produce IL-17 and IL-22. In this workshop, we would like to discuss the development, signaling pathways, cell-cell interactions and functions of a variety of innate immune cells.

Excessive Reactive Oxygen Species (ROS) blocks IL-17A+ γδT cells and subsequent innate immunity required for efficient clearance of Streptococcus pneumonia (Spn)

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Excessive oxidative stress is associated with a deficiency to control pathogenic microbes that can colonise the airways. Streptococcus pneumonia (Spn) is particularly resistant to oxidant-dependent killing mechanisms and infection can be life threatening for immunocompromised people, children under the age of 2 and elderly sufferers of chronic lung diseases such as Chronic Obstructive Pulmonary Disease (COPD). We aimed to elucidate the impact of excessive oxidative stress on innate immune pathways important for the clearance of Spn. A mouse model of high oxidative stress, extracellular-superoxide dismutase 3 (SOD3) deﬁcient mice were used to investigate the mechanism by which oxidative stress compromises pneumococcal clearance. The recruitment of innate immune subsets and expression of their effector molecules were assessed at day 2, and pneumococcal load in the airways was assessed at day 7. At day 2, SOD3KO mice recruited signiﬁcantly less neutrophils (SOD3KO: 53,252.27 ± 11,908 vs WT: 341,935.95 ± 106,166.30 cells, p < 0.05) into the airways as a consequence of a failure to expand IL-17A+ γδ T cells which were proportionally signiﬁcantly reduced (0.15% ± 0.07% WT vs 0.08% ± 0.016 SOD3KO, p < 0.05). The lung mucosa of SOD3-deﬁcient mice also had a signiﬁcant reduction in IL-23 and IL-1β. Since inﬂammatory macrophages are a major source of these cytokines, CD11b and CD11c were used to track monocytes/macrophage populations, where SOD3-deﬁciency failed to recruit signiﬁcant numbers of monocytes in response to the bacteria (5,844.69 ± 3,710.37 cells). In contrast, WT mice robustly recruited monocytes (96,661.96 ± 40,595.63 cells) in response to increased production of CCL2, which was signiﬁcantly reduced in SOD3 deﬁcient mice (SOD3KO: 1,018.76 ± 366.79pg/ml vs WT:2,744.04 ± 532.77pg/ml, p < 0.05). As a consequence, signiﬁcantly higher pneumococcal load (2 log increase, p < 0.05) was observed in SOD3-deﬁcient mice at day 7. Overall, this study demonstrated that excessive oxidative stress fails to initiate the IL-17A+ γδT cell response and the subsequent inﬂammatory neutrophil/macrophage recruitment required to eﬃciently eradicate Spn.

The role of NK cell-derived interferon-γ in anti-viral immune responses

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Upon viral infection interferons (IFN), including IFN-α/β and IFN-γ, constitute the ﬁrst line of defense. Although IFN-γ is mostly known to play an important role during bacterial infection, it also is a key player during anti-viral responses. However, it is still largely unknown to which extent different cell types contribute to the production of protective IFN-γ in virus infections. To address the cellular origin of protective IFN-γ in vaccinia virus (VACV) infection, we created IFN-γST/ST ST mice, in which the IFN-γ function can be reconstituted in a Cre-dependent manner. To generate mice in which only NK cells can produce IFN-γ we intercrossed IFN-γST/ST mice with Ncr1-Cre+/- mice. The speciﬁcity of NK cell-derived IFN-γ reconstitution was veriﬁed by ex vivo stimulation of Ncr1-Cre+/- IFN-γST/ST derived splenocytes and
subsequent intracellular IFN-γ staining by FACS as well as qPCR analysis of FACS-sorted cell types. Of note, during homeostasis no detrimental effect was detected in such mice. To analyze the importance of NK cell-derived IFN-γ in vivo, C57BL/6, IFN-γ<sup>ST/ST</sup> and Ncr1-Cre<sup>ff</sup>/IFN-γ<sup>ST/ST</sup> mice were intravenously VACV infected and survival was monitored. Interestingly, upon VACV infection NK cell-derived IFN-γ responses sufficed to promote survival and to control the virus load in infected organs. Furthermore, in VACV infected Ncr1-Cre<sup>ff</sup>/IFN-γ<sup>ST/ST</sup> mice two waves of IFN-γ responses were detected, which both were reduced when compared with WT mice. This NK cell-derived IFN-γ response controlled the overall cytokine milieu and modulated myeloid cell function upon VACV infection.

Taken together, our results indicate that NK cell-derived IFN-γ responses are sufficient to regulate innate and adaptive immune responses upon VACV infection and to mediate survival.

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**We-WS15-4**

**T cell factor-1 is a Critical Factor in Determining Natural Killer and Group 1 Innate Lymphoid Cell Fate Decisions**

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Protection against infections and malignancies depends on the coordinate development and cytokine production of immune cells. T cell factor 1 (TCF-1) is a transcriptional regulator of both adaptive T cells and innate lymphoid cells (ILC), but how this factor drives their development remains unclear. We found that TCF-1 acted in the thymus as a critical checkpoint to limit the expression of signature NK cell genes. De-repression of this thymic checkpoint resulted in the development of thymic-derived NK-like cells that could reconstitute the entire peripheral compartment and were capable of preventing tumour establishment. We also show that TCF-1 is required downstream of the common ILC progenitor to establish the ILC1 lineage by directing ILC1-specific progenitors cells, but it was not required for maintenance of mature ILC2 or ILC3 cells. Collectively, our data reveal the complexity of the role of TCF-1 as a molecular switch regulating the fate decisions in controlling adaptive T cells vs NK cell fate and ILC1 development.

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**We-WS15-5**

**Terminal differentiation of tissue-resident ILC2 occurs in peripheral tissue**

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It is a well-known fact that various cytokines and interferons secreted from effector cells play a crucial role in achieving various complicated functions in a living body. On the other hand, the effector cells secreting the humoral factors are often assumed to be only a small part of the population. Therefore, techniques to distinguish secretion activity at the single cell level, e.g. intracellular FACS and Elispot assay, are widely used. Recent single cell studies have revealed that secreted cytokines from cells are heterogeneous in terms of amount and variety even when the same stimulus for secretion response is given to the cells considered to be identical. These facts concerning secretion...
heterogeneity also highlighted the new issues of how to overcome heterogeneity to maintain the robustness of the organism and how to make clinically meaningful measurements from fluctuating features. Here we propose a new technology to continuously monitor cytokine secretion activity of individual cells over a wide period of time, “live cell imaging of secretion (LCI-S)”. This method is based on our previously reported technology (Sci. Rep., 4, 4736, 2014; Cell Rep., 8, 974-982, 2014), where we could continuously monitor sandwich fluorimunnoassay on total internal reflection fluorescence illumination microscopy without any washing steps. We improved LCI-S for long-term continuous measurement of secretion activities by realizing the essential perfomance for clinical evaluation, i.e. long-term stability of the measurement for several days or more, simultaneous multi-specimen or multi-condition measurement and multi-cytokines measurement. Furthermore, we successfully recovered each cell exhibiting characteristic secretion activity and analyzed gene expression by single cell RNA-seq. We applied our LCI-S to several type of cytokine secretion response including inflammasome associated IL-1β production of macrophage and type 2 cytokine production of type 2 innate lymphoid cells.

We-WS15-7

Regulation of lipid metabolite-mediated IL-4 production in group 2 innate lymphoid cells

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Interleukin (IL)-4 plays a crucial role in type 2 immune responses such as immunity against helminth infection and allergic responses through the induction of IgE class-switching in B cells and Th2 differentiation. TH2 cells and basophils/mast cells are known as IL-4-producing cells and express antigen recognition receptors TCR and IgE, respectively. IL-4 production is initiated by the engagement of these receptors with antigen, leading to antigen-specific immune responses.

Group 2 innate lymphoid cells (ILC2s), are a new type of innate lymphocyte that are known to regulate type 2 immune responses. ILC2s rapidly produce large amounts of IL-5 and IL-13 in response to IL-33 or IL-25, suggesting that ILC2s regulate the initiation of ‘antigen-non-specific’ type 2 immune responses. Although ILC2s highly express IL-4 mRNA as well as IL5 and IL13, IL-4 production by ILC2s is difficult to detect at the protein level in vitro and in vivo. Recently, cysteinyl leukotriene D4 (LTD4) was reported to induce ILC2 activation including IL-4 production, suggesting that IL-4 production in ILC2s is regulated by lipid metabolites. However, the molecular mechanisms that regulate IL-4 production in ILC2s and the physiological function of ILC2-derived IL-4 are unknown.

We found that blockage of calcium signaling completely inhibited LTD4-induced IL-4 production whereas Ionomycin induced IL-4 production, suggesting that the calcium signaling pathway is required for IL-4 production in ILC2s. Further, ILC2s from a protease allergen-induced asthma mouse model showed higher IL-4 production than ILC2s from naïve mice under LTD4 stimulation. ILC2s from IL33-deficient mice lacked LTD4-induced IL-4 production. These data indicate that ILC2s acquire the ability to produce IL-4 though IL-33-mediated activation in vivo. Taken together, LTD4-mediated calcium signaling and the IL-33 signaling pathway cooperatively regulate IL-4 production in ILC2s in an antigen-independent manner and ILC2s may regulate antigen nonspecific type 2 immune responses by alarmin and lipid mediators.

We-WS15-8

Neuronal regulation of group 2 innate lymphoid cell responses and type 2 inflammation

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The type 2 inflammatory response is induced by the exposure to helminth infection, allergens, venoms and other infectious and environmental triggers. The hallmark of type 2 responses include activation of T helper type 2 cells and release of type 2 cytokines such as interleukin (IL)-4, IL-5, IL-9 and IL-13, production of immunoglobulin E, and the activation and accumulation of multiple immune effector cells such as basophils, mast cells and eosinophils. This cascade of immunologic events is associated with goblet cell hyperplasia, elevated mucus production and induction of smooth muscle contractility. While recent studies identified group 2 innate lymphoid cells (ILC2s) as potent sources of type 2 cytokines, the molecular pathways controlling ILC2 responses are incompletely defined. Here, we demonstrate ILC2s selectively express the β2 adrenergic receptor (β2AR), the receptor for neurotransmitter norepinephrine, and reside in intestinal tissue in close proximity to adrenergic neurons. β2AR-deficiency resulted in exaggerated ILC2 responses and inflammation following helminth infection. Conversely, β2AR agonist-treatment was associated with impaired ILC2 responses and reduced inflammation in vivo. Mechanistically, we demonstrate that the β2AR pathway is an ILC2-intrinsic negative regulator of ILC2 responses through inhibition of cell proliferation and effector function. Collectively, these data provide the first evidence of a neuronal-derived negative regulatory circuit that limits ILC2-dependent type 2 inflammation at mucosal sites.

We-WS15-9

ILT cells integrate mesenchymal cell-derived RANKL signals essential for lymph node organogenesis

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Development of mammalian secondary lymphoid tissue is a programmed process governed by the sophisticated cytokine network, especially TNF family cytokines such as RANKL and Lymphotoxins (LTs). Lymphoid Tissue Inducer (LTi) cell is a member of group 3 innate lymphoid cells (ILC3). In the absence of LTi cells, development of all the secondary lymphoid tissue is impaired.

Previous studies showed essential role of RANKL on the development of lymph node (LN) but not on the development of Gut-Associated Lymphoid Tissues (GALTs). However, the difference in the usage of RANKL signal during organ development between LNs and GALTs remained to be elucidated.

In this study, we first identified RANKL-expressing mesenchymal cells in both LNs and GALTs using RANKL-reporter mouse system. We also proved that mesenchymal stromal cell plays a predominant role as a source of RANKL during LN formation. We further proved that RANK, a receptor for RANKL on LN-resident LTi cells transduced signals
IL-23 is a pro-inflammatory heterodimeric cytokine comprised of the IL-12p40 and IL-23p19 subunits. Similarly, the receptor for IL-23 is composed of two subunits, IL-12Rβ1 and IL-23R. Our group has previously demonstrated IL-23 promotes tumour initiation, growth and metastases using IL-23p19 gene-targeted mice or anti-IL-23 neutralizing antibodies. Although we expected that IL-23p19-deficient (IL-23p19/-/-) and IL-23R-deficient (IL-23R-/-) mice would phenocopy each other, surprisingly, we found that IL-23R-/- mice had lower levels of lung metastases compared to IL23p19/-/- mice using three different experimental lung metastasis models (RM-1, B16F10, LWT1). Importantly, antibodies blocking IL23R was also more efficacious than antibodies neutralizing IL-23p19 in suppressing experimental B16F10 lung metastases. Our preliminary data suggest this increased anti-tumor efficacy was due to an increase number of DNAM-1 expressing NK cells in the lungs of B16F10 tumor-bearing IL-23R/-/- mice compared to similar groups of IL-23p19/-/- mice. Using IL-23R GFP PKI reporter mice, experiments are currently underway to identify the IL23R expressing cell(s) in the lungs of B16F10 tumor-bearing mice that suppress NK cell function and its mechanism of action. These data suggest blocking IL23R maybe more effective than neutralizing IL23 in the suppression of metastases.

Evening Symposium Invited Speaker Abstracts

Mo-ES1-1

Innate sensor-mediated signaling for interferon induction during viral infection

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Cellular senescence is triggered by various distinct stresses and characterized by a permanent cell cycle arrest. Senescent cells secrete a variety of inflammatory factors, collectively referred to as the senescence-associated secretory phenotype (SASP). The mechanism(s) underlying the regulation of the SASP remains incompletely understood. Here we define a role for innate DNA sensing in the regulation of senescence and the SASP. We find that cyclic GMP-AMP synthase (cGAS) recognizes cytosolic chromatin fragments (CCFs) in senescent cells. The activation of cGAS, in turn triggers the production of SASP factors via Stimulator of interferon genes (STING), thereby promoting paracrine senescence. We demonstrate that diverse stimuli of cellular senescence engage the cGAS-STING pathway in vitro and we show cGAS-dependent regulation of senescence upon irradiation and oncogene activation in vivo. Our findings provide insights into the mechanisms underlying cellular senescence by establishing the cGAS-STING pathway as a crucial regulator of senescence and the SASP.

Mo-ES1-2

Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence

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Interferon (IFN) has long been used as an antiviral therapy to treat patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. In the case of chronic hepatitis C infection, peg-IFN plus ribavirin therapy is able to eradicate the virus in about half of genotype 1b HCV-infected patients. The eradication rate was much improved following the advent of protease inhibitors. Later development of direct acting antiviral agents (DAAs), such as NS5A and polymerase inhibitors, has led to very high sustained virological response (SVR) rates without requiring IFN.

In contrast, treatment of HBV infected patients has not achieved the same success. Forty-eight weeks of peg-IFN alpha is administered to both HBe antigen (HBeAg)-positive and HBeAg-negative patients. For patients treated with 180 micrograms of peg-IFN alpha for 48 weeks, HBeAg seroconversion and HBV DNA negativity were seen in 19.5% of 41 HBeAg-positive patients, whereas suppression of HBV DNA to less than 4.3 log/ml was obtained in 93.1% of 29 HBeAg-negative patients. However, such suppression continued for more than 24 weeks in only 37.9% of patients. HBs antigen seroconversion was observed in less than 10% of treated patients. The recently introduced peg-IFN beta has shown comparable-to-superior anti-viral activity against HBV.

The role of IFN in treatment of HCV-infected patients has nearly come to an end due to the efficacy of interferon-free alternatives, whereas IFN continues to play a role in treatment of HBV infection. However, as the effect of IFN against HBV is limited, combination therapies with cytokines or other compounds should be developed to improve future prospects for treatment of HBV.

Mo-ES1-3

Double-Stranded RNA in Lactic Acid Bacteria Prime Protective Immunity via Interferon-beta

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The bromodomain protein BRD4 binds to acetylated histones and activates transcriptional elongation of many genes. BRD4 occupies the TSS, gene body and numerous enhancers, including super-enhancers. We investigated the role of BRD4 in the development and activity of
Mo-ES1-4

Chromatin binding factor BRD4 directs development of hematopoietic stem cells and regulates inflammatory responses in macrophages through super-enhancers

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Recent great progress in studies of innate immunity has revealed that an array of pattern recognition receptors (PRRs) are involved in the detection of microbes to activate innate responses. In particular, during viral infection, virus-derived nucleic acids serve as major microbe-associated molecular patterns (MAMPs), which are sensed by certain PRRs, leading to the activation of their downstream signaling pathways. In most cases, such viral sensors induce interferon (IFN) gene activation and suppress blood cancer growth and reduce inflammation. These drugs are, thus believed to offer new therapeutic avenues to complex diseases. We show that Vav-Cre mediated Brd4 deletion in early embryos blocks the generation and differentiation of hematopoietic stem cells (HSCs). As a result, all immune cells, i.e., lymphocytes and myeloid cells as well as red blood cells were markedly reduced in Brd4 KO embryos leading to death in utero at around day 17-18. This and additional data with later Brd4 deletion indicate that BRD4 is required for proliferation of developing immune cells at various stages. Brd4 deletion in post-mitotic macrophages displayed a less dramatic phenotype: induction of inflammatory cytokines and chemokines by LPS was only partially affected in Brd4 KO macrophages. ChIP-Seq analyses showed that BRD4 clusters on super-enhancers located near the genes controlling the myeloid lineage and inflammatory responses. Together, BRD4 affects every step of immune cell development and exerts complex regulatory influences on inflammation.

Mo-ES1-5

Chronic hepatitis virus infection and interferon

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The small intestine harbors a substantial number of commensal bacteria and is sporadically invaded by pathogens. Double-stranded RNA (dsRNA) of one major commensal species, lactic acid bacteria (LAB), triggered IFN-β production from dendritic cells (DCs) and protected mice from experimental colitis. Further, we clarified that IFN-β production not only improves mucosal immune-homeostasis but also systemic Th1 immunity. We demonstrated that IFN-β secreted in response to LAB enhanced IFN regulatory factor 1 (IRF1) and IRF7 mRNA, which contribute to Il12p35 expression. Dendritic cells from type I IFN-receptor deficient mice fail to enhance IFN-γ producing T cells upon recognition of LAB. Thus oral administration of LAB enhances systemic Th1 immunity and suppresses Th2 immune responses. These results identify TLR3 as a sensor to small intestinal commensal bacteria and the resultant induction of IFN-β contribute to the two phases of protective immunity, i.e. maintenance of immunological homeostasis and enhancement of Th1 cellular immunity.

Mo-ES2-1

ILC2s: A window into the evolutionary role of allergic immunity

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Memory CD4+ T helper (Th) cells are central to long-term protection against pathogens, but they can also be pathogenic and drive chronic inflammatory disorders. To develop more effective vaccines and strategies to regulate chronic inflammatory diseases, it is important to understand the mechanisms underlying the generation and maintenance of immunological memory. In 2011, we identified a highly pathogenic IL-5-producing memory Th2 cell subset in allergic airway inflammation. Based on these data, we propose a new model called “Pathogenic Th population disease induction model” in the pathogenesis of Th1/Th2/Th17 diseases (Nakayama et al. Ann. Rev. Immunol. 2017). We have extended our research, and found that the pathogenic Th2 cells (Tpath2 cells) are a distinct cell population generated in vivo, and express high levels of IL-33 receptor component, ST2 (Endo et al. 2017). We have extended our research, and found that the pathogenic Th2 cells (Tpath2 cells) are a distinct cell population generated in vivo, and express high levels of IL-33 receptor component, ST2 (Endo et al. Immunity, 2015). These newly identified memory type Tpath2 cells are CD44+CD69+CD62LloCXCR3loCCR4+CCR8+IL-7Ra+ST2+CD4 T cells. A similar Tpath2 cells were identified in the patients of eosinophilic esophagitis and atop dermatitis (Prussin et al JACI 2016). More recently, we identified the functional ligand for CD69 that is expressed on the pathogenic T cells. In lymphoid tissues, CD69 regulates cellular retention via inhibition of S1P1 expression and requires no specific ligands to function. In contrast, we identified that myosin light chain (Myl) 9 and 12 are new functional ligands for CD69. Within inflamed mouse and human airways, platelet-derived Myl9/12 localize on the luminal surface of blood vessels and form intravascular net-like structures. Moreover, blockade of the CD69-Myl9/12 interaction ameliorates allergic airway inflammation (Hayashizaki et al. Science Immunol, 2016, Kimura et al. Immunological Reviews, 2017). Thus, the Myl9/12-CD69 interaction is a key event in the recruitment of activated lymphocytes in inflamed tissues and could be a therapeutic target for intractable airway inflammatory diseases.
Mo-ES2-2

**IL-5-producing ILC2s and eosinophils in the development of pulmonary arteriopathy**

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Memory CD4^+^ T helper (Th) cells are central to long-term protection against pathogens, but they can also be pathogenic and drive chronic inflammatory diseases. To develop more effective vaccines and strategies to regulate chronic inflammatory diseases, it is important to understand the mechanisms underlying the generation and maintenance of immunological memory. In 2011, we identified a highly pathogenic IL-5-producing memory Th2 cell subset in allergic airway inflammation. Based on these data, we propose a new model called “Pathogenic Th population disease induction model” in the pathogenesis of Th1/Th2/Th17 diseases (Nakayama et al. Ann. Rev. Immunol. 2017). We have extended our research, and found that the pathogenic Th2 cells (Tpath2 cells) are a distinct cell population generated in vivo, and express high levels of IL-33 receptor component, ST2 (Endo et al. Immunity, 2015). These newly identified memory type Tpath2 cells are CD44^+^ CD62LCD CXCR3^+^ CCR4^+^ CCR8^+^ IL-7R^a^ ST2^+^ CD4 T cells. A similar Tpath2 cell was identified in the patients of eosinophilic esophagitis and atopic dermatitis (Prussin et al JACI 2016). More recently, we identified the functional ligand for CD69 that is expressed on the pathogenic T cells. In lymphoid tissues, CD69 regulates cellular retention via inhibition of S1P1 expression and requires no specific ligands to function. In contrast, we identified that myosin light chain (Myyl) 9 and 12 are new functional ligands for CD69. Within inflamed mouse and human airways, platelet-derived Myl9/12 localize on the luminal surface of blood vessels and form intravascular net-like structures. Moreover, blockade of the CD69-Myl9/12 interaction ameliorates allergic airway inflammation (Hayashizaki et al. Science Immunol, 2016, Kimura et al. Immunological Reviews, 2017). Thus, the Myl9/12-CD69 interaction is a key event in the recruitment of activated lymphocytes in inflamed tissues and could be a therapeutic target for intractable airway inflammatory diseases.

Mo-ES2-3

**Memory-type pathogenic Th2 (Tpath2) cells in airway inflammation**

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Interleukin(IL)-5 regulates various functions and survival of eosinophils, and in mice the maintenance of B-1 cells. Local production of IL-5 in lung is associated with eosinophil accumulation and the exacerbation of inflammatory responses whereas eosinophils can also promote the regeneration of damaged tissues. In addition to T helper type 2 (Th2) cells, group 2 innate lymphoid cells (ILC2s) rapidly produce Th2 cytokines including IL-5 in response to IL-33, IL-25 and TSLP derived from epithelial and other cells. We have generated an IL-5 reporter mouse and revealed that IL-5-producing ILC2s resides in lung and that IL-33 induces IL-5 production by ILC2s, resulting in eosinophil accumulation in lung. IL-33 is one of critical mediators in allergic reactions. In addition, accumulating evidences suggest that IL-33 is also involved in pathogenesis of several connective tissue diseases, in which pulmonary arterial hypertension (PAH) is known as refractory and fatal complications. We investigated the consequences of chronic IL-33 stimulation and discovered a previously unrecognized mechanism that initiates pulmonary arterial remodeling. Repeated administration of IL-33 more than four weeks resulted in expansion of ILC2s and eosinophil accumulation around arteries and occlusive arterial hypertrophy in lung, but not in spleen, liver or kidney. The occlusive arteriopathy was ameliorated in IL-5 or eosinophil-deficient mice, but not in Rag2-deficient mice, indicating IL-5 from ILC2s and eosinophils play pivotal roles in the pathogenesis. Administration of iloprost, an analogue of prostacyclin and vasodilator used in therapy for PAH, repressed the expansion of IL-5-producing ILC2s and pulmonary arteriopathy. We have identified several genes strongly induced in lung by chronic IL-33 administration, and will discuss the roles in lung remodeling and functions.

Mo-ES2-4

**Eosinophilia, Interleukin-5, and Eosinophil-Related Diseases**

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Shortly after the discovery of the eosinophil, increased numbers were identified in blood and tissues of numerous diseases, especially asthma. An association with anaphylaxis suggested a reparative eosinophil function, a dominant hypothesis for many years. However, the potent cytotoxic and cytokinimulatory properties of granule proteins, particularly major basic protein (MBP1), argued that the eosinophil contributed to tissue damage; MBP1 tissue deposition was found in diseased tissues, often in the absence of intact eosinophils. Electron microscopy showed that eosinophils undergo cytolysis with release of intact granules into diseased tissues. The discovery of interleukin-5 (IL-5) as a key cytokine stimulating eosinophil production along with increased IL-5 levels in disease established its importance in driving eosinophil activity in disease. The hypothesis implicating the eosinophil as a mediator of disease activity has now been strengthened by demonstration that humanized monoclonal antibodies to IL-5 (mepolizumab and reslizumab) benefit patients with asthma by reducing exacerbations and by increasing pulmonary function. Notably, even in eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome), a life-threatening disease, treatment with mepolizumab produced definite benefit. Although these findings strengthen the eosinophil hypothesis, perhaps the most compelling data comes from studies with antibody to the IL-5 receptor alpha chain that ablates eosinophils through an antibody-dependent cytotoxic reaction. Treatment with such an antibody benefits asthma, and, because it destroys eosinophils, support for the eosinophil hypothesis is strengthened further. Clinicians concerned with treatment of eosinophil-related disease currently have effective therapies to block IL-5, mepolizumab and reslizumab, thus inhibiting its actions on eosinophils. Presently, we do not know whether one may have a decisive advantage over the other generally or disease-specifically, and a current task for clinical investigation is to determine their effectiveness and relative benefits in eosinophil-related diseases.

Despite evident roles for immunity orchestrated by Th1 cells and Th17 cells in host defense, the role for Th2 cells remains enigmatic, as helminths are highly adapted and poorly controlled by type 2 immunity in humans and animals. The discovery of innate lymphoid cells, ILC2s, that programatically express type 2 cytokines, enables a fresh look at the evolutionary role of this response without regard for antigen specificity. ILC2s develop during fetal life, position in peripheral organs, and express IL-5 following birth. ILC2s express tissue-specific transcriptomes, which identify key signals that control their activation in distinct tissues. For example, small intestine ILC2s constitutively express the receptor for IL-25, even in germfree mice, thus identifying a
key role for this cytokine in regulating ILC2 activity in this tissue. As such, ILC2s reveal a tissue-specific geography that begins to uncover their key role in organ-specific homeostasis through response to local signals that enable dynamic responses to sustain tissue and systemic health.

Mo-ES3-1

Negative Regulation of Cytokine and Interferon Expression

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The cytokine response requires tight regulation of gene and protein expression to ensure no excessive production or prolonged activity of cytokines occurring in acute and chronic inflammation. Negative control mechanisms exist and include both transcriptional and post-transcriptional processes. Among post-transcriptional mechanisms control of cytokine mRNA stability by AU-rich elements which cis-acting elements in the 3’UTR. Ribonuclease L (RNase L) is a part of the IFN system and possesses strong activity against viral mRNAs but has also been proposed as a negative feedback player in IFN response targeting specific cellular mRNAs. Also, Type-I IFN is known to have anti-inflammatory action and inhibits the NF-κB pathway, a key element in negative control of gene expression of pro-inflammatory cytokines. In this work, we identified a novel repressor-like protein, the pyrimidine 5’-nucleotidase (NT5C3A) which is an enzyme of nucleotide catabolism. NT5C3A acted as a negative player of interferon and cytokine expression. Specifically, NT5C3A as an intracellular IFN-stimulated gene (ISG) limits inflammatory cytokine production by a cascade of events that involve IRF-1, SIRT1, SIRT6, histone deacetylation, and targeting NFκB interaction with the promoter. Our studies here suggest a novel role for NT5C3A as an anti-inflammatory mediator by inhibiting cytokine expression in a process that involves NFκB inhibition and NAD+-dependent chromatim modifications during IFN and the cytokine response.

Mo-ES3-2

The priming effect of β-catenin to NF-κB p65 for interleukin 6 production via TCF4-mediated signaling in macrophage

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Interleukin 6 (IL-6) plays a central role in host defense and acute inflammatory responses. In this study, we found that there is a complex cross-regulation between NF-κB p65 and β-catenin pathways for IL-6 production. The β-catenin/NF-κB p65 complex that is translocated to the nucleus was combined with primary TCF-4, and then the NF-κB p65 was sequentially bound to the domain of NF-κB p65 on IL-6 promoter. Importantly, the β-catenin is an essential factor in the production of IL-6 via NF-κB pathways. In contrast, impaired β-catenin signaling decreases IL-6 cytokine production for early immune response. Furthermore, the Ctnnb1loxp/loxp/LysMcre+/- conditional KO mice did not increase the level of IL-6 cytokine in plasma and CD169(+) macrophage by LPS. Additionally, TCF-4 acted as a co-activator of NF-κB p65 in potentiating the production of cytokine IL-6 in macrophage. Thus β-catenin could play an important role in IL-6 cytokine production via the priming effect for NF-κB p65.

Mo-ES3-3

Syk-CLRs and TLR2 are critical for dengue virus-induced NET formation and thrombocytopenia

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Thrombocytopenia is a critical factor to predict the onset of dengue hemorrhagic fever. CLEC5A (also known as MDL-1) and CLEC2 are Syk-coupled myeloid C-type lectins receptors (Syk-CLRs) expressed in macrophages/neutrophils and platelets, respectively. We have shown that dengue virus (DV) activates NALP3 inflammasome and induces proinflammatory cytokines from macrophages via CLEC5A, and blockade of CLEC5A is able to attenuate DV-induced hemorrhaging shock and lethality. In this study, we further found that DV activate platelets via CLEC2, and DV-activated platelet-derived microparticles (DV-PMPs) induced ‘neutrophil extracellular trap’ (NET) formation and proinflammatory cytokine release via co-stimulation of CLEC5A and TLR2. Furthermore, co-incubation of DV with neutrophils and platelets simultaneously not only resulted reduced platelet counts, but also enhanced vascular permeability change in vitro. Furthermore, blockade of both CLEC5A and TLR2 not only attenuated thrombocytopenia, but also protect mice from DV-induced lethality in vivo. Thus, CLEC2-activated PMP plays a critical role in DV-induced NET formation and proinflammatory cytokine production, and blockade of CLEC5A and TLR2 simultaneously is a promising approach to prevent DV-induced thrombocytopenia and lethality in the future.

Mo-ES3-4

The role of Mucin-2 and its monosaccharides in regulation of mucosal immunity

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Introduction: Beneficial commensal microorganisms and potential pathogens continuously challenge mucosal immune system. A number of physiological adaptations protect IECs from bacterial invasion such as glycoprotein layer of heavily glycosylated mucin-2 secreted by goblet cells. Deficit of the mucin layer could lead to the pathogenesis of numerous inflammatory conditions, including food allergies, IBD and intestinal cancer. Here we studied the effect of mucin layer depletion on microbiome, inflammation and macrophage programming using Mucin-2 knockout mice. We have also investigated the effect of its monosaccharides on the phenotypes observed.

Methods: The study was conducted at the Center for Genetic Resources of Laboratory Animals at the ICG SB RAS (RFMEFI61914X0005 and RFMEFI621114X0010) using specific pathogen free C57BL/6 Muc2−/− controls and C57BL/6 mice. Microbial communities of mouse fecal samples were analyzed by Shotgun sequencing and Single-Strand Conformation Polymorphism of 16S rRNA gene. We evaluated the effect of Mucin-2 depletion alone or in combination with the addition of Mucin-2 derived monosaccharides on the mucosal immune system. This included cytokine expression (INF-gamma, TNF-alpha, IL1-alpha and beta, IL-10, IL-12, IL-17, etc.) and histological analysis of the colon.
Results: The changes observed in intestinal flora of Muc2−/− mice were associated with up-regulation of the inflammatory cytokines, generation of IgG against own intestinal bacteria and development of Th2 and Th17 response as a result of continuous contact between microflora and IECs. Fusoc and sialic acid restored gut flora such as Bacteroides spcies, especially after antibiotics therapy. However, only fusoc, but not sialic acid, suppressed Th2 and Th17 immune response with the decrease of M2 type macrophages and the increase of Th1 response in combination with proinflammatory factors.

Conclusion: Mucin-2 derived monosaccharides per ce or through regulation of the intestinal flora could modulate mucosal immunity in the experimental model of IBD.

Work was supported by RFBR grant 15-04-07653.

Mo-ES3-5

Growth hormone-IGF1 axis and Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease become the first metabolism problem in the whole world in recent decades. Low growth hormone (GH) production and/or hepatic GH resistance has also reported to patients with nonalcoholic fatty liver disease (NAFLD). GH and IGF1 replacement can resolve the fatty liver condition in obese rodents and in GH-deficient patients. Those suggest that GH plays a key role in regulating hepatic lipid processing.

However, questions regarding how GH mediates the regulation of hepatic lipid metabolism still remain. Therefore, our laboratory has generated a mouse model with the adipose tissue(AT) -specific GHR knockout (ADGHRKO) by cross Adiponectin cre mice with GHR floxed mice. Combined with high-fat feeding at the same time, the effects of AT-specific GHR gene disruption on the body lipid metabolism was explored comprehensively.

In the current study, we found AT-specific disruption of GHR significantly increased female body weight after 3 mo. but no difference in male. Subcutaneous WAT of male ADGHRKO mice were significantly larger than that of the control littermate whereas several internal organs were significantly smaller such as heart and liver, which may result in the unchanged body weight of male mice. In addition, subcutaneous, gonadal and perinephric WAT of female ADGHRKO mice were increased significantly compared to the control mice.

After a High Fat Diet (60% kcal from fat) treatment for 16 weeks, the ADGHRKO mice were predisposed to diet-induced obesity, but their glucose tolerance and insulin sensitivity were slightly better than the control (LL) littermate mice. ADGHRKO mice also showed the lighter level of severity of the fatty liver which was demonstrated that GHR in adipose tissue may play an important role in regulating adipocyte function and metabolism, the relevant mechanism is yet to be further analysis.

Mo-ES3-6

Antigen specific immunotherapy for autoimmune disease targeting dendritic cells

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New therapies targeting innate immune cytokines have and continue to revolutionise our capacity to treat autoimmune diseases, like rheumatoid arthritis (RA) and inflammatory bowel disease (IBD). Immunotherapies are now revolutionising treatment of certain cancers. However, these treatments are expensive, are generally not curative and may have side effects. Furthermore, no immunotherapies have survived phase 3 trials to reach the market for Type 1 Diabetes (T1D).

In a phase I clinical trial, intradermally-injected autologous DCs exposed to citrullinated peptides and the NF-kb inhibitor, BAY11-7082, reduced circulating effector T cells and increased the ratio of regulatory to effector T cells in RA. However, simpler strategies are desirable for widespread clinical use. We developed antigen-specific liposomal nanoparticle immunotherapy which passively targets and modifies dendritic cells in situ. The liposomes co-encapsulate a disease-specific peptide and an NF-kb inhibitor. Models of inflammatory arthritis and autoimmunity diabetes demonstrate antigen-specific suppression of disease development and severity, providing proof-of-concept for translation towards RA and T1D clinical trials. In this talk I will discuss cellular and cytokine-mediated mechanisms involved in the induction of antigen-specific tolerance using liposome immunotherapy.

Tu-ES4-1

Pathological contribution of an inflammatory chemokine CCL3 in chronic myeloid leukemia as a stem cell inhibitor

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Accumulating evidence suggests that an inflammatory chemokine, CCL3, has multiple functions in hematopoietic system besides its pro-inflammatory activities. CCL3 can in vitro inhibit the proliferation of hematopoietic stem/progenitor cells (HSPCs). Based on this unique function, CCL3 is alternatively called as “stem cell inhibitor”. Thus, CCL3 can potentially influence the homeostasis of HSPCs. In sharp contrast, CCL3 hardly affects leukemia initiating cells (LiCs) in chronic myeloid leukemia (CML) although they share several characteristic capabilities including self-renewal and cellular quiescence, with normal HSPCs. Thus, CCL3 can be a potent mediator to induce the dominant proliferation of LiCs in the CML BM. However, there is a dearth of studies precisely demonstrating the major cellular source of CCL3 in BM and the contribution of endogenously-produced CCL3 to the CML pathophysiology.

Basophilia is a frequently observed hematological abnormality in CML, but its pathophysiological roles are undefined. Herein, our meticulous examination of CCL3-expressing cells in CML BM revealed that basophil-like leukemia cells are a major cellular source of CCL3. Moreover, CCL3-expressing basophil-like leukemia cells accumulated in CML BM and basophil-derived CCL3 preferentially acted on the normal HSPCs, resulting in their suppressed proliferation. As a consequence, LiCs expanded dominantly in BM during the initiation process of CML. Indeed, the ablation of CCL3 or the depletion of basophils markedly retarded the CML development in mouse CML model. These observations would imply that intra-BM basophil expansion can favor leukemia-tropic hematopoiesis in CML by providing CCL3, a potent inhibitor of normal hematopoiesis and that basophil-derived CCL3 can be a novel target molecule for the treatment of CML.
Tu-ES4-2

Chemokine and oxysterol regulation of immune cell migration and metabolism

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Helper T cells move to the interface of the T cell zone and B cell follicles shortly after activation, a relocation event that is important for their ability to support T cell-dependent antibody responses. While CXCR5 is well established to play a critical role in promoting T cell entry into follicles, the guidance mechanisms responsible for T cell positioning at the B-T zone interface have been less understood. EBI2 (GPR183) is a chemotactant receptor that responds to the oxysterol 7a,25-dihydroxycholesterol (7a,25-HC) and guides the movements of activated B cells. We recently found that EBI2 is unregulated on activated CD4 T cells and promotes their movement to the B-T zone interface in response to 7a,25-HC. As well as promoting interactions between cognate T cells and B cells, we find that this positioning fosters interactions with activated CD4+DCIR2+ dendritic cells (cDC2s). Positioning of sentinel cDC2s in the spleen is found to require the action of two EBI2 ligands, 7a,25-HC and 7a,27-HC. Following activation by certain stimuli, cDC2s rapidly migrate to the B-T zone interface. This occurs due to upregulation of both CCR7 and EBI2 and the integrated action of these two chemotactant receptors. Our work on the requirements for EBI2 ligand production led us to identify a role for the precursor oxysterol, 25-HC, in regulation of macrophage sterol metabolism, inflammasome activation and IL-1b production. In ongoing work we are studying how 25-HC influences macrophage metabolism in a way that limits inflammasome activation. We are also testing whether 25-HC acts as an intercellular communication molecule to regulate the metabolic state of responder cells. Our ongoing work in these areas will be presented.

Tu-ES4-4

Chemokine-dependent and -independent mechanisms of T cell immune surveillance

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Rapid migration of naïve T cells through lymph node (LN) parenchyma and non-lymphoid tissue (NLT) is critical for detection of antigens and effective immune responses. Here we use in vitro and in vivo imaging to dissect the mechanics of naïve T cell migration in LN and determine how chemokine receptor CCR7, integrin LFA-1 and cortical actin contribute to intranodal locomotion. We find that T cells undergo periodic shape oscillations, where elongated shapes coincide with forward translocation and increased actin flow, which is quantitatively tuned by the strength of chemokine signaling. LFA-1 does not control cytoskeletal dynamics but creates tangential friction between actin cortex and environment, without adhesively confining the cells to lymph node stroma. In contrast to naïve T cells, we found that memory T cells migrating through NLT, such as salivary glands, do not require chemokines for efficient F-actin treadmilling or integrin coupling. Rather, integrin-independent intrinsic cell shape changes are sufficient and necessary for T cell surveillance of epithelial and non-epithelial compartments. The unexpectedly distinct dependencies of naïve and memory T cells on chemokines and integrins for efficient immune surveillance probably reflect the homogeneous structure of lymphoid organs versus the highly divergent NLT structure in terms of physical and biochemical properties.

Tu-ES4-5

Specific features of Tregs migrated from skin and colon to the draining lymph node in the steady state and under inflammation

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We have demonstrated importance of elucidating futures of cells correlated with spatiotemporal information about cellular movement by using the mice expressing the photoconvertible protein Kaede and KikGR. We reported that Foxp3+ regulatory T cells (Tregs) migrating from the skin to the draining lymph node have a strong immunosuppressive effect on contact hypersensitivity (CHS) response. To elucidate the role and characteristics of Tregs subsets in CHS response in more details, we utilized a combination of single-cell real-time PCR high-dimensional gene expression profiling data with KikGR mice. We found that although immunosuppressive genes Cdla4 and Tgfbl1 were expressed in the majority of Tregs, Il10-expressing Tregs were rare and the majority of them co-expressed Gzmb and displayed Th1-skewing. Furthermore, Gzmb-/Il10-expressing Treg subset was gated with CD43+CCR5+. In addition, CD43+CCR5+CXCR3+ Tregs highly expressed skin-tropic chemokine receptors CCR4 and CCR8, preferred to retain within inflamed skin, and had superior in vivo inhibitory function. These results suggested that even if only present in small numbers, highly activated Tregs that co-express Gzmb and Il110 and that have the capacity to remain in inflamed tissue are likely to be clinically relevant due to the role of these molecules in the control of excessive immune responses. Taken together, the identification of a rare Treg subset co-expressing multiple immunosuppressive molecules and having tissue-remaining capacity offers a novel strategy for the control
of skin inflammatory responses.

In addition, requirement of elucidation of cellular movement between gut and another parts of body is increasing for revealing relation of gut immune system to systemic immune responses. Thus, I also introduce here that Tregs in caecum and ascending colon migrated to the distal part of mesenteric lymph node in an S1PR1-dependent manner in the steady state and dynamic changes of spatiotemporal regulation of colonic Tregs under inflammation.

Tu-ES5-1

Pro-inflammatory cytokine therapy in rheumatoid arthritis and other inflammatory/autoimmune diseases

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Rheumatoid arthritis (RA) is a chronic and systemic autoimmune disease with synovitis and progressive joint destruction as key features. Pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-6 (IL-6) and others, are involved in the development of the disease.

The effects of these pro-inflammatory cytokine have been reported in some clinical trials and pre-clinical autoimmune/inflammation models. The most recent information on these topics is summarized.

Tu-ES5-2

In vitro pharmacological action of biologic agents visualized by intravital bone imaging

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During the last decade, intravital optical microscopy has launched a new trend in the field of biology. By using this advanced imaging technique we have established a new system for visualizing in situ behavior of a diversity of living cells within intact tissues and organs. Among them, we succeeded in visualizing the various dynamic phenomena within bones and joints, where various kinds of immune cells are produced and functioning although poorly analyzed by conventional methodology such as histological analyses with decalcified sections. We have so far identified the real modes of migration, differentiation and function of bone-destroying osteoclasts, special kind of macrophages responsible for bone and joint erosions. This novel technique does not only help us to understand the dynamic nature of living cells and tissues in situ, but identifies in vivo pharmacological actions of several new drugs. Here we show our recent studies for analyzing bone-protecting actions of different biological agents treating RA by directly visualizing drug-induced cellular behaviors in vivo. Such trials would be useful for classifying various biological agents based on their respective pharmacological effects in vivo.

Tu-ES5-3

The significance of RA treatment by IL-6 signaling inhibition learned from the translational research

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Appropriate disease modifying anti-rheumatic drugs (DMARDs) can inhibit inflammation and improve clinical outcome in RA patients, and we aim at remission with DMARDs for them. Better development for diagnosis tools will be expected, and preventive care, preemptive therapy and personalized healthcare will be the next candidates of treatment strategies for the future. However, at this stage, we need to consider the maximization of RA treatment using approved DMARDs.

Methotrexate (MTX), which is one of synthetic DMARDs, is the most frequently used in Japan. Meanwhile, there is possibility of the risk to affect an influence to various organs via MTX using, although MTX remains the ‘anchor drug’ in RA treatment. It is important for us to understand whether the efficacy of biologic DMARDs treatment with MTX is more meaningful or not. We described the efficacy of MTX in combination with TNF inhibitor was superior to that of TNF inhibitor monotherapy in the JESMR study. Additionally, the SURPRISE Study with Tocilizumab (TCZ) which inhibits IL-6 signaling, is a multicenter, prospective, randomized, open-label study compared the MTX plus TCZ with TCZ monotherapy for RA patients who have inadequate response to MTX. The remission rate of combination therapy using DAS28-ESR was higher than that of TCZ monotherapy at 24 weeks. On the other hand, there was no significant difference among two groups at 52 weeks. It suggests TCZ might be able to taper the dose of MTX if remission is achieved with combination therapy after 24 weeks.

So far, we have demonstrated not only clinical data of TCZ, but also translational data using blood samples. Our research suggests the changes of serum cytokine and several subsets of peripheral cells are not the same in RA patients treated with different biological DMARDs.

We will highlight our studies by focusing on the significance of IL-6 inhibition.
Selective suppression of IRF5 activity by Lyn in the TLR-MyD88 pathway restrains the development of SLE-like disease

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The transcription factor interferon regulatory factor-5 (IRF5) plays an important role in Toll-like receptor (TLR)-mediated innate immune responses, whereas it contributes to the pathogenesis of systemic lupus erythematosus (SLE). However, little is known about the mechanism regulating the extent of IRF5 activation, especially the negative regulatory mechanism. Lyn, a Src family kinase, is also implicated in human SLE, and Lyn−/− mice develop an SLE-like disease. Here we show that Lyn selectively inhibits the activity of IRF5 in the TLR-MyD88 pathway, thereby restraining the development of autoimmunity. Interestingly, Lyn inhibited IRF5 in a kinase activity-independent manner; it bound to IRF5 and inhibited ubiquitination and phosphorylation of IRF5. Consistently, these post-translational modifications of IRF5 were significantly enhanced in TLR7/9-stimulated Lyn−/− bone marrow-derived dendritic cells (BMDCs), resulting in the boost of IRF5-dependent type-I IFN induction. Moreover, DCs freshly isolated from Lyn−/− mice exhibited phosphorylation and enhanced nuclear translocation of IRF5. These results suggest that Lyn is constitutively activated in vivo in DCs, if Lyn is lost. Importantly, even monoallelic ablation of the Ifi5 gene was sufficient to alleviate the hyper-production of type-I IFNs in TLR7/9-stimulated Lyn−/− BMDCs, and to ameliorate the development of SLE-like symptoms in Lyn−/− mice. Taken together, our results identify Lyn as a critical suppressor of IRF5 in the TLR-MyD88 pathway, and implicate that the selective control of IRF5 activity may contribute to better therapeutics for SLE. We will also show our recent results of high-throughput-screening of small molecular compounds that inhibit IRF5 transcriptional activity.

Memory-type ST2+CD4+ T cells participate in the steroid-resistant pathology of eosinophilic pneumonia

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The lung has an unique epithelial barrier system to protect host from continuous invasion of various harmful particles including viruses and bacteria. Interleukin (IL)-33, an epithelial cytokine, is released from epithelial cells in the lung and drives the type 2 immune response by activating ST2-expressed immune cells in a number of allergic diseases. However, the pathogenic roles of memory-type ST2+CD4+ T cells in such lung inflammation has been unclear. Here we showed that intratracheal administration of IL-33 induced the substantial increase of numbers of tissue-resident memory-type ST2+CD4+ T cells in the lung. Eosinophilic lung inflammation developed sequentially accompanied by enhanced production of IL-5 and IL-13. T cell-deficient Foxn1nu mice and NSG mice exhibited ameliorated eosinophilic inflammation induced by IL-33. Dexamethasone treatment showed small effects on both the cell number and function of memory-type ST2+CD4+ T cells. Taken together, our study provides novel insight into the pathogenesis of eosinophilic lung disease, showing that memory-type ST2+CD4+ T cells are involved in IL-33-induced eosinophilic inflammation and elicited steroid-resistance.

A long noncoding RNA regulates the switch between macrophage differentiation and inflammation

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Macrophages are critical effector cells of the innate immune system essential for controlling infection and maintaining tissue homeostasis. At the cellular level, pathogen-response involves recognition by Toll-like receptors (TLRs) and complex intracellular signaling cascades that result in induction of an inflammatory program. Perturbations to these signaling pathways can have devastating consequences, leading to diseases, such as Rheumatoid Arthritis and Cancer. Macrophages arise from monocytes in a differentiation process that is tightly regulated, involving many microRNAs, proteins and stage-specific expression of transcription factors. Long non-coding RNAs (lncRNAs) represent the largest group of RNA produced from the genome and are described as transcripts greater than 200 nucleotides in length that lack protein-coding ability. LncRNAs are rapidly emerging as critical regulators of a broad range of biological processes including genomic imprinting, development, and cancer. We sought to identify novel lncRNAs involved in monocyte to macrophage differentiation. We generated comprehensive RNA-sequencing data sets from primary healthy human monocytes, differentiated macrophages and identified hundreds of lncRNAs differentially expressed during differentiation. We characterized one lncRNA, called GAPLINC, which is dramatically induced over one-thousand-fold transitioning from monocyte to macrophages. GAPLINC is localized to the cytoplasm, but does not associate with polysomes. Interestingly, this lncRNA is rapidly downregulated from monocyte to macrophages. GAPLINC is localized to the cytoplasm, but does not associate with polysomes. Interestingly, this lncRNA is rapidly downregulated upon TLR stimulation suggesting a connection to inflammatory pathways. Knocking down GAPLINC in primary human macrophages results in up-regulation of infection-related genes, suggesting this lncRNA may negatively regulate inflammatory pathways. Overexpression of GAPLINC suppresses the inflammatory response and promotes proliferation. Here we reveal an intriguing role for a lncRNA in regulating the switch between macrophage differentiation/proliferation and the downstream inflammation pathways.
Pathogens that gain entry into the CNS elicit a uniquely tailored response from the immune compartment by modulating cytolytic and inflammatory functions that confer viral clearance with minimal brain damage. Neurotropic viruses such as vesicular stomatitis virus (VSV) infect neurons in the olfactory epithelial barrier and pass via sensory axons directly into brain. Within the olfactory bulb, VSV-infected neurons contact a variety of CNS neurons; however, the infection rarely invades the brain. While type I interferon responses are crucial for initial viral control, little is known about how the local adaptive immune response prevents fatal viral neuroinvasion. In this study, we sought insights into how cytotoxic lymphocytes prevent distal spread of infection from the olfactory bulb. Without T cells, virus containment is compromised and VSV traverses caudally to hindbrain regions. Utilizing intravital two-photon microscopy and other techniques, we characterized the mechanism by which T cell interactions within the olfactory bulb facilitate barrier protection. Interestingly, chimeric mice deficient in MHC class I on brain-resident cells, as well as microglia-depleted mice, showed reduced antiviral T cell calcium signaling upon target cell engagement, suggesting that microglia can cross-present viral antigen and indirectly facilitate viral clearance from adjacent virally infected neurons. To understand what protective signals T cells might provide the brain, we generated VSV expressing Cre recombinase. VSV-Cre infection of floxed IFNAR or floxed IFNγR mice revealed that while continued IFNAR signaling is critical, IFNγR expression was dispensable for VSV clearance. In addition, IFNγ and perforin deficient mice are similarly resistant, while TNFα deficient animals have increased VSV susceptibility. This study has revealed a novel mechanism of T cell-mediated viral control in neurons that involves engagement of uninfected resident myeloid cells that have acquired antigen and provides barrier protection against a virus attempting to enter the CNS via the nasal route.

Tu-ES6-9

Type-I interferon mediated degradation of microRNAs is sequence and length dependent

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Innate immune activation by pathogens promotes global transcriptional changes in infected cells, promptly affecting the levels of messenger RNAs (mRNAs) and non-coding RNAs such as microRNAs (miRNAs). As such, induced miRNAs cause rapid down regulation of mRNA targets, thereby controlling the duration and intensity of the immune response. Interestingly, there is evidence that upon type-I interferon (IFN) stimulation select miRNAs can be actively degraded, including the pro-inflammatory miR-221 and miR-222. However, the impact of these decreased miRNAs on IFN responses is currently not known.

In this work, we initially observed by RT-qPCR that the levels of miR-221, and not that of the co-expressed miR-222, were decrease by >80% following Toll-like receptor (TLR) 3/4 stimulation, but not that of other TLRs. This specific decrease of miR-221 was type-I IFN dependent, and ablated in IFN-α/β receptor 1 deficient bone marrow-derived macrophages (BMDMs). Unexpectedly, miR-221 decrease was restricted to its longer isoforms, a phenomenon also observed for miR-222. RNA sequencing (RNAseq) carried out on human fibroblasts treated with type-I IFN confirmed these observations, identifying a group of other miRNAs for which the longer isoforms were similarly impacted. Significantly, we identified a core motif in these miRNAs directly regulating their stability, upon IFN-β stimulation, which can be attributed to the exoribonuclease Pnpt1. In addition, the RNAseq data revealed an overall decrease >70% of miR-222 molecules with IFN-β.

Collectively, these findings suggest that sequence and length-dependent miRNA degradation helps control the overall abundance of miR-221/222, and their pro-inflammatory function during type-I IFN responses.

ICIS Awards Session Abstracts

We-Awards-2

1st Place Milstein Young Investigator Award Lecture: Defining group 2 innate lymphoid cell tissue niches

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Group 2 innate lymphoid cells (ILC2) are tissue resident cells that are key instigators of type 2 allergic immune responses. ILC2 can promote beneficial type 2 immunity in the context of helminth infection, but are also key contributors to the allergic pathology associated with asthma and atopic dermatitis. ILC2 are developmentally deposited in tissues where they locally proliferate, and recent work suggests their contribution to physiologic tissue responses during tissue development and tissue remodeling. Our group’s prior work indicates adipose tissue ILC2s are key coordinators of a mixed regulatory/type 2 immune response which promotes adipose tissue function and is protective in models of obesity and type 2 diabetes. However, the cells and signals that regulate ILC2 in tissues are only beginning to be explored. In adipose tissue and elsewhere, the IL-1 family cytokine IL-33 is a dominant positive regulator of ILC2 function. As such, we have pursued the regulation and cellular sources of IL-33 that are proximal to tissue-resident ILC2. Using high-resolution imaging and genetic approaches, our studies suggest ILC2 are maintained in specific micro-anatomic tissue niches that are defined by subsets of non-hematopoietic IL-33 expressing cells. By defining these ILC2 niches, we hope to better understand the cells and signals that regulate ILC2 so that we may ultimately exert control over both beneficial and pathologic type 2 immune responses.

We-Awards-4

ICIS President’s Lecture: From Type I IFN to HMGB1 and other DAMP molecules: Regulators of immunity, inflammation and cancer

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At the time we initiated our studies on the molecular characterization of cytokines, namely interferons and interleukins, the conventional wisdom was that cytokines were soluble factors secreted upon activation of cells by various stimuli, such as upon virus infection. It was in this context that we discovered the type I IFN gene family and, subsequently, a family of transcription factors, termed interferon regulatory factors (IRFs), as crucial regulators of type I IFN and other cytokine systems. The IRF family is now recognized for its broad role in immunity and oncogenesis.

Subsequently we encountered another class of cytokine-like...
molecules called damage-associated molecular patterns (DAMPs) that function intracellularly and extracellularly as soluble factors to exert seemingly unrelated biological activities. High-mobility group box protein1 (HMGB1) is a typical DAMP molecule that has been extensively studied for its protective and pathogenic functions.

In this lecture, I will present data on how HMGB1, which we originally identified as a general sensor of immunogenic nucleic acids, is released from the nucleus and how it functions to regulate inflammation and cancer metastasis. Our recent data indicate that HMGB1 exerts its function via a mechanism that is distinct from what has been widely appreciated heretofore. If time permits, I will also present our results on other DAMPs that may also participate in the regulation of inflammation and cancer.

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