treatment revealed that the treatment of IFN-γ up to 24 hours suppressed the IL-4 + TNF-α-induced CCL11 expression, whereas the CCL11 expression was enhanced 3 days after the treatment.

Conclusions: These results uncovered previously unsuspected contribution of IFN-γ to the fibroblasts in allergic inflammatory milieu in terms of the change in production of certain chemokines. In other words, the antagonistic function of IFN-γ to Th2 cells at the early phase may represent only a small part. The intracellular signaling and IFN-γ-dependent secondary events are needed to be explored to explain the long-term effect or the late phase phenomenon after IFN-γ administration.

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Hyperosmolar Conjunctival Provocation Test (HCPT) in the Evaluation of Ocular Symptoms
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Background: Non-allergic environmental factors may cause ocular symptoms in atopic and non atopic subjects, which are known as nonspecific conjunctival hyperreactivity (CHR). This study aims to investigate the presence of CHR to the HCPT in subjects with ocular symptoms.

Methods: 63 adults with ocular symptoms (itching, red eyes or tearing) were selected and tested for allergy to house dust mites and grass pollen by skin prick tests (ALK Abelló) and serum specific IgE (ImmunoCAP-Phadia). They were considered atopic if these tests were positive to at least one allergen and non atopic if tests were negative. HCPT with 10-fold serial diluted glucose solutions was performed in all subjects until it produced conjunctival redness. Digital images were analyzed by 2 investigators (MD and technician) registering redness of the challenged eyes in red and the total area of contra-lateral eyes in blue using the fine brush tool (software GIMP 2.6.5). The number of red dots of the affected eye (%) was compared to the number of blue dots of the control eye.

Results: TPCH was positive in 33/38 atopic subjects (87%) and in 4/25 non atopic (16%). Most reactions occurred at the 40% glucose solution. Sensitivity was 87% and specificity 84% (P < 0.0001). There was a significant correlation (96.5%, Pearson, P < 0.0001) between the number of red dots reported by investigators in 23 digitalized images.

Conclusions: TPCH identifies CHR in both atopic and non atopic subjects. Atopic subjects exhibit CHR more frequently than non-atopic subjects. Digital images may be useful for grading ocular hyperemia in TPCH.

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Tear IFN-G is Increased After Sublingual Immunotherapy in Allergic Conjunctivitis Patients and Correlates With Clinical Improvement
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Background: Despite success of sublingual immunotherapy (SLIT) in the treatment of allergy diseases, more research is needed related to ocular allergy. Thus, the aim of this work was to analyze the ocular microenvironment provided by tear cytokines in allergic conjunctivitis (AC) patients treated with SLIT and to correlate tear and serum cytokines with ocular findings.

Methods: 19 AC-patients were included in this study. AC diagnosis was based on a clinical history and full ophthalmologic examination according to the diagnosis standards of the American Academy of Ophthalmology. Routine immunological studies were performed to corroborate allergic status. Negative coproprostasitic results were documented. This study was approved by Scientific and Ethics Committees if Institute of Ophthalmology “Conde de la Valenciana,” Mexico City and all subjects gave their informed consent to obtain samples. Tear and serum samples were collected to determine cytokines IL-2, IL-4, IL-5, IFN-γ, TNF-α, IL-10 by cytometric bead arrays (CBA), following manufacturer’s instructions.

Results: After 6 months of treatment with SLIT we observed significant higher IFN-γ concentration, without significant changes in IL-2, IL-4, IL-5, TNF-α or IL-10. We observed significant clinical improvement since 3 months of treatment and it was maintained until the end of 6 months. Clinical improvement correlated with IFN-γ concentration.

Conclusions: Clinical outcome in AC-patients treated with SLIT could be tear IFN-γ dependent.

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CYTOKINES AND CHEMOKINES

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Hypoxia-Inducible Factor 1 (HIF-1) Transcription is a “Signalling Driver” for Allergic Inflammation, Host Innate Immune Defence and Leukaemia Progression
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Background: Hypoxia-inducible factor 1 is a transcription complex that plays a pivotal role in cellular adaptation to low oxygen availability, which occurs during allergic responses, host immune defence and leukaemia progression. We investigated the role of HIF-1 in cellular adaptation to stress associated with different types of pathological reactions of immune cells. We studied IgE-dependent responses of human mast cells and basophils, Toll-like receptor (TLR)-mediated innate immune reactions of human myeloid cells and stem cell factor (SCF)-mediated responses of hematopoietic cells of myeloid lineage.

Methods: LAD2 human mast cells, primary human basophils, and THP-1 human myeloid cells were used for investigations of FcεRI, TLR ligand and SCF-induced responses. Quantitative real-time PCR, Western blot analysis, ELISA, fluorometry, luminometry and fluorescence microscopy were employed to run the assays.

Results: We observed that HIF-1 activation is differentially regulated in the cases of pro-allergic, TLR-dependent and SCF-induced cellular responses. While PI3K/mTOR and MAP kinase pathways were the major contributors to HIF-1 activation during allergic/SCF-dependent responses, TLR-mediated processes occurred mostly via redox-dependent mechanisms. Experiments with HIF-1α (the inducible subunit regulating HIF-1 transactivation) knockdown cells demonstrated that HIF-1 plays a crucial role in the expression of the primary angiogenic cytokine VEGF and controls intracellular energy metabolism by regulating glycolytic metabolic activity.

Conclusions: The HIF-1 transcription complex supports not only the survival of immune cells (mast cells, basophils, myeloid cells) in pathological environments but also determines their abilities to generate pro-allergic, pro-inflammatory as well as pro-angiogenic cytokines over sustained periods.
62 Keratinocytic Thymic Stromal Lymphopoietin Plays an Important Role in Epicutaneous Sensitization and the Atopic March
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**Background:** Atopic dermatitis (AD or eczema) often precedes the development of asthma and allergic rhinitis in atopic subjects, a phenomenon known as atopic march. An important role of epicutaneous (e.c.) sensitization has been recognized in the atopic march; however, the factors involved in e.c. sensitization remain poorly understood. Our previous studies using mouse models have shown that induced overexpression of Thymic Stromal Lymphopoietin (TSLP) in keratinocytes not only triggers an AD [Li, M. et al. Proc Natl Acad Sci U S A. 2006;103:11736–11741] but also aggravates experimental asthma induced by systemic sensitization and airway challenge of ovalbumin (OVA) [Zhang Z, et al. Proc Natl Acad Sci U S A. 2009;106:1536–1541], suggesting that TSLP represents an important factor linking AD to asthma. However, whether keratinocytic TSLP is essentially required for developing e.c. sensitization and triggering the atopic march remained to be determined.

**Methods:** We develop a mouse model in which e.c. sensitization of OVA through tape-striped skin is followed by intranasal challenge to induce an allergic asthma. TSLP+/−/ mice (in which TSLP is selectively ablated in epidermal keratinocytes at adult stage) or TSLP+ mice (in which keratinocytic TSLP overexpression is induced by topical application of MC903, a low-calcemic vitamin D analog) are subjected to this mouse model.

**Results:** Upon OVA e.c. treatment, TSLP+/−/ mice develop a defective allergen sensitization evidenced by decreased production of OVA-specific IgE and IgG1 and a reduction of the secretion of Th2 and Th17 (but not Th1) cytokines by in vitro OVA stimulated splenocytes. TSLP+/−/ mice also exhibit a decreased OVA-induced skin inflammation. Finally, upon intranasal challenge, TSLP+/−/ mice develop a less severe airway allergic inflammation and a reduced airway hyperresponsiveness. In contrast, overproduction of keratinocytic TSLP boosts the e.c. sensitization and triggers an aggravated asthma.

**Conclusions:** Our results demonstrate an important role of keratinocytic TSLP in developing epicutaneous sensitization, generating allergic skin inflammation and triggering the atopic march. Thus, blocking the expression or activity of keratinocytic TSLP could be helpful to limit epicutaneous sensitization and prevent the atopic march. This study is supported by CNRS, INSERM, ARI and ANR projects (07-PHYSIO-002-01 and JCJC-1106-01).

64 Effect of Formoterol on Eosinophil Trans-Basement Migration Induced by Interleukin-8-Stimulated Neutrophils
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**Background:** Neutrophils are often increased in the airways of either chronic severe disease or acute exacerbation of asthma. Neutrophils migrated in response to interleukin-8 (IL-8) may lead eosinophils to accumulate in the airways of asthma. Although eosinophils are considered to be harmful, Neutrophils and eosinophils were co-administered orally into mice, while OVA alone could induce oral tolerance. To evaluate the contribution of various cytokines, we used interleukin-17 (IL-17) or IL-23 knockout (KO) and wild type (WT) mice as control.

**Results:** Here we demonstrate that gamma delta T cells in the intestinal mucosa, as well as the cytokines interleukin-23 (IL-23) and IL-17, have pivotal roles to suppress the induction of serum OVA specific immunoglobulins and anaphylaxis in the food allergy model. The expression of IL-23, which was derived mostly from mucosal macrophages, and IL-17 levels were elevated after CT and OVA sensitization, and this induction of IL-17 was dependent on IL-23.

**Conclusions:** These data, together with analysis of mice genetically disrupted for IL-17 and IL-23, suggest that IL-23 suppress the food allergy, whereas IL-17 has an important role in the anaphylaxis shock. Moreover, depletion of gamma delta T cells exacerbates the food allergy. We propose that T lymphocytes, including gamma delta T cells, could be a therapeutic target for mitigating the allergic response that evokes the anaphylaxis shock.

63 Pivotal Role of Intestinal Interleukin-17-Producing Gammadeltat Cells in the Food Allergy
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**Background:** Food allergy is a serious health problem, which affect 5% of children in westernized countries and evoke life-threatening hypersensitivity, termed anaphylaxis shock. Type 2 helper T cell (Th2) response and immunoglobulin E (IgE) has been implicated in the progression of food allergy, but the roles of specific lymphocyte subpopulations and cytokines remain to be clarified.

**Methods:** The mucosal adjuvant, cholester toxin (CT) and ovalbumin (OVA) were co-administered orally into mice, while OVA alone could induce oral