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Session I: T cells and T cell subsets in autoimmune rheumatic diseases

I.1. T-cells can present intracellular enterobacterial antigens to a MHC-unrestricted cytotoxic T-cell clone.

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Reactive arthritis can occur after gastrointestinal infection with enterobacteria such as Yersinia and Salmonella, or urogenital infection with Chlamydia. We and others have shown that the immune response against the triggering microorganisms is mediated by MHC-class II-restricted CD4+ and MHC-class I-restricted CD8+ T-lymphocytes (CTL), which recognize bacterial or autoantigenic structures. Some of these bacteria-specific CD8+ CTL are HLA-B27 restricted. In this study, we have isolated and characterized CTL clones from a patient with Yersinia-induced reactive arthritis that lysed bacteria infected target cells in a MHC-unrestricted fashion. These clones recognized autologous and allogeneic Yersinia-infected B-LCL-cells but did not lyse the infected MHC-class I deficient Daudi-cell-line nor NK-sensitive K562 cells. Previous studies have demonstrated that activated T cells express B7(CDS0) which is a feature of professional antigen-presenting cells to interact with CD28/CILTA 4 expressed on T cells. Such T cells can process and present soluble antigens and induce proliferation of other antigen specific T cells. Here we show that one of our MHC-unrestricted enterobacteria-specific CD8+ T cell clones (J.P 5.104) specifically lysed autologous and allogeneic Yersinia-infected CD4+ and CD8+ T cells. Clone J.P 5.104 was even able to kill itself when infected with Yersinia or Salmonella. Our findings confirm previous data that also T-cells can serve as non-professional antigen-presenting cells. Cytotoxic T cells such as J.P 5.104 which recognize bacterial antigen on other T cells could provide a new mechanism of antibacterial cytotoxicity in reactive arthritis.

I.2. The combination of T-helper-2 cytokines IL-10 and IL-4 strongly prevents mononuclear cell induced cartilage damage

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Cartilage damage in RA is largely dependent on the production of proinflammatory cytokines like IL-1 and TNF-α. The production of these cytokines by synovial macrophages is regulated by T-helper cells (Th). Th 1 cell activity has been described to dominate in RA joints. Th 1 cells are characterized by production of IFN-γ and can specifically be induced and activated by bacterial antigens. We have shown that stimulation of Th 1 activity (IFN-γ, no IL-4) within RA synovial fluid (SF) MNC with bacterial antigen is accompanied by the subsequent production of IL-1 and TNF-α and consequently induction of cartilage damage. These effects could be inhibited by IL-4. In the present study we evaluated the capacity of the immunosuppressive Th 2 cytokine IL-10 in combination with IL-4 to prevent peripheral blood (PB) MNC and SFMNC induced cartilage damage and proliferative responses. Th 1 activity within isolated RA PBMC and SFMNC was stimulated with bacterial antigen. Stimulated MNC were treated with IL-10 (0.2, 2 and 20 ng/ml), IL-4 (20, 200 and 2000 pg/ml) and combinations of IL-10 and IL-4. After 4 days proliferative responses were measured. Conditioned media of these cultures were tested on proteoglycan (PG) synthesis of healthy articular cartilage.

IL-10 and IL-4 significantly and dose-dependently restored the inhibited cartilage PG synthesis induced by activated PBMC and SFMNC. IL-10 and IL-4 were additive in this respect. The bacterial antigen induced proliferation of SFMNC was also inhibited dose dependently by IL-10 and IL-4, IL-10 being more potent. The bacterial antigen induced PBMCN proliferation was inhibited dose dependently by IL-10. By contrast, IL-4 stimulated this induced proliferation of PBMCN. IL-10 was capable to inhibit this IL-4/bacterial antigen induced proliferative response. An explanation for the discrepancy in responsiveness to IL-4 may be found in a relative large number of naive cells in PB (≈ 50%) compared to SF (＜ 10%). IL-4 may cause skewing of antigen treated naive cells towards Th2 cell activity accompanied with enhanced proliferation, particularly in PB. IL-10 in contrast to IL-4 is known to downregulate MHC class II molecules on antigen presenting cells, which in both PB and SF will result in suppressed T cell proliferation. The anti-inflammatory capacities of IL-10 on PBMC in combination with prevention of cartilage damage induced by SFMNC indicates IL-10 to be a novel cytokine with therapeutic potential.

I.3. Selective binding of IL-2 expanded lymphocytes from rheumatoid nodules: evidence for recirculation of memory T-cells between articular and extra-articular manifestations in rheumatoid arthritis.

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Background: Rheumatoid arthritis, an autoimmune disease characterized by a polyarthritis as by well as by extra-articular manifestations such as rheumatoid nodules, is believed to be T-cell mediated. The precise relationship between the development of the extra-articular manifestations and the arthritis is yet unclear: are the same immune cells that cause arthritis also involved in the formation of rheumatoid nodules? Previously, we demonstrated a marked oligoclonality among IL-2 expanded T-cells from rheumatoid nodules. Moreover, TCR-sequencing from these IL-2 expanded T-cells revealed highly numerical similarities.
identical CDR3-regions, suggesting an antigen driven activation. We assume that IL-2 stimulation induces a clonal expansion of those T-cells that are preactivated in the in vivo situation, and may therefore reflect those cells that are immunologically relevant for the immune process.

**Aim of the study:** To evaluate the homing characteristics of IL-2 expanded T-lymphocytes (CD45RO+) from rheumatoid nodules.

**Methodology:** In vitro bindings assay on fresh frozen tissue sections (modified Stamper-Woodruff assay) was performed with lymphocytes expanded from a rheumatoid nodule (patient DKL): 500,000 lymphocytes were applied on each tissue section. Binding was quantified using a microscopic image processing system (Zeiss, Germany). Results are expressed as a/ the number of adherent lymphocytes/tissue area, b/ the relative number of lymphocytic aggregates (>5 lymphocytes) and c/ relative area of adherent lymphocytic aggregates.

**Results:** Remarkable binding of expanded T-cells was noted on rheumatoid nodules and on rheumatoid synovium, in contrast to control tissues such as skin or tonsil. Adherence of memory T-cells occurred typically in focal aggregates. Binding experiments gave the same results either on autologous or heterologous substrate. (see Table I.)

**Conclusion:** These data indicate selective binding of IL-2 expanded T-cells derived from rheumatoid nodules on rheumatoid nodule and rheumatoid synovium and are therefore suggestive for recirculation of memory T-cells between articular and extra-articular manifestations in rheumatoid arthritis. Interestingly, in vitro adherence of nodular memory T-lymphocytes was superior to rheumatoid synovium, compared to rheumatoid nodules.

### Table I:

| Origin of tissue section | Synovium WM | Synovium VL | Nodule DKL | Nodule DLJ | Tonsil DKL |
|--------------------------|-------------|-------------|------------|------------|------------|
| Lymphocytes/mm²          | 96.63       | 51.4        | 15.3       | 16.97      | 0.58       |
| Lymphocytic aggregates/mm²| 2.72        | 1.9         | 0.20       | 0.25       | 0          |
| Area of aggregates/total area | 1/57        | 1/286       | 1/1066     | 1/814      | 0          |
| Area of tissue analysed  | 15.43 mm²   | 1.1 mm²     | 35.2 mm²   | 72.79 mm²  | 47.92 mm²  |

To study this issue in more detail, synovial tissue specimens of 11 patients with RA and 4 patients with osteoarthritis (OA) were stained for IFN-γ and its specific receptor. The level of expression of IFN-γ in synovial tissue was compared with that in two well defined T cell mediated immunological reactions, namely delayed type hypersensitivity (DTH) reactions of the skin and tonsils. Finally, the percentage of IFN-γ producing T lymphocytes was determined using double staining techniques. Staining for IFN-γ and its receptor could be detected in all patients with RA and 4/4 and 2/4 patients with OA respectively. The intensity of the staining for IFN-γ in RA and OA was lower when compared with DTH-reactions and tonsils. The percentage of CD3⁺ -cells being positive for IFN-γ was around 1% of RA, whereas in DTH reactions this was >90% and in tonsils around 30%.

The low frequency of IFN-γ positive T cells in this group of patients with RA questions the immunological activity of these cells in the ongoing inflammatory processes in the inflamed joint. Our preliminary data indicate that, at specific sites, groups of cells may be found that contain higher number of IFN-γ positive T lymphocytes, possibly suggesting localized immunoreactivity.

### I.5. T cells from patients with systemic lupus erythematosus (SLE) have a diminished capacity to express CD40 ligand (CD40L).

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SLE is characterized by a polyclonal B cell activation and the development of autoantibodies. A key event for the activation and differentiation of B cells is a stimulation signal through their CD40 surface receptors, which is given by physical contact with T cells expressing the ligand for CD40 (CD40L). The present study was performed to investigate the regulation of CD40L expression on SLE T cells. For that purpose PMBC and purified T cells were activated with ionomycin and PMA as well as with insolubilized anti-CD3 antibodies. The expression of CD40L, CD25 and CD71 was measured before activating the cells as well as after 2, 6 and 20 hours of stimulation. CD40L expression was detected by a construct of the extracellular portion of CD40 conjugated with the human IgG1 heavy chain, CD25 and CD71 by monoclonal antibodies by flowcytometry and activated cell sorter analysis. There was no significant expression of either one of the three markers before activation. After 2, 60 and 20 h stimulation with anti-CD3 significantly few-
er T cells from SLE patients than from normal donors expressed CD40L, whereas no difference was found for CD25 and CD71. Ionomycin/PMA also induced a higher CD40L expression in normal T cells up to 6 hours after activation. After 20 h CD40L was still stable in SLE but decreased in normals to levels comparable to SLE T cells. The tendency to a more sustained activation of positive cells and the peak fluorescence intensity were inactivated PBMC. Our data demonstrate an altered capability of SLE T cells to express CD40L on their surface, which may play an important role for the development of autoantibodies.

I.6. Decreased expression of BCL-2 in synovial fluid lymphocytes from patients with rheumatoid arthritis.

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Proto-oncogene bcl-2 has the ability to block apoptosis of several cell types. Bcl-2 transgenic animals demonstrate increased B and T cell survival and enhanced immune responses. Some animals develop autoimmune disease resembling human systemic lupus erythematosus (SLE). In human SLE the expression of bcl-2 protein has been shown to be increased in peripheral blood (PB) lymphocytes. In this work we studied the expression of bcl-2 protein in PB and synovial fluid (SF) lymphocytes and synovial tissues derived from patients with rheumatoid arthritis (RA).

The expression of bcl-2 protein was uniformly high (> 90% of cells positive) in PB lymphocytes and similar in RA vs. controls when studied by flow cytometry. Interestingly, the expression of bcl-2 in SF lymphocytes was significantly lower than in RA or control PB lymphocytes (p < 0.001). Both the proportion of positive cells and the peak fluorescence intensity were reduced. This difference was seen in total lymphocytes and T cells. Cytokines IL-2, IL-4 or IL-10, which have previously been shown to affect bcl-2 expression in lymphoid cells, had no marked effect on the bcl-2 expression. When synovial tissues from RA, reactive arthritis and osteoarthritis were stained with anti-bcl-2 mAbs, no significant differences were observed in the staining pattern or the proportion of bcl-2 positive inflammatory cells between different diseases.

In conclusion, we could not demonstrate any abnormal expression of bcl-2 which would lead to the accumulation of lymphoid cells in RA. This suggests different regulation of lymphocyte apoptosis in RA and SLE. The decreased bcl-2 expression in SF lymphocytes is probably due to the activated status of these cells since T cell priming has been shown to be associated with a decrease in bcl-2 expression.

I.7. In vivo migration of human lymphocytes to human rheumatoid synovium engrafted on SCID mice.

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We engrafted subcutaneously 20 SCID CB17 mice with synovial tissue obtained after surgery of patients with RA or osteoarthritis. Human blood lymphocytes (PBL) from 5 RA patients (ACR criteria) and 5 healthy controls were isolated on Ficoll gradient. Twenty millions HMPAO-99 mTC labelled lymphocytes were injected intra-peritonealy (IP) 3 weeks after synovial engraftment. Total mice scintigraphy was performed 20 hours post-injection, and a region of interest (ROI) of 200 pixels was determined in the area of the human graft. Total radioactivity was obtained from each mouse. Immunohistology of graft and spleen were available in all cases. TNFα and IL-1 were injected in ten mice 24 hours before scintigraphy.

Results: Retention of lymphocytes by engrafted human rheumatoid synovium was reflected by the increased activity obtained in a ROI of synovial graft 2123 ± 958 cpm versus 496 ± 50 in a ROI of muscle graft, (p < 0.01) and 521 ± 40 cpm when osteoarthritis synovium was engrafted (p < 0.01). Results were the same when injected PBL originate from RA or normal subjects. The effect TNFα and IL-1 on the lymphocyte homing, and on adhesion molecules expression in synovial tissue is currently being studied.

Conclusion: Increased radio-labelled lymphocytes tissue retention was observed within engrafted rheumatoid synovium in SCID mouse. Lymphocytes attachment to synovial tissue was increased in RA versus osteoarthritis.

I.8. Proliferation of T-cells, macrophages, PMN and fibroblast-like cells in the synovial membrane (SM) of rheumatoid arthritis (RA) patients.

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Controversial findings about the proliferation of inflammatory and fibroblast-like cells in the RA SM range from very low rates (approx. 1%) to very high ones (70%). In the present study, the proliferation rates in the lining layer of the RA synovial membrane (SM) were assessed by immunohistochemical investigations using the markers proliferating nuclear antigen (PCNA; mAb PC10) and Ki-67 (mAb MIB-1); the rates were compared to those occurring in osteoarthritis (OA), joint trauma (JT), as well as in synovial sarcomas (SS). The nature of the proliferating cells was analyzed by double-staining experiments with T-cell (CD3), macrophage (CD68), and PMN markers (CD15).

The expression of PCNA, also observed in non-cycling cells, indicated proliferation rates in the lining layer ranging from 29.7 ± 3.7% (mean ± SEM) in JT, 48.6 ± 4.5% in OA, and 23.8 ± 9.9% in RA, to 67.2 ± 3.1% in SS. The proliferation marker Ki-67 (mAb MIB-1), not expressed in the G0 phase and...
Table: CDR3 sequences of the overexpressed idiotypes

| Patient | Ratio | Valpha2       | CDR3         | Jalpha         | Calpha         |
|---------|-------|---------------|--------------|----------------|----------------|
| DVA     | 8/11  | SATYLCAVA     | MINAGNML     | TFGGGTRLMVKP  | HIQNP          |
| 1/11    | SATYLCVV    | NIGAGYSTL     | TFGKGTMLLVSP | DIONP          |
| 1/11    | SATYLCAV    | KSDNDM        | RFGAGTRLTVKP | NIQNP          |
| 1/11    | SATYLCAV    | RGGTST        |              |                |
| DKL     | 11/12 | FCASS         | LMGALN       | GELFFGEGSRTLVL| Ed             |
| 1/12    | FCASS    | FGGSVDS       |              |                |

therefore more tightly restricted to proliferating cells, was positive in 0.8 ± 0.1% of JT, 1.1 ± 0.2% of OA, 0.6 ± 0.2% of RA lining cells, and 20.0 ± 2.8% of SS cells. The differences for Ki-67 between SS and all other alterations as well as between RA and OA were significant (p < 0.01, U-test). Ki-67 was only expressed in some lymphocytes in germal centers of RA lymphoid aggregates, but not in macrophages or PMN; in the RA lining layer virtually only fibroblast-like cells expressed this proliferation marker.

Our study confirms that T-cells in the RA SM undergo very limited and spatially confined proliferation. The proliferation rates of fibroblast-like cells in the RA lining layer are very low, in particular in comparison to a sarcoma at the same anatomical location.

1.9. Molecular analysis of T cell receptor rearrangement in IL-2 expanded lymphocytes from rheumatoid nodules suggests an antigen-driven T cell activation.

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Aim of the study: The aim of this study was to explore the molecular diversity of T cell receptors among IL-2 expanded lymphocytes from rheumatoid nodules. Using anti-TCR monoclonal antibodies, we previously demonstrated oligoclonality among these cells, leaving the possibility of an antigen- or superantigen-driven activation.

Materials: IL-2 expanded lymphocytes from the rheumatoid nodules of 2 patients (DVA and DKL) were analysed for TCR rearrangements. RNA was extracted after 2 weeks of expansion.

Results: In patient DVA, strong overexpression of Valpha2 was observed among the expanded lymphocytes. The Valpha2 sequence was amplified with PCR, cloned and sequenced (Table). Patient DKL showed overexpression of the Vbeta8 idiotype in the majority of expanded cell lines; the Vbeta8 sequence was obtained in the cell line with the strongest Vbeta8 overexpression (table).

Conclusions: IL-2 expansion of T cells from rheumatoid nodules generates oligoclonal cell lines with an identical CDR3 region sequence in the large majority of the clones. This strongly suggests an antigen-driven T cell activation in situ.

I.10. Application of an immunohistochemical method for cytokine detection to the study of in vivo and in vitro production of cytokines in arthroscopically obtained synovial tissue from RA patients.

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We have applied a recently developed immunohistochemical method for analysis of cytokine production in tissue specimens to investigate synovial cytokine production in RA from two aspects; (i) ongoing in vivo cytokine production in various phases of RA; (ii) potentials to modulate cytokine production from biopsy specimens in vitro. In staining the sections, we used carefully selected cytokine-specific antibodies detecting 19 different cytokines after fixation of the specimens with paraformaldehyde and using saponin to permeabilize cellular membranes. For analysis of in vivo cytokine production in the synovium, biopsies were obtained arthroscopically at different phases of development of RA. The immunohistochemical method yielded reproducible and distinct staining patterns, where the cytokines accumulated mainly in the Golgi apparatus of producing cells, indicating that the method preferentially detects local synthesis rather than cytokine uptake. The cytokine pattern varied considerably between different biopsies; monokines such as TNF-alfa and II-1 dominated in almost all specimens: there was, however, also a variable appearance of T-cell derived cytokines, with some specimens producing appreciable amounts of IFN-gamma, II-2, II-12 as well as II-4 and II-10. There was also in many specimens a strong staining for all three TGF-beta subsets. We were mainly struck by the large variability in cytokine staining, and are currently characterizing variations in staining patterns between different parts of the same synovium as well as between biopsies from the same site obtained at different time points.

The staining technique is currently being used also for functional in vitro assays of intact biopsy specimens, which are subjected to stimulation both by molecules which may modify cytokine production or action and by different specific antigens. Recent results from these studies will be presented.
I.11. Serum sIL-2R level as a marker of rheumatoid arthritis activity.
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Serum sIL-2R levels were measured in elderly onset rheumatoid arthritis patients in order to verify whether sIL-2R level was a good marker of systemic and local activity in the elderly onset rheumatoid process, as well. ORA was defined as RA starting after the age of 60. RA was defined according to the revised ARA criteria 1987. Activity parameters: ESR, CRP, Ritchie count and a total activity score consisting of 13 variables were measured and correlated. sIL 2R was assayed by an ELISA kit (DAKO). sIL 2R level significantly increased in ORA compared to the healthy elderly controls. sIL 2R showed a highly significant correlation with ESR, CRP and the total activity score, still significant with the Ritchie count. The highest sIL 2R levels were found in the seropositive classical form of ORA. Thus, sIL 2R seems to be a good marker of RA activity, reflecting the importance of TH1 type immune activation in the elderly onset process.

I.12. Immobilized anti-CD3 antibody activates T-cell clones to induce interstitial collagenase, but not tissue inhibitor of metalloproteinases, in monocytic THP-1 cells and dermal fibroblasts.
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Both rheumatoid arthritis (RA) and osteoarthritis (OA) are associated with pathologic changes resulting from the degradation of components of the extracellular matrix. We have now investigated whether direct cell to cell contact between activated paraformaldehyde fixed T-cell clones obtained from synovial tissue of patients with OA or RA, and monocytic cells or dermal fibroblasts influenced the balance between the matrix degrading enzyme interstitial collagenase and its specific inhibitor TIMP (tissue inhibitor of metalloproteinases) produced by these cell types. PHA/PMA-activated fixed T-cell clones or their membranes strongly induced the production of collagenase both in monocytic THP-1 cells and fibroblasts. In contrast, only low levels of TIMP were induced in THP-1 cells and no change of TIMP expression was observed in fibroblasts as a result of stimulation with PHA/PMA-activated T-cell clones or T-cell membranes. Anti-CD3 activated T-cell clones stimulated the production of collagenase both in THP-1 cells and fibroblasts, whereas TIMP levels were not influenced. Collagenase production in THP-1 cells induced by anti-CD3 activated T-cell clones was (1) dependent on the dose of anti-CD3 used to stimulate the T-cells, (2) initiated only when CD3 was crosslinked and (3) inhibited when cyclosporin A was included during T-cell activation. Our data collectively indicate that activated T-cells in contact with monocytic cells or fibroblasts may alter the balance between interstitial collagenase and its specific inhibitor TIMP. This selective induction of a mediator profile representative of matrix breakdown as a result of target cell interaction with activated T-cells may be an important factor in the local process of tissue destruction which characterizes OA and RA.

I.13. Mode of action of inhibitory/protective MHC modules
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| SPECIFICITY | MODE OF ACTION |
|-------------|----------------|
| Epitope-specific | Antigen presentation: epitope capture or interference |
| Tolerance: MHC molecule creates a hole in the repertoire |
| Mimicry: disease-inducing epitope mimics certain previous environmental priming epitopes, and thus favours Th2 activation |
| Differential locus expression |
| Density: low MHC density favours Th2 activation |
| Location: preferential expression in B cells favours Th2 activation |
| Differential allele expression |
| Density: low MHC density favours Th2 activation |
| Location: preferential expression in B cells favours Th2 activation |
| Independent of epitope binding to the inhibitory MHC molecule |
| The inhibitory MHC molecule contributes a peptide which binds to another MHC molecule and so activates inhibitory/protective T cells |

The shaded area indicates the territory which I propose to defend.

I.14. Regulatory cytokine production profiles of infiltrating cells from rheumatoid synovium.
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CD 4 T cells have been classified according to their cytokine profiles. However, such classification has been more difficult to reconstitute when looking at the cytokine produced by the RA synovium itself. We compared the production of cytokines by pieces of synovium, by the cell suspension obtained after digestion of the synovium and by T cell clones. In the culture supernatants of RA synovium pieces, no IL 10, IL 4, IFN γ could be detected, a result contrasting with the high levels of proinflammatory cytokines such as IL 1, TNF α and IL 6. However, a spontaneous secretion of IL 10 and IFN γ could be detected at the single cell level with the ELISPOT assay in the cell suspension obtained after digestion of the synovium. Monocytes represented the major source of IL 10. In part because of the secretion of endogenous IL 10, incubation of RA synovium with IL 10 reduced proinflammatory cytokine production, al-
though its effect is not as potent as that of IL-4. IL-1 Ra produc-
tion was increased with IL-4 and IL-13 but not with IL-10. When
looking at the cytokine profiles of T cell clones, surpris-
ingly, a large proportion of the CD4+ cells from synovium
or synovium fluid, producing large amounts of IFN-γ, also pro-
duced significant levels of IL-10. Additional patterns were also
observed including typical Th2 profiles. Conversely, γε T cell
clones, producing large amounts of IFN-γ, also produced sig-
nificant levels of IL-4 but no IL-10, suggesting a possible pro-
tective role for γε T cells.

These results indicate a major role of cell to cell interactions
inside the inflammatory reaction. The T cell derived cytokine
production may be sufficient to induce potent local effect. The
inhibitory effect of IL-10 and IFN-γ on each other production
and function may explain the absence of secretion when one se-
creting cells are in close contact.

I.15. Detection of IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-
12/p40, IFN-γ, TNF-α, and TGF-β1 in the synovial fluid of pa-
ients with osteoarthritis, rheumatoid arthritis and miscella-
neous other forms of arthritis.

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many.

It has become clear in the past years that cytokines play a crit-
ical role in the pathogenesis of arthritis. Several monokines
have been detected in the synovial fluid of patients with vari-
sous forms of arthritic disease whereas the role of lymphokines
such as IL-4 and IFN-γ and immunoregulatory cytokines like
IL-10 and IL-12 is not yet understood. The aim of this study
was to compare the amount of IL-1α, IL-1β, IL-4, IL-6, IL-
8, IL-10, IL-12/p40, TNF-α, TGF-β1, and IFN-γ in the synovial
fluid of 43 patients with osteoarthritis (O.A.), 28 patients with
rheumatoid arthritis (R.A.) and 17 patients with miscellaneous
other forms of arthritis (M.A.) using cytokine specific ELLA-
SAs. Since some samples exhibited non-specific activities all
cytokines were measured in the presence or absence of cytokine
specific neutralizing antibodies. Our data show that these
cytokines can be detected in O.A., R.A. and M.A. Compared
to patients with O.A. all cytokines were found in much higher
levels in patients with R.A. and M.A. With regard to the T helper
lymphokines IL-4 and IL-10, all T helper lymphokine pat-
terns (Th0, Th1 and Th2) could be observed. The presence of
IL-4 and/or IFN-γ was associated with different monokine pro-
files in patients with R.A. and M.A., indicating that different
immunoregulatory mechanisms are active in both subgroups.
Surprisingly, high levels of IL-10 were associated with higher
monokine but lower IFN-γ levels in all patients indicating that
this cytokine may not be active as a suppressor of monocyte
activity in vivo. In addition, no suppression of cytokine levels
was observed in synovial fluids with higher amounts of TGF-β1.
In conclusion, our data demonstrate that monokines (IL-1, IL-6,
IL-8, TNF-α), lymphokines (IL-4, IFN-γ) and immunoregula-
tory cytokines (IL-10, IL-12/p40, TGF-β1) can be detected in
the synovial fluid of patients with R.A. and
M.A. as compared to patients with O.A. Different cytokine
profiles are detectable in distinct subgroups of patients which
may reflect differences in etiology and stage of disease. The
measurement of cytokines in the synovial fluid may provide a

I.16. Production of cytokines by T cells in RA synovial
membranes.

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It is generally assumed that chronic overproduction of mono-
kines, particularly IL-1 and TNF-alpha (TNF-a), contributes
to the pathophysiology of RA. Much less is known about cyto-
kines which are produced by activated T cells such as IL-2,
IL-4, and IFN-gamma (IFN-g). In order to investigate produc-
tion of cytokines by T cells in RA we studied synovial mem-
branes (SM) from 8 RA and 3 OA patients by immunohis-
tochemistry using monoclonal antibodies to CD3, CD4, CD8,
CD45R0, CD68, HLA-DR, and IL-2R and affinity-purified
polyclonal antibodies to the cytokines IL-2, IL-4, IL-6, TNF-a
and IFN-g. For the characterization of cytokine producing cells
a peroxidase/alkaline phosphatase double labelling technique
was employed.

Most tissue sections derived from RA SMs were heavily infltr-
tated with clusters of CD3+ T cells most of which expressed
HLA-DR and CD45R0. The ratio of CD4+ :CD8+ cells was
approximately 3:1. Using IL-2R expression as activation mark-
er less than 0.5% of all T cells seemed to be activated. How-
ever, in some clusters up to 2% of CD3+ T cells expressed IL-
2R and in such clusters cytokine producing T cells were ob-
erved with similar or even somewhat higher frequency. IL-2,
IL-4, and IFN-g were produced both by CD4+ and CD8+ cells.
These cells stained also positive for TNF-a and IL-6, two
cytokines which were mainly produced by non T cells. These
cells stained also positive for TNF-a and IL-6, two cytokines
which were mainly produced by non T cells. Interestingly, T
cell cytokines were also detected in some CD68+ cells which
could represent a subset of activated T cells; this observation
demands further investigation. As expected, in specimens from
OA patients T cells were usually much less abundant than in
those from RA patients and neither IL-2R expression nor cyto-
kines could be detected.

Taken together, in SMs from RA patients production of IL-2,
IL-4, IL-6, IFN-g and TNF-a was observed in both CD4+ and
CD8+ T cells. Assuming antigen specific activation of these
cells their presumptive role in the pathophysiology of RA must be
considered.
I.17. Expression of the activation marker CD27 on synovial T-cells in RA.

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Objective: To investigate the activation state of T-cells in rheumatoid arthritis (RA) using the recently defined marker CD27. It has been demonstrated before and chronically activated T-cells become CD27 negative (CD27-).

Methods: Peripheral blood and synovial fluid mononuclear cells (PBMCN and SFMNC) as well as biopsies of synovial tissue (ST) from the knee were obtained from RA patients and patients with osteoarthritis (OA) as controls. The expression of CD27 on T-cells and subsets of T-cells was studied by FACS analysis and immunohistochemistry.

Results: Comparison of the mean percentages of CD27- PBMCN did not reveal differences between RA (n=9) and OA (n=8). In RA an increase in the mean percentage of CD27- cells was found for CD4+ SFMNC compared with PBMCN (n=7). This was, however, also found in the CD4 + SFMNC of OA patients (n=2). Similar results were obtained when the mean percentages of CD27- cells were determined in the CD45RA- subsets.

The mean percentages of CD27- T-cells in ST were also similar for RA (n=7) and OA (n=6).

Conclusion: An increase in chronically activated CD27- T-cells could be demonstrated in patients with RA.

I.18. The TCR/CD3-complex is downmodulated from CD4+ cells in vivo during anti-CD4 therapy of SLE patients.

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Anti-CD4 antibodies have been introduced in the therapy of rheumatic autoimmune diseases to affect selectively CD4+ positive cells. Up to now, seven patients with otherwise intractable SLE have been treated with the monoclonal anti-CD4 antibody MAX. 16H5 (murine IgG1) in a clinical trial (0.3 mg/kg body weight over 120 min. at 7 consecutive days). Nevertheless, the underlying mechanism is still a matter of debate. T-cell depletion is neither necessary nor sufficient for the clinical efficiency. Recently, we reported a downmodulation of CD4 molecules from targeted cells within four hours after antibody infusion followed by a subsequent reexpression of lost surface-associated CD4 molecules after 10-14 hours. We therefore analyzed whether this effect can interfere with other surface molecules, especially CD3 and TCR II. Unexpectedly, CD4+ lymphocytes from three out of five patients removed their TCR and CD3 molecules from cell surface within 4-6 hours after infusion and reexpressed them after about 20 hours. The transient lack of CD3+CD4+TCR+ lymphocytes is not due to elimination of these cells from the circulation and not to steric hindrance of anti-CD3 and anti-TCR binding. Taken together, there is first evidence that MAX. 16H5—though directed against CD4—may act via modulation of important surface molecules such as CD3/TCR complex which may have profound effects on signal transduction and consecutive T-lymphocyte activities.

I.19. Differential effect of IL-10 on the expression of GM-CSF/IL-4 induced antigen presenting molecules.

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IL-10 down regulates constitutive and IFNγ+ or IL-4 induced expression of HLA class II molecules on monocytes. These molecules are expressed in abundance in the rheumatoid synovium despite the presence of IL-10, suggesting that the induction of class II molecules by cytokines other than IFNγ or IL-4 may be IL-10 resistant. Indeed the induction of class II molecules by GM-CSF or GM-CSF/IL-4 is not inhibited by IL-10 at up to 10 ng/ml. In contrast, IL-10 completely downregulated the expression of CD1 molecules on the same preparation of monocytes. CD1 molecules are also induced by GM-CSF/IL-4 and known to present bacterial antigens to CD4+CD8- T lymphocytes. Class II and CD1 expression was determined by flow cytometry analysis and further confirmed in functional studies. Our results thus show a dichotomy in the effect of IL-10 on the modulation of antigen presenting molecules. Moreover they suggest that the postulated immunoregulatory effect of IL-10 in autoimmune diseases such as RA may be more sophisticated than assumed. Indeed persistent HLA class II expression, combined with direct inhibitory effects on T lymphocytes may create an ideal environment for the induction of anergy of self-reactive T cells.

I.20. Synovium of rheumatoid arthritis patients is enriched in fully differentiated CD4+CD45RBdimCD27-memory T cells.

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Expression of CD45RB and CD27 permitted four subsets of CD4+ T cells to be identified: CD45RBbrigh/CD27dim, CD45RBbright/CD27+, CD45RBbrigh/CD27+ and CD45RBdim/CD27-. When analyzed for the capacity to provide help for B cells, higher Ig levels were induced by CD45RBdim/CD27- cells compared to the CD45RBdim/CD27+ population, whereas CD45RBbright cells were poor inducers of Ig synthesis. Mitomycin C treatment markedly enhanced helper activity of each subset except CD45RBbright/CD27- cells. CD45RBdim cells remained the most efficient stimulators but no difference was detected between CD27+ and CD27- cells. These results indicate that, within the CD4+ memory T cell population, an increase of helper activity associates with the shift from a CD45RBbright to a CD45RBdim phenotype, whereas the loss of CD27 is paralleled by a reduction of suppressive capacity. The subsets also differed in their capacity to proliferate and secrete cytokines. Both CD27- subsets exhibited decreased sustained proliferation and uniquely secreted IL-4, with the CD45RBdim/CD27- subset producing the greatest quantities of IL-4. No differences in IL-2 and IFN-γ production were found. IL-10 secretion increased with the acquisition of a CD45RBdim phenotype and, within the CD45RBdim cells, with the loss of CD27. The different capacity of CD4+ mem-
In vivo

1.21. The anti-CD4 monoclonal antibody Max.16H5 induces a switch from Th1 cytokines to Th2 cytokines in human peripheral blood mononuclear cells.

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In vivo treatment of animals with monoclonal antibodies against CD4 prevents autoimmune diseases and may induce tolerance to allogeneic organ transplants. In humans anti-CD4 antibodies were successfully used in the treatment of autoimmune diseases and graft rejection. However, the mechanisms of CD4-mediated tolerance induction have not been clearly understood. (for review: Sabinis et al., 1991). We investigated whether the anti-CD4 monoclonal antibody Max.16H5 could induce a switch in the cytokine pattern of T cells reactive to allogeneic lymphoid cells or to soluble recall antigens. Peripheral blood mononuclear cells from healthy donors were treated with Max. 16H5 before stimulation with different antigens or allogeneic T cell-depleted irradiated cells. After 8 days the cells were restimulated with the corresponding antigen without Max.16H5. Cytokines were determined in the supernatants during the primary and secondary stimulation. Whereas IL-2 and γ-IFN were always markedly suppressed, an increase was observed in IL-4 and IL-10 concentration, especially after restimulation. These results might indicate that anti-CD4 treatment not only causes an inhibition of T cell activation and thereby suppression of Th1 cytokines. It may, in addition, actively induce an antigen specific Th2 dominated response, which might be important in suppressing Th1 like response and thus perhaps induce a tolerant state in vivo.

Session II: The role of autoantibodies in autoimmune rheumatic diseases

II.1. Maturation of rheumatoid factors by a non-autoimmune mechanism.

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To test whether a chronic infection would change the low avidity-, germline encoded RF repertoire that is found in normal individuals to the high avidity-, somatically mutated one that is characteristic for RA, we analyzed the RFs produced by EBV transformed mononuclear B cells established from 11 representative individuals: 4 with rheumatoid arthritis (RA), 3 with tuberculosis (TB) and 4 age-matched normal controls. Fifty eight RFs were analyzed for specific activity (IU-RF/mg) for the Fc part of IgG and their interaction with Tetanus Toxoid (TT) or DNA (polyspecificity). Furthermore, we sequenced the V-D-J heavy chain region of 13 (9TB-/4RA-) RFs. The RF repertoire of normal individuals comprised of low affinity RFs of which the majority (15/17) was polyspecific. The interaction of these polyspecific antibodies with DNA, TT as well as with Fc was inhibited either by increase of the ionic strength to 0.3-0.5 M NaCl or by addition of the polyanion Dextran Sulphate (DS) indicating that all antibodies interacted with a similar anionic epitope shared by the three antigens. In contrast, the RF repertoire in RA patients comprised of high affinity, monospecific (16/21) RFs. In the individuals with a history of mycobacterial infections, one patient diagnosed three months before blood sampling had a normal polyspecific (4/5) repertoire, while the other two, who had suffered from numerous recurrences through their life, had a high affinity, monospecific (9/15) RA-like repertoire. Moreover, these RFs were encoded by somatically mutated V genes showing that after chronic infections, RFs differ significantly from RFs in normal individuals and might become indistinguishable from the RFs that are found in RA.

II.2. Screening for ANA – Comparison of immunofluorescence and ELISA Assay.

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Antinuclear antibodies (ANA) are important in the diagnosis and care of patients with autoimmune diseases. In many laboratories testing for ANA is done by an immunofluorescence assay (IFA) using HEP-2 cells as a substrate. Although a lot of effort has been put into standardization of this method, there is a great interlaboratory variation. In recent years several ELISA testkits for ANA are commercially available, intended to replace the IFA as a screening method. We compared 3 of these kits (I, B, D) with our IFA results on HEp-2 cells cultivated in our laboratory.

Samples of 154 patients were tested, 123 women and 31 men. The average age was 54 years (7-89). About 50% were suffering from inflammatory rheumatic diseases (RA, JCA, PSA etc.), 35% from systemic autoimmune diseases (SLE, scleroderma etc.), and 15% from a degenerative disease. For this study samples were chosen according to there IFA patterns, in order to cover the whole spectrum of ANA. 45 showed a homogeneous, 17 a speckled, 35 a nucleolar, 23 a centromere, and 20 seldom or mixed patterns. 14 sera were negative for ANA by IFA. All samples with titers > 1:160 in the IFA were tested for ENA by immunoblot. All samples with centromere and nucleolar pattern were rechecked with a commercially available IFA. All three ELISA tests showed a good cocordance for samples with a speckeled and homogeneous pattern, if only titers > 1:160 were considered.

The absorbance of the individual samples in the ELISA assay correlated only poorly with titer of the IFA for all patterns. Nucleolar and centromere patterns are recognized in 52/57% only in test I, 60/96% in test B and 63/91% in test D.
The sensitivity, specificity of the individual tests and the correlation with ENA results are discussed and correlated to the specific diseases of the patients.

II.3. Ligation of CD40 influences the function of human Ig Secreting B Cell hybridomas both positively and negatively.
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Ligation of CD40 is known to play a role in B cell activation and Ig heavy chain class switching. However, the effect of engaging this B cell surface molecule on Ig production and cell proliferation after initial activation and class switching has not been defined. To examine this issue, the effect of ligation of CD40 on the function of a battery of human Ig secreting hybridomas was examined. B cell hybridomas were generated by fusing activated peripheral blood B cells with the SP8-4 non Ig secreting fusion partner. Resulting hybridomas were cloned and characterized for surface phenotype and function. All hybridomas expressed CD40. These hybridomas were stimulated with either recombinant baculovirus expressed membrane bound CD40 ligand or a soluble murine CD40 ligand/CD8 construct in the presence or absence of various cytokines. Growth of all cell lines tested was dramatically inhibited when presented with large concentrations of CD40L. This response was blocked by a soluble CD40-Ig construct. Lower concentrations stimulated proliferation and Ig secretion of 7/11 IgM but 0/11 IgG or IgA secreting hybridomas. An increase in homotypic adhesion was induced by ligation of CD40 regardless of the concentration of CD40 ligand employed or the effect on proliferation or differentiation. These results indicated that ligation of CD40 signals Ig secretion human hybridomas to alter their growth patterns and Ig secretion. The functional impact of CD40 ligation varies with extent of CD40 engagement and whether the hybridoma had undergone switch recombination or differentiation.

II.4. 68k autoantigen recognised by autoreactive antibodies and T cells in RA.
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A 68k autoantigen is recognised by autoreactive antibodies and T cells in RA. Anti-68k-specific antibodies could be detected in 66% of 167 RA patients, but in only 1% of 97 patients with other rheumatic diseases and not in 55 healthy controls. Thus the sensitivity of the novel autoantibody is 66%, its specificity is 99%.

The 68k antigen shows an IP of 5.1, is O-glycosylated and located in the endoplasmatic reticulum or cytoplasm. It is detectable in all tissues investigated.

The 68k antigen was applied in T cell proliferation assays to investigate whether T cells specific for this antigen could be identified. The antigen was added to PBMCs of healthy and RA individuals in a nitrocellulose-bound form. For PBMCs the proliferative response, measured via 3H-thymidine incorporation, peaked on day 10, in cells isolated from inflamed synovium on day 3. - 70% of 27 RA patients harboured T cells proliferating upon 68 presentation.

Monoclonal antibodies directed against one of either HLA-DP, -DQ or -DR allele were additionally applied in order to block respective antigen presentation. Comparing proliferation responses to the 68k antigen with and without monoclonals against HLA-DP, 63% of 27 RA patients showed an increased stimulation in the presence of the anti-DP antibody. This is indicative for DP restricted suppressor T cells with the potential to inhibit 68k-reactive T cells in an antigen specific fashion.

II.5. Anti-P protein antibodies in systemic lupus erythematosus: correlations with clinical and serological data.
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Anti-P protein antibodies have been reported to be correlated with psychiatric symptoms in SLE, but other studies have not confirmed this relationship and hypothesize other associations. Nevertheless the importance of these autoantibodies has been underlined due to the observation that they may cross-react with protein epitopes expressed on the cellular membrane.

Recently we developed an ELISA using a multiple antigen peptide as antigen, which we demonstrated to be far more reliable, sensitive and specific than the detection methods usually employed. In this study, 420 sera from 119 patients with SLE (25.2%) were tested for anti-P protein antibodies using our ELISA, and the results were compared with a set of clinical symptoms and with certain immunologic specificities recorded at the time of the study.

79 sera from 30 patients were found to be positive for anti-P antibodies. Statistically significant correlations were found between the presence of anti-P antibodies and the presence of malar rash (no. of sera tested simultaneously for antibodies and for the presence or absence of this sign: n = 292, p < 0.01), cutaneous vasculitis (n = 289, p < 0.01), leukopenia (n = 278, p < 0.01), anti-Sm antibodies (n = 321, p < 0.01), and the lack of renal involvement (n = 320, p < 0.01). We found no correlation with the presence of discoid rash, oral ulcers, arthritis, serositis, or neurologic involvement, nor with the presence of antinuclear, anti-DNA, anti-RNP, anti-SSA or anti-SSB antibodies. Correlations with psychiatric symptoms could not be determined since the retrospective analysis did not allow us to adequately evaluate these manifestations.

In conclusion, this study indicates that anti-P protein antibodies are linked to a number of signs and symptoms in SLE, and therefore may be of great diagnostic and clinical relevance. Prospective studies to confirm these preliminary results are being planned.

II.6. Antibodies against HIV-1/2 proteins in SLE: correlation with antibodies to intracellular antigens.
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Autoimmune phenomena have been reported in patients with HIV infections and, on the other hand, reactivity of the sera from patients with systemic rheumatic diseases, such as sys-
tcemic lupus erythematosus (SLE), with antigens of HIV has been observed. The possibility of molecular mimicry is one of the explanations. Commercial ELISA tests were used to screen for reactivity of 194 sera from SLE patients with HIV 1/2 antigens.

Reactive sera were then tested by immunoblotting with HIV-1 proteins. All the sera were examined by standard methods on the presence of particular autoantibodies to intracellular antigens. Including immunoblotting with cytoplasmic and nuclear antigens of HeLa cells respectively. Antiphospholipid antibodies were analysed by ELISA. 31 (15.9%) samples showed anti-HIV 1/2 reactivity in ELISA. Of these the reactivity with HIV 1 proteins by immunoblotting was observed in 22 (11.3%). Frequency of the positive reaction was as follows: with p18 (10.6%), p24 (12.7%), p34 (12.7%), p51 (10.6%) and gp 160 in 1 serum (2.1%). By retesting the sera of reactive patients fluctuation of reactivity and sometimes persisting reactivity was observed. No strict correlation with the specificity of autoantibodies against intracellular antigens was observed. So different antibodies in sera of patients with SLE most probably co-exist and reflect a pleura of immune anomalies in this disease.

II.7. Phosphorylation of complement component C3 by platelets activated by immune complexes isolated from patients with SLE.

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Recent studies have shown that phosphorylation of different components of the complement and coagulation systems by protein kinases secreted from activated human platelets result in pronounced alterations of the functions of these components. Upon activation by heat aggregated IgG (HAGG) or ADP, platelets release a casein kinase I-like protein kinase which phosphorylates C3. C3b and C3dg in the C3d-domain of the protein; close to the internal thiol ester. This phosphorylation leads to decreased susceptibility to cleavage of C3b by factor I which might influence both convertase functions and interactions with different complement receptors.

In the present study, we describe phosphorylation of complement component C3 after activation of platelets with immune complexes (ICs) obtained from sera of patients with SLE. ICs were isolated from 10 pathological sera by precipitation with PEG 6000. By gel filtration it was confirmed that the precipitates contain high molecular weight (> 10^7 kDa) material. HAGG, and PEG precipitates from sera of 10 blood donors served as controls. Washed platelets were activated with HAGG or PEG precipitated ICs at 22°C for 15 min. After centrifugation, the supernatants were incubated with [γ-32P]ATP and purified C3 for 15 min at 37°C. Alternatively, the platelets were incubated with [32P]PO4 for 60 min at 37°C to label the intracellular pool of ATP. After washing, the labelled platelets were mixed with purified C3 and activated with precipitated ICs of HAGG. In both experimental set ups, the samples were analysed by SDS-PAGE and the degree of phosphorylation of C3 was assessed using a phosphoimagor. The analysis shows that the pathological ICs activate platelet in a similar way as HAGG, which leads to release of a protein kinase which phosphorylates C3 in the a-chain.

In conclusion, sera from SLE patients contain ICs which might mediate phosphorylation of C3 by activating platelets. Since C3 is one of several plasma proteins which are substrates for platelet protein kinases, it is likely that phosphorylation represent an important mechanism by which serum proteins are regulated. This regulatory mechanism might be involved in the pathogenesis of SLE.

II.8. Differential regulation of rheumatoid factor production by B cells from healthy individuals or patients with rheumatoid arthritis.

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The presence of rheumatoid factor (RF) in the circulation is a characteristic phenomenon in patients with rheumatoid arthritis (RA). There is evidence that the RF produced in RA patients is structurally and functionally distinct to that induced in normal subjects where RF production is a transient event. To compare the regulation of RF production by B cells derived from RA patients and healthy individuals we have studied IgM-RF production in a T-B cell co-culture system. Antibody production by B cells was induced by either anti-CD3-stimulated T helper cell activity or staphylococcal enterotoxin D (SED)-induced T-B cell interaction. For this purpose we have selected a T cell clone, K15, that exhibited helper capacities in the presence of SED and anti-CD3. Clone K15 is a synovial fluid autoreactive Vβ19+ CD4+ T cell clone isolated from the inflamed joint of a RA patient. Both, the anti-CD3 and SED-driven T-B co-culture systems led to production of IgG and IgM by B cells from healthy individuals and patients with RA. Equal quantities of IgM-RF were produced by B cells from healthy individuals in the presence of anti-CD3 and SED. However, IgM-RF production was differentially regulated in B cells isolated from patients with RA. In RA B cells only minimal quantities of IgM-RF were produced in the SED-driven system, whereas a marked increase in IgM-RF production was observed in the anti-CD3-driven system. These results indicate that distinct regulatory mechanisms exist in the regulation of RF production in normal subjects and in patients with RA.

II.9. Epitope mapping of human immunoglobulin G Fc portion.

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Rheumatoid factor (RF) is known to be associated with autoimmune diseases, in particular, with rheumatoid arthritis. Nevertheless, its relevance to the pathological autoimmune status is not yet clear. It is not known whether the overall repertoire of the fine epitope specificity of RF differs in healthy people and patients with rheumatoid arthritis and other autoimmune disorders. An epitope map of human IgG Fc portion could be an useful tool to investigate this question. The aim of this work was to construct an epitope map of human IgG Fc, including conformational antigenic determinants. 12 monoclonal antibodies to Fc portion of human IgG molecule were produced. All antibodies reacted with common (non-subclass specific) conformational epitopes, present only on un-
damaged IgG $\gamma$-chains. The cross-reactivity with some xenogenic IgG was also established. We examined the interaction of all possible combinations of antibodies in two-side sandwich and competition ELISA. Based on these data the relative epitope map of conformational determinants was drawn. To localise the exact epitope positions on the three-dimensional model of IgG molecule the competition of each antibody with $S$. aureus protein A (SpA) for IgG binding was studied. We also determined the efficiency of antibody reaction in sandwich ELISA with anti-$\gamma$-chains antibodies, which was supposed to be dependent on the distance between the epitope and the Hinge region. Putting together the results of our experiments and the published data concerning the location of SpA-binding site, subclass-specific amino acids positions and homology regions with xenogenic IgG we have drown an epitope map of IgG $\gamma$-portion and situated it on the three-dimensional model of human IgG.

II.10. Anti-RNP antibodies are associated with lower incidence of renal disease in systemic lupus erythematosus.

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Ninety-eight Finnish SLE patients, who have been followed and treated in Turku University Central Hospital between Jan 1, 1989 and Dec 31, 1991, were studied. All patients fulfilled four or more ARA criteria for SLE. There were 85 female and 13 male patients, their mean age was 43 years, and mean follow-up time 10.7 years. The aim was to find clinical associations of different serum autoantibodies, and their combinations, in SLE.

In these SLE patients, there was a statistically significant association of anti-RNP and anti-Sm antibodies with Raynaud’s phenomenon ($p < 0.001$) and a significant negative association of anti-RNP with renal disease ($p=0.022$). Pericarditis was found more often in patients with anti-SS-B ($p=0.012$) or anti-SS-B+SS-A ($p=0.007$). AIHA and deep vein thrombosis were associated with aCL antibodies ($p=0.014$ and 0.049, respectively).

We conclude that determination of autoantibodies is helpful in evaluation of the risks of many clinical manifestations of SLE. This study confirms the negative association of anti-RNP antibodies with renal disease in SLE.

II.11. The low affinity Fc$\gamma$RII and Fc$\gamma$RIIIb on PMN are differentially regulated by CD45 phosphatase.

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Human polymorphonuclear neutrophils (PMN) play an important role in inflammation and are implicated in pathogenesis of rheumatoid arthritis. Tissue damage in articular joints is due to rheumatoid factor immune complex activation of PMN via Fc$\gamma$ receptors. The multivalent ligation of the constitutively expressed low affinity receptors Fc$\gamma$RII (CD32) and Fc$\gamma$RIIIb (CD16b) leads subsequently to enzyme release and induction of the respiratory burst. Recent studies have indicated that tyrosine phosphorylation precedes other known signals in Fc$\gamma$R signal transduction and can be regulated by CD45. Since protein tyrosine kinases (PTKs) are linked to Fc$\gamma$RII, and glycosylphosphatidylinositol (GPI)-molecules were shown to associate with PTK and CD45, we tested the ability of CD45 to modulate signal transduction through low affinity Fc$\gamma$ receptors on PMN. We employed luminol-dependent chemiluminescence, which directly reflects the activity of the NADPH-oxidase, for determining the respiratory burst. $[Ca^{2+}]_i$ was measured in PMN loaded with the intracellular fluorescent indicator fura-2/AM. Using mAb fragments, engagement of the GPI-linked Fc$\gamma$RIIIb results in a different pattern of $[Ca^{2+}]_i$ flux than that observed after cross-linking Fc$\gamma$RII. Even though each of the two Fc$\gamma$Ris induces comparable increases in $[Ca^{2+}]_i$, induction of the respiratory burst is significantly higher in the case of Fc$\gamma$RIIIb. Pretreatment of PMN with a mAb f (ab')2 fragment recognizing CD45 phosphatase followed by co-cross-linking Fc$\gamma$RII or Fc$\gamma$RIIIb and CD45 suppresses Fc$\gamma$R-induced calcium mobilization in a dose-dependent manner. Engagement of Fc$\gamma$R II together with Fc$\gamma$RIIIb demonstrates a synergistic contribution of PMN activation, but differs in its susceptibility to inhibition by CD45 mAb. While CD45 mAb abolishes Fc$\gamma$RII as well as Fc$\gamma$RIIIb-induced calcium elevation, combined cross-linking of both receptors even with subsaturating concentrations of mAb is not significantly reduced by CD45 mAb. Pretreatment with CD45 has different effects on respiratory burst triggered by Fc$\gamma$RII and Fc$\gamma$RIIIb engagement. Only the Fc$\gamma$RII-stimulated respiratory burst is abolished, whereas the Fc$\gamma$RIIIb-triggered response is almost unaffected despite the lack of $[Ca^{2+}]_i$ mobilization. These data indicate differences in the signal transduction cascade depending on Fc$\gamma$R involvement. The tyrosine phosphatase CD45 exerts different regulatory effects on low affinity Fc$\gamma$ receptors, providing further evidence for an important role of tyrosine phosphorylation in PMN activation. The better understanding of signal transduction pathways initiated by Fc$\gamma$R activation may help to therapeutically influence arthritic processes.

II.12. Antihistone antibodies in SLE: Specificity of Anti-Histone-H1-IgG and their correlation of sequence motives within the C-terminal regions of human H1-subtypes.

S. Kuhn, R. Hoffmann, L. Cebeceauer, W. Rapp and M. Zeppezauer.

Anti-Histone-Antibodies (AHA) from sera of SLE-patients were characterized by means of synthetic peptides within the C-terminal regions of all known human somatic histone H1-subtypes (H1a, H1b, H&c, Hld) by ELISA-technique. 50 per cent of H1-positive sera (H1-PCA-extract from calf thymus) show activities with certain sequences of the tested peptides. All peptides recognized in these tests contain common sequences of the tested peptides. One of these boxes, AAKPK or a variant of it, and the peptide KPAAKAAAKKK were tested successfully in inhibition ELISA tests to examine their ability to bind AHA as free antigens. Additionally the short peptides were coupled with EDC on a carrier protein (BSA) and tested as immobilized antigens.

These motives are considered as potential DNA-binding motives and their CD-spectra show helical structures in TFA/water and by presenting anions or short DNA-sequences. Two of the most interesting sera were selected to purify their IgG-fractions on a protein G affinity column. The purified IgG fractions of both sera show a high activity (but not higher than...
their parent sera) against all peptides recognized by the sera (both diluted 1:250 in 1% BSA).

### II.13. Natural autoantibodies and their physiologic serum inhibitors.

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Autoimmune diseases are characterized by the presence of autoantibodies (AA) from sera of healthy individuals, therefore called natural autoantibodies (NAA).

Objectives: to detect the presence of various NAA in sera and IgG fractions from healthy individuals.

Methods: IgG fractions were purified on Sepharose-anti human-IgG or Protein-A from sera of healthy donors, having no autoantibody activity, as measured by an ELISA assay.

Results: All the IgG fractions, after elution, reacted with a variety of autoantigens (phospholipids, PDH, DNA, thyroglobulin). Competition assay confirmed the specificity of the binding of the purified IgG fractions to their antigens. The binding to the autoantigens was completely inhibited by either whole normal human serum or sera from various animal species. The binding to negatively charged phospholipids was completely inhibited by β2-glycoprotein I.

Conclusions: Our results support the notion of the existence of IgG NAA in healthy individuals.

### II.14. Rheumatoid factor B cells selectively present synovial fluid antigens to T cell clones isolated from inflamed joints of patients with rheumatoid arthritis (RA).

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The histopathological features of rheumatoid joint inflammation suggest that an antigen driven activation of T cells plays a central role in the onset and/or perpetuation of the inflammatory process. However, the disease-associated antigens responsible for the activation of T cells in the joint are unknown. We found that inflamed joints of RA patients harbour CD4⁺ CD8αβ⁺ T cells able to proliferate in a HLA-restricted manner to antigens present in the autologous synovial fluid (SF). Some of these SF-reactive T cell clones responded specifically to autologous and not to allogeneic SF, whereas others revealed responsiveness against a limited number of allogeneic SF samples. This (restricted) specificity of T cells towards autologous antigens is indicative for heterogeneity of the epitopes recognized and argues against ubiquitous joint constituents as the relevant antigens recognized by the SF autoreactive T cells. Accordingly, we could not detect any reactivity of the SF-reactive T cells to ubiquitous antigens like synoviocyte membrane extracts, chondrocyte extracts and the protein calreticulin. To account for the (restricted) patient specificity we have to consider either exogenous antigens or polymorphic antigens as the relevant antigens. No reactivity was observed with exogenous antigens such as bacterial proteoglycans and Mycobacterium tuberculosis. Furthermore, we were not able to find responsiveness in the presence of (polymorphic) IgG purified from autologous SF. However, a role for an IgG-binding protein could not be excluded. Therefore, we tested the antigen presenting capacity of rheumatoid factor positive (RF⁺) versus RF-EBV transformed B cell lines isolated from the peripheral blood of RA patients. We found that RF⁺ B cells were capable of efficient presentation of SF antigens towards SF reactive T-cells, whereas the nonspecific B cells were less effective. This result suggests that the relevant antigen is selectively captured by the RF⁺ B cells and therefore could be present in the SF in the form of an immune complex. Hence, the presence of polymorphic molecules such as (auto)antibodies could facilitate efficient presentation of ubiquitous (auto)antigen and serve an indirect role in T cell responsiveness.

### II.15. Is Cathepsin D an autoantigen recognized in patients with rheumatoid arthritis?

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Collagen type II is regarded as a possible autoantigen in rheumatoid arthritis (RA). However, attempts to clone T cells responsive to human collagen type II frequently lead to the establishment of T cell clones responding to porcine pepgin, which is present in collagen preparations as a contaminant. Recently we showed that a pepsin-reactive T cell clone established from a patient suffering from mixed connective tissue disease was autoreactive towards human cathepsin D, presented as an autoantigen by macrophages. We now analyse the relevance of cathepsin D as autoantigen in patients with RA. Cathepsin D is an aspartic protease with 65% homology to porcine pepgin. It is a lysosomal enzyme involved in the degradation of proteins and in antigen processing. We detected cathepsin D in synovial fluids of RA patients by western blots using rabbit antibodies specific for human cathepsin D. These antibodies also recognized cathepsin D in the cytoplasm of ethanol fixed human granulocytes as demonstrated by indirect immunofluorescence. Further experiments indicate the presence of T and B cell responses to cathepsin D in RA patients.

### II.16. Antinuclear antibodies in an adult population sample.

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Among tissue autoantibodies detected routinely by indirect immunofluorescence test, antibodies to nuclear antigens represent the most common finding in different unselected study groups. As rule, the incidental finding of low titer antinuclear antibodies (ANA) is not further evaluated and the antigenic specificities are not characterized. In the present investigation we put the aim to study the ANA incidence in an Estonian adult population sample and to characterize the ANA specificities revealed. All 1745 inhabitants at 15 years or older from a village Karksi-Nuia were asked to donate the venous blood for antibody studies. A total of 1461 (84%) persons were investigated using indirect immunofluorescence ANA test with rat kidney and liver as antigen substrates (serum dilution 1:10). Our results showed
that 51 persons (44 females) had strong ANA reaction. Sera from all but one of these 51 persons were further studied by indirect immunofluorescence test using ANA test procedure on HEp-2 cell substrate from Immuno Concepts (Sacramento, CA). Thirty three persons had ANA reaction on HEp-2 cell substrate at the serum dilution 1:10, whereas only 8 sera were shown to contain antibodies at dilution 1:40 which is suggested for screening by Immuno Concepts. The next reactivity patterns were revealed: nucleolar - 2; speckled - 3; homogeneous - 3 (including 2 with antibodies to dsDNA). No autoimmune diseases were diagnosed so far in 7 persons. A 49-year-old lady with nucleolar ANA had rheumatoid arthritis. Thus, our results showed the higher specificity of HEp-2 assay for ANA detection if compared to ANA assay with rodent tissues and showed that although more specific, still HEp-2 assay reveal ANA without clinical evidence of systemic autoimmune disease. Whether the antigenic targets for ANA reactivities are similar to that in systemic autoimmune diseases should be further studied.

II.17. Identification of antinuclear and antinucleolar antibodies in patients with progressive systemic sclerosis (PSS) / polymyositis (PM) overlap syndrome.

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Introduction: Progressive systemic sclerosis (PSS) may be associated with an asymptomatic increase of muscle enzyme levels (CPK and aldolases). However muscle weakness and/or EMG or histopathological alterations are seldom associated with criteria for PSS. This subset of patients define a PSS/PM overlap syndrome.

Objective: The purpose of this study was to identify the different antinuclear autoantibodies in sera from patients with the PSS/PM overlap syndrome and to correlate these findings with the prognosis of this subset of PSS.

Patients and methods: Clinical and biological charts of a series of 220 patients with PSS were checked and a subgroup of 18 patients (14 F; 4 M) with PSS/PM overlap syndrome was isolated. These 18 sera were tested for ANA by both indirect immuno-fluoresence test using ANA test procedure (CPK and aldolases). However muscle weakness and/or EMG or histopathological alterations are seldom associated with criteria for PSS. This subset of patients define a PSS/PM overlap syndrome.

Results: Antinuclear antibodies were present in 13/18 samples (78 p. cent) with two major patterns, nucleolar (n=7), homogeneous (n=5) and one minor pattern: speckled (n=1). Autoantibodies to defined antigens were identified with WB in 8/13 cases: 2 are anti-U1 RNP ab (11 p. cent), associated with anti-SSB ab in case 1, one is anti-KU ab (5.5 p. cent), 5 are anti-Pm/Scl ab (28 p. cent), and in 3 cases antibodies to unidentified specificities were detected (16.5 p. cent). A total of 11/18 sera (61 p. cent) were positive for ANA using WB. Among sera with a negative IIF test for ANA were identified antigens (30 kDa + 40 kDa) and one gave a negative WB test. Two of five sera with a negative IIF test for ANA were positive for WB test: anti-JO1 ab (1 case) or unidentified specificities (60 kDa + 65 kDa 1 case).

Conclusion: The most prevalent ANA among patients with a PSS/PM overlap syndrome correspond to anti-Pm/Scl antibodies specific for a nucleolar antigen. The presence of anti-Pm/Scl antibodies is correlated with a rather good prognosis among patients with PSS.

II.18. Complete congenital heart block is associated with increased autoantibody titers against calreticulin.

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Neonatal lupus erythematosus (NLE) is a rare syndrome, which was first described as a distinct clinical and pathological entity in 1954 by MCViustion and Schoch. The potentially severe and permanent manifestation of NLE, congenital complete heart block (CCHB), was first recognised in 1945 by Plant and Steven. CCHB is associated with the presence of anti-Ro/SS-A antibodies. Now it is clear that calreticulin, a calcium binding glycoprotein of the endoplasmic reticulum with C-terminal KDEL-sequence, is not part of the Ro/SS-A ribonucleoprotein complex. In this study anti-calreticulin autoantibody responses in serum samples from sixteen infants with CCHB and in a control group were analysed. Calreticulin from human placenta was purified according to Van Nguyen et al. (J. Biol. Chem. 264, 1989, 17494-17501). Specific enzyme-linked immunosorbent assays and Western-blot analysis were performed. ELISA: The native Calreticulin (5 mg/ml) in 34 mM Na2CO3 - 16 mM NaHCO3, pH 10.6 was added to 96 well polystyrol microtiter plates (50 ng/well) and ELISA was performed as previously described (Eur. J. Clin. Invest. 24, 1994, 248-257). Seven of the sixteen sera with CCHB contained IgG-antibodies. Three sera of those with IgG-antibodies had also IgM-antibodies. One serum contained anti-calreticulin-IgM-antibodies only. In the non-CCHB group there was one serum with IgG and one serum with IgM-antibodies. When calreticulin was processed by SDS-PAGE and immunoblotted onto nitrocellulose, neither sera of the CCHB or non-CCHB nor a control sera showed a positive reaction with calreticulin (sera dilution 1:200). Calreticulin was more a molecular chaperon function than involvement in calcium storage, but anticalreticulin antibodies might be involved in development of some but not all cases of CCHB. The finding of IgM autoantibodies does not support the hypothesis of a passively acquired autoimmune disease of CCHB.

II.19. Fine specificity and quantity of anti-Ro antibodies in 31 prospectively followed patients with SLE and SJS.

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Objective: We studied 31 consecutive anti-Ro positive systemic lupus erythematosus (SLE) and Sjögren syndrome (SJS) patients to assess the possible clinical impact of a change in the fine specificity and/or titer of anti-Ro antibodies.

Methods: Thirty-one consecutive anti-Ro positive patients, 12 with SLE, 15 with primary SJS and 4 patients with SLE and secondary SJS, were subjected to clinical and laboratory examinations every 3 months. SLE activity was determined by ECLAM index, and extraglandular manifestations were used as
criteria for Sjögren syndrome’s activity. Western blot (IB) was used to determine the anti-Ro60 and anti-Ro52 specificities, and counter-immunoelectrophoresis for anti-Ro titers. All collected sera were stored and tested at the same time to avoid interassay discrepancies.

Results: The titers of anti Ro antibodies were significantly lower (p < 0.05) and less constant in SLE sera than in pSJS sera. Significant rise in anti-Ro titer, preceding the relaps of the disease was observed only in two patients with high titers of anti-Ro antibodies: renal involvement in one SLE patient and leukopenia in one SJS patient. The significant fall in anti-Ro titer as a consequence of steroid therapy was found only in one patient with primary SJS. Anti-Ro60 occurred in all 19 SJS patients (primary and secondary), but anti-Ro52 was present at the same time in only 7 of them. IB analysis of all follow-up samples from SLE patients showed the presence of anti-Ro60 alone in 2, anti-Ro52 alone in 3, but 3 sera, although CIE positive, were persistantly IB negative. The shift from anti-Ro52 to anti-Ro60 was found only in one SLE patient without any evident clinical association. In one SLE patient anti-Ro52 antibodies arisen after stopping of cyclophosphamide therapy.

Conclusions: The change in anti-Ro titers and/or anti-Ro60/ anti-Ro52 specificity during the two years follow-up study of SLE and SJS patients showed only very rare shifts or change in firstly detected specificity of anti-Ro associated with certain clinical observation. This was through also for the raise and fall of anti-Ro titers which followed the specific disease course in only 3/31 cases.

II.20. Possible predictive value of ELISPOT assay for the production of IgG anti-C1q in SLE nephritis.
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Anti-C1q antibodies are commonly found in SLE sera, and their occurrence associates with nephritis development. To investigate the possibility that an active process in peripheral blood (ongoing anti-C1q antibody production) would mirror a likewise active process in the kidney (biopsy verified nephritis), we evaluated the anti-C1q ELISPOT method as a marker of active kidney involvement in SLE in a pilot study.

Methods: Fourteen female patients with active SLE were investigated for the presence of anti-C1q sport forming cells (SFC) in peripheral blood. All patients were untreated or treated with low doses of corticosteroids at the time of blood sampling. IgG, IgA, and IgM anti-C1q SFC were enumerated, together with total number of Ig-producing cells.

Results: Seven patients had biopsy verified nephritis at the time of blood sampling. Six out of the remaining patients had active SLE based on clinical and laboratory parameters, but no evidence of renal involvement. High numbers of anti-C1q SFC were detected in 4/7 nephritic patients, all of whom had active proliferative glomerulonephritis (GN) WHO class III/IV. The three remaining nephritic patients showed low numbers of anti-C1q SFC. Two of these had membranous GN without active histopathological findings. One patient with a low number of IgA anti-C1q SFC had proliferative nephritis WHO class IV. Mean number of anti-C1q SFC were 47 (range 9-95) in patients with proliferative nephritis. In the non-nephritic group, 6/7 patients had no or low numbers of anti-C1q SFC. The seventh patient had pronounced hematuria and slight proteinuria at the time of investigation; kidney biopsy was never performed. Mean number of anti-C1q SFC in patients with no signs of renal involvement was 3.5 (0-11).

Conclusion: Ongoing IgG anti-C1q production may be a marker of active renal disease in SLE. These data support the idea that detection of an active process in the circulation may be used as a mirror of an ongoing active pathological process in a distant target organ.

II.21. Mi-2 autoantigen - A Novel 218 kD helicase encoded on chromosome 12.
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The IgG fraction of a serum positive for anti-Mi-2 in agaragel double diffusion (anti-Mi-2 reference sera were kindly provided by I.N. Targoff, Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City) of a patient suffering from dermatomyositis (DM) was used to screen a HeLa cDNA expression library. Alignment of the DNA sequences of positive clones from these experiments and those obtained by RACE protocols revealed a 6417 bp long cDNA encoding a 217,989 D protein of 1912 aa which is structurally related to the family of helicases. A polyA+ RNA from HeLa and HEp-2 cells of about 8 kb in length hybridized with the cDNA probe in Northern blots. The encoding gene was localized on chromosome 12p by FISH technique.

A 56 kD recombinant protein fragment (rMi-2) was synthesized in E. coli and was used for antibody production in rabbits and as antigen in ELISA and immunoblot techniques for screening of human sera. Affinity purified rabbit antibodies against rMi-2 and the human anti-Mi-2 antibodies showed an indistinguishable nuclear immuno-fluorescence pattern, immunoprecipitated the same protein from 32S-methionine-labeled HEp-2 cell extracts, and reacted with an identical, SDS-PAGE separated and blotted natural protein of a somewhat larger size (about 250 kD) compared to the predicted molecular weight. Twelve sera positive for anti-Mi-2 in agaragel double diffusion from well-documented DM patients all recognized rMi-2 in ELISA and immunoblots. No reaction with rMi-2 was seen in 234 sera of 94 SLE patients (provided from the German multicenter SLE study), in 30 sera of patients with rheumatoid arthritis, and 188 healthy control persons. Of 901 ANA positive sera (HEp-2 cells, titer > 1:80) five showed a borderline and three a positive reaction with rMi-2. The data suggest that the main target of anti-Mi-2 antibodies represents a new helicase.

II.22. The rheumatoid arthritis-specific ‘antikeratin antibodies’ and antiperinuclear factor are the same autoantibodies directed to filaggrin.
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The so-called ‘antikeratin antibodies’ (‘AKA’) and the antiperinuclear factor (APF) are the most specific serological mark-
ers of rheumatoid arthritis (RA). Using indirect immunofluorescence, 'AKA' label the stratum corneum of various cornified epithelia and APF the keratohyalin granules of human jugal (cheek buccal mucosa) epithelium. We recently demonstrated that 'AKA' recognize human epidermal filagrin.

Here, we report the identification of the major APF antigen as a diffuse protein band of 200-400 kD. This protein is seen to be closely related to human epidermal (pro)filaggrin since it was recognized by 4 antifilaggrin mAbs specific for different epitopes, and since the APF titer of RA sera was strongly correlated to their AKATA titer and related to their immunoblotting reactivity to filaggrin. Immunoabsorption of RA sera on purified epidermal filaggrin resulted in the abolition of their reactivities to the keratohyalin granules of jugal epithelial cells and to the 200-400 kD antigen. Moreover, antifilaggrin autoantibodies, i.e. 'AKA', affinity-purified from RA sera, were shown to immunodetect the 200-400 kD antigen and to stain the jugal keratohyalin granules.

These results indicate that 'AKA' and APF are largely the same autoantibodies. They essentially recognize human epidermal filaggrin and (pro)filaggrin-related proteins of jugal epithelium. Identification of the epitopes recognized by these autoantibodies, which we propose to name antifilaggrin autoantibodies (AFA), will certainly open new paths of research into the pathophysiology of RA and will perhaps provide information on its etiology.

II.23. Immune tolerance to T cell determinants from autoantibodies to DNA delays development of murine lupus.

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In systemic lupus erythematosus (SLE), Th cells have been shown to upregulate production of anti-DNA antibodies (Ab), subsets of which cause nephritis. How are these Th cells activated and to which determinants? Here, we have focused on the interaction between Ig-derived peptides and T cells, and their potential involvement in disease pathogenesis in a murine SLE model, (NZB x NZW) F1 (BWF1) mice. We have found that prior to the onset of clinical disease, BWF1 (but not MHC-matched normal mice) develop spontaneous T cell autoimmunity to V<sub>H</sub> region determinants of syngeneic anti-DNA monoclonal autoantibodies (mAb), but not to peptides from a mAb to a foreign antigen. These Ig peptides bind to MHC class II molecules and activate different Th subsets: A6H p34 binds I-E<sup>δ</sup> and elicits a Th0 (or Th1 + Th2) cytokine pattern; A6H p58 binds I-E<sup>δ</sup> and I-E<sup>ε</sup> and induces Th2 cytokine secretion; and A6H p84 binds I-A<sup>ε</sup> and elicits Th1 pattern. Immunization with peptides in adjuvant or adoptive transfer of a peptide-specific T cell line in young BWF1 mice upregulates anti-DNA Ab production and clinical disease. To determine the effect of tolerance induction to these Ig determinants, we administered 300ug to 1 mg of individual peptides, either singly or in combinations, to prenephritic BWF1 mice. We found that intravenous treatment with a combination, to prenephritic BWF1 mice. We found that intravenous treatment with a combination of determinants prolonged survival (p < 0.05), delayed the onset of nephritis (p < 0.05) and decreased serum anti-DNA (p < 0.01) compared to BWF1 mice treated with control peptides. This beneficial clinical effect was associated with profound unresponsiveness in peptide-specific T cell proliferation and peptide-specific Ab production. Thus, in SLE, the presence of autoreactive T cells against V region determinants of autoantibodies may represent an important mechanism initiating/sustaining development of disease, perhaps using the mechanism of determinant spreading. Immune tolerance of Th1 and Th2 cells to such determinants delays development of autoimmunity in murine lupus. The effect of tolerance to these determinants in established disease is being examined.

II.24. Anti-RA33 autoantibodies recognize major epitopes in the RNA binding region of hnRNP-A2 (RA33).

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The A2 protein of the heterogeneous nuclear ribonucleoprotein (hnRNP-A2) is a nucleic acid binding protein of 341 amino acids. Its N-terminal half consists of two adjacent RNA binding domains (RBD I and RBD II), the C-terminal half contains a long glycine rich "auxiliary" domain. This protein is targeted by anti-RA33 autoantibodies from patients with RA, SLE and MCTD.

For epitope mapping studies truncated versions of recombinant hnRNP-A2 (RA33) were generated and their antigenicity tested by immunoblotting employing 31 sera from anti-RA33 positive patients with RA (n=12), SLE (n=10), and MCTD (n=9). All but one serum recognized a fragment terminating at amino acid 212, and 24 sera were also reactive with a fragment terminating at position 182 which comprised both RBDs but lacked glycine-rich sequences. Removal of RBD I from this fragment did not affect binding of most RA and SLE sera whereas 6 MCTD sera became non reactive. However, even small deletions at both ends of RBD II had strong effects on binding of all other sera: Thus, a fragment terminating at amino acid 170 was recognized by 8 sera only (2 RA, 2 SLE, 4 MCTD) and N-terminal deletions of RBD II completely abolished reactivity of all sera. In additional experiments, the reported binding preference of A2 for the oligonucleotide (UUAGGG), was investigated. These studies showed that the complete RBD II was required for binding of this sequence. However, inhibition of oligonucleotide binding by autoantibodies could not be observed.

Taken together, sequences at both ends of RBD II seem to be indispensable for binding of both nucleic acids and autoantibodies, particularly for those of RA and SLE patients. Antibodies from most MCTD patients require RBD I specific sequence. Anti-A2/RA33 antibodies seem to be directed to structural epitopes located in functionally important regions of the antigen.

II.25. No evidence for an independent role of anti-heparan sulfate reactivity in anti-DNA associated lupus nephritis.

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The presence of anti-heparan sulfate (HS) reactivity in serum is closely related to the occurrence of nephritis in patients with
were tested for anti-DNA (Farr assay) and anti-HS reactivity. In addition, we tested single serum samples of another 24 patients obtained during a renal disease exacerbation and 22 sera of patients without nephritis. The sera of all patients were tested for anti-DNA (Farr assay) and anti-HS reactivity (ELISA). We confirmed that SLE patients during renal exacerbations have a significantly higher anti-HS reactivity than patients without nephritis (p < 0.003). In addition, patients with nephritis also had higher titers of anti-DNA antibodies during renal exacerbations than during non-renal exacerbations (p < 0.01). A correlation between anti-DNA and anti-HS reactivity was observed (r=0.40, p < 0.02) which in itself explains the correlation between nephritis and anti-HS reactivity. Comparing sera from nephritis and non-nephritis patients matched for anti-DNA titer, we found no difference in anti-HS reactivity, and therefore must conclude that the anti-HS reactivity is a direct reflection of anti-DNA reactivity.

II.26. The IgG binding site of human FcγRIIIB receptor involves C' and FG loops of the membrane-proximal domain. A. Tamm, A. Kister*, R.E. Schmidt. Department of Immunology, Hannover Medical School, 30625 Hannover, Germany; *Laboratory of Immunobiology, Dana Farber Cancer Institute, Boston, MA, USA.

The low affinity Fcγ receptors are believed to play an important role in the pathogenesis of rheumatoid arthritis and leukocytoclastic vasculitis through their interaction with immune complexes. FcγRIII (CD16) and FcγRII (CD32) belong to the immunoglobulin superfamily and bind the Fe part of IgG. To define the conformational sites on human FcγRIIIB essential for the interaction with IgG, chimeric FcγRIIIB/FcεRI receptors were constructed. The amino acid sequence of the human high affinity receptor for IgE, FceRI, reveals high homology (41% of the extracellular part) with FcγRIIIB, but FceRI does not interact with IgG. Thus, the regions of potential β-turns as the more probable determinants for binding and, subsequently, single amino acids on the second Ig-like domain of FcγRIIIB, were replaced with the equivalent residues on FcεRI sequence in order to diminish the IgG-binding capacity of the chimeras. 293 cells transfected with chimeric receptors were subjected to binding with hIgG1 and four residues were found to be involved in interactions with IgG. The putative three-dimensional model of the membrane-proximal domain of FcγRIIIB demonstrates these amino acids on the loop connecting C' and C" β-sheets (Gln 126), on F β-sheet (Arg 156) and on FG loop (Lys 162, Val 164) which are conformationally located on the same surface of the domain. Lys 162 and Val 164 are also recognized by the ligand-binding inhibitory monoclonal antibody 3G8. Understanding the molecular basis of interactions of Fcγ receptors with immunoglobulins may provide new possibilities for peptide treatment by blocking receptor – ligand interaction.

II.27. Detection and identification of Ro60kD and La48kD disease specific epitopes: performance using synthetic peptides. A.G. Tzioufas1, J.G. Routsias2, Efthimia Giannaki2, Mary Sakarellos-Daitiotsi2, C. Sakarellos2, H.M. Moutsopoulos1. 1Dept of Pathophysiology, Medical School, Univ. of Athens and 2Laboratory of Organic Chemistry, University of Ioanna-Greece.

Sera from patients with systemic autoimmune disease, often contain autoantibodies against Ro/SSA and/or La/SSB ribonucleoprotein particles, composed of small RNA's in conjunction with at least 3 protein components: Ro52kD, Ro60kD and La 48kD. In the present study the linear antigenic epitopes of both Ro60kD and La 48kD antigens were investigated, using overlapping 22 mer and 20 mer peptides respectively, covering the entire sequence of the autoantigens, according to the multipin epitope mapping strategy (Geysen's method). IgG was purified by protein A Sepharose from 10 systemic lupus erythematosus patients sera (SLE), 10 primary Sjogren's syndrome sera (primary SS) and 7 normal blood donors. Autoantibody specificity was tested in all sera by counterimmunoelectrophoresis, ELISA and Western blot. 5 sera (4 from SLE and 1 from primary SS patients) contained only antibodies to Ro60kD, 4 sera (all from primary SS patients) had autoantibodies to both Ro60kD and La 48kD and 9 sera (5 from SLE and 4 from primary SS) had autoantibodies directed against La 48kD and anti Ro52kD and/or Ro60kD. Two sera, 1 from primary SS with anti Ro52kD and 1 from SLE with anti Ro52kD and anti Ro60kD were also tested as disease controls for Ro60kD and La 48kD epitope mapping respectively. All IgG fractions were tested against the synthetic peptides by ELISA. It was found that IgG from patients with SLE preferentially recognize the 169-190 segments of Ro60kD protein and the 145-164 and 289-308 segments of the La protein. In contrast IgG from primary SS patients reacted selectively, with the 211-232 protein of Ro60kD and 311-320 and 349-368 fragments of La protein.

Restriction of the epitopes was performed by aminoacid subtraction from both sides of the initial active fragments. The exact length of the epitopes and their sequence are appeared in the table.

| SLE epitopes | Primary SS epitopes |
|-------------|---------------------|
| Ro60kD 175-185 aa (NGNSKHDLLEF) | 220-232 aa (VETEKLKYLEAV) |
| La 48kD 147-154 aa (HGAFKGSI) | 310-318 aa (VTVEVEGVEKEKLKI) |
| 291-302 aa (NGNLQRKEVT) | 349-364 aa (GSGKGVQFGKKTTF) |

In conclusion, anti Ro60kD and anti La 48 kD antibodies from SLE and primary SS sera, are directed against different linear epitopes on these molecules. The diagnostic significance and biologic role of these epitopes are under investigation.
II.28. Characterization of human variable immunoglobulin domains directed to U1RNA-associated autoantigens, selected from a synthetic- and patient-derived combinatorial library.

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Patients with SLE and SLE-overlap syndromes often produce autoantibodies directed to U1RNA-associated proteins [1]. In this study we characterized human antibody fragments to U1RNA-associated autoantigens selected from a synthetic combinatorial library and a patient-derived combinatorial spleen library. Using phage technology, autoantigen binding Fab fragments were isolated from a large human synthetic combinatorial Fab library (titer $6.5 \times 10^9$), containing all human heavy and light chain germline genes with synthetic random CDR3 domains [2]. From this library three different Fab clones (A1, A2 and A3) directed to the UIRNA-associated A protein (U1A) and one Fab clone (C1) directed to the U1RNA-associated C protein (U1C) were selected. The first combinatorial library derived from a spleen of an autoimmune patient was also screened with the U1A and U1C autoantigens. A single-chain variable fragment (scFv) against the U1A protein (spA1) was selected from this library. All VH and VL genes were sequenced and germline gene usage was determined. The isolated Fabs and scFv react specifically with recombinant proteins in ELISA and on Western blot with U1A contained in a HeLa total nuclear extract. Using protein-A-coupled Fabs A1, A3 and scFv spA1, we were able to precipitate U1 ribonucleoprotein complexes (U1RNP) out of a $35S$-methionine labeled HeLa nuclear extract. Epitope mapping was performed with in vitro translated wt and N- or C-terminal truncated proteins. In case of U1A protein two different epitopes are recognised by different clones. Clone A2 recognises the C-terminal end of U1A (aa 203-282) and cross-reacts with a U2RNA-associated autoantigen, U2B$, which is $86\%$ identical with the U1A protein in its C-terminal domain. The other two anti-U1A Fabs, A1 and A3, as well as the scFv spA1, need also a more central domain of U1A (aa 118-282) for their recognition, and appear not to cross-react with U2B$^\text{a}$. In competition-ELISA assays, anti-U1RNP patient sera were able to compete with Fabs as well as scFv on binding to U1A. The heavy chain of the anti-U1A spA1 clone uses the germ-line gene DP65, and has 8 somatic mutations randomly distributed throughout the variable region. Two out of the three anti-U1A Fabs (A1 and A3) use the same germ-line gene for their heavy chain, and indeed, appear to recognise the same epitope on U1A protein. In normal individuals, this DP65 gene (VH4 family) is only used in about $1\%$ of the heavy chain sequences. This suggests that, in case of the U1A protein, this synthetic library mimics the in vivo autoantibody repertoire.

1. Klein Gunnewiek J.M.T., W.J. van Venrooij. Manual of biological markers of disease (eds. W.J. van Venrooij & R.N. Maini), B3.1, 1-20 (1994) ISBN 0-7923-2219-3.
2. Griffiths A.D. et al. EMBO J. 13, 3245 (1994).

II.29. IgM anti-U1RNP antibodies characterize patients with systemic lupus erythematosus.

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Anti-U1RNP antibodies occur in patients with mixed connective tissue disease (MCTD), systemic sclerosis (SSc), systemic lupus erythematosus (SLE) and other ill defined connective tissue disease syndromes. The isotopic specificity of these antibodies and the association with the disease status has not been extensively studied.

Purpose: To study the isotopic expression of anti-U1RNP antibodies in SLE and MCTD.

Methods: Sequential sera of patients positive for anti-U1RNP antibodies by counter immuno-electrophoresis (CIE) (31 with SLE, 36 with MCTD) were tested for IgG and IgM anti-U1RNP antigen by ELISA using affinity purified antigen. Since the U1RNP antigen contained also Sm antigenic determinants the ELISAs's results were confirmed by RNA precipitation. IgG RNA precipitation was carried out by coupling the IgG after incubation of the sera with nuclear Hela cellular extracts to protein -A-sepharose (pAS) coated particles. IgM RNA precipitation was carried out after removing the whole IgG of the serum by two cycles of pAS precipitation and then coupling the IgM-anti-U1RNP to protein A-sepharose particles using as a ligand a rabbit IgG with anti-human IgM activity. The presence of human IgM and the absence of human IgG from the third fragment was confirmed by SDS-polyacrylamide gel electrophoresis and Western Blotting using anti-human IgG and anti-human IgM alkaline phosphatase conjugated sera. The RNAs were electrophorised in 10.5% acrylamide - bisacrylamide gels and stained with ethidium bromide.

Results: All sera were positive for IgG-anti-U1RNP, with 12 of the 36 MCTD and 21 of the 31 SLE patients possessed IgM anti-U1RNP (p < 0.001). Sequential sera samples were tested from 23 MCTD and 17 MCTD patients (a mean of one sample per patient per year for a period of 4 years). The IgM anti-U1RNP reactivity was found in the 2 of the 17 MCTD and 17 of the 23 SLE-patients (p < 0.0001).

IgG RNA precipitation indicated that all the sera precipitated U1RNP's 11% of the MCTD sera, precipitated also U2, U4, U5, U6 RNAs. IgM RNA precipitation indicated that the IgM from MCTD sera do not precipitate URNAs, while in 50% of SLE sera IgM RNA precipitation indicated that the IgM from SLE-patients precipitated Sm antigenic determinants. Concordance between ELISA and RNA precipitation was 80%.

Conclusion: IgM-anti-U1RNP antibodies characterize patients with SLE, while IgG-anti-U1RNP characterize patients with MCTD.

II.30. Anti-Fcγ receptor activity in primary Sjögren's syndrome.

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Fc-gamma receptor (FcγRIII) is expressed as a phosphatidyl inositol-anchored protein in polymorphonuclear leukocytes (PMN) and constitutes a class of receptors structurally distinct from FcγRI and FcγRII. Anti-FcγR autoantibodies might
account for the defective FcγR-mediated clearance by the reticuloendothelial system as described in patients with primary Sjögren’s syndrome (pSS). The sera from 66 patients with pSS were examined for the presence of such autoantibodies by an enzyme-linked immunosorbent assay (ELISA), using recombinant human FcγRII as the substrate. Cell-free FcyRII was also detected by an ELISA. Anti-FcγRII autoantibodies were found in 28 of the patients (33%). Twenty-three of these sera did not react with autologous PMN nor NA1+/NA2-/NB1+, NA1-/NA2+/NB1+, and NA1+/NA2+/NB1-cells from normal volunteers, whereas they bound to FcyRII eluted from the same PMN in ELISA and Western blotting. The fine specificity of autoantibodies from 10 sera was established by inhibition with a preparation of FcγRII plus FcγRII, and two ELISAs using FcγRI and FcγRII as the substrates, respectively: autoantibodies from one serum recognized the three receptors, from six with FcγRII in addition to FcγRIII, and from three sera were specific for the latter receptor. None of these sera reacted with FcγRII- and FcγRII-carrying cells. Cell-free FcyRII b (from PMNs), but negligible amounts of FcyRIIa (from Natural Killer cells), were detectable in the patient sera. The membrane expression of CD15, an early activation marker of PMN, was diminished, while that of three PMN late activation markers was markedly enhanced. Taken together, these results suggest that autoantibodies are produced following the shedding of FcyRIIb upon RMN activation. A credible candidate for this activation is IgG-containing immune complexes (IC). Binding of autoantibodies and/or cell-free FcyRII to IC may prevent their capture by the reticuloendothelial system.

II.31. Neurological dysfunctions associated with anti-phospholipid antibodies (aPL).

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aPL Abs have been associated with various neurological dysfunctions in patients with the anti-phospholipid syndrome (APS), secondary to lupus, or as a primary syndrome (PAPS). The direct pathogenic role of these antibodies in neurologic impairment is still under investigation. An experimental model of APS was established in BALB/c mice (J Clin Invest 1992, 89, 1558-1563) and in C5HeB mice. Mice immunized with a pathogenic monoclonal human anti-phospholipid antibody (H-3), developed high titers of aPL Abs in their sera, 2-5 month after injection. The aPL titers were evaluated by enzyme-linked immunoassay (ELISA). A series of neurological reflexes for rodents (Acta Neurobiol Exp 1980, 40, 999-1003) were tested five months after APS induction. An open field system was used for behaviour assessment of the mice. Mice induced with APS developed high titers of aPL in the sera, mean O.D. ±2SD 1.73±0.15, compared to 0.025±0.01 for control mice immunized with irrelevant antibody. Clinically, thrombocytopenia and fatal resorptions were found in the former group. Significant neurological dysfunctions were also demonstrated in the APS mice. Placing reflex, which examined vision was performed with 33% accuracy, (p<0.001), postural reflex – 60%, (p<0.05), grip test – 73% (p=0.05) in comparison to 100% in control mice. In preliminary tests these mice exhibited changes in locomotion parameters in an open field system.

Conclusions: High aPL serum levels correlate with neurological impairment in the experimental model of APS mice.

II.32. Renal involvement in antiphospholipid syndrome - description of six patients and induction of renal injury in acid mice.

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Renal involvement in antiphospholipid syndrome (APLS) both clinically and experimentally by inducing the lesion in SCID mice via transfer of lymphocytes from a patient. We present here six patients with APS and renal involvement. Primary APS associated renal lesions were induced in SCID mice by transferring peripheral blood lymphocytes (PBLs) from one of the patients with primary APS and membranous glomerulopathy to SCID mice.

The clinical manifestations of the six patients with APS and renal involvement entail hypertension (n=4), acute renal failure (n=1), chronic renal failure (n=3) and proteinuria (n=6). Renal biopsy was performed in five of the six patients. In three of them, typical thrombotic microangiopathy was demonstrated and in two, pathological changes resembling membranous glomerulopathy were disclosed. The latter lesions were not reported so far in primary APS. The SCID mice transferred with PBLs of one of the patients developed anti-phospholipid antibodies (aPL) and renal lesions reminiscent of those of the patient.

This group of patients with APS with renal involvement illustrate the diversity of the clinical symptoms and pathological abnormalities seen in this syndrome. The successful induction of renal injury in the SCID mice reinforces the apparent role of aPS in the pathogenesis of the renal involvement.

II.33. Libman sacks endocarditis: the possible pathogenic role of anti-cardiolipin antibodies deposited at the valve subendothelium.

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Background: The Libman-Sacks non-bacterial endocarditis was described in patients with Systemic Lupus Erythematosus (SLE) to denote valvular deformities in the heart. The cardiac dysfunction may require valve replacement. Recently, an association between valvular defects and the presence of anti-phospholipid antibodies (aPL) in serum was confirmed. The aim of
this study was to evaluate the pathogenetic role of anti-cardiolipin antibodies (aCL) in Libman-Sacks endocarditis.

Methods: The deposition of aCL and complement components in the valves was ascertained by immunoperoxidase staining employing direct binding assays with anti-pathogenic idiotype of aCL and competition assays. Aortic, tricuspid and mitral valves from patients with primary and secondary anti-phospholipid syndrome (APS) to SLE were analyzed.

Results: A linear subendothelial deposition of aCL, C1q and C3 components were demonstrated in valves from patients with APS. No deposition was noted in the control valves derived from non-related conditions (atherosclerosis, subacute bacterial endocarditis - SBE), or valves with similar histopathological findings from patients without aPL.

Conclusions: The deposition of anti-cardiolipin antibodies with complement components may contribute to the deformities reported in the Libman-Sacks endocarditis. It is still unknown if this subendothelial deposition is a primary event due to a specific presentation of phospholipids in the valves or secondary to an additional insult.

Session III: Reactive arthritis and Lyme disease.

III.1. Examination of sacroiliac biopsies from ankylosing spondylitis patients with immunohistologic and in situ hybridisation techniques.

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To investigate mechanisms involved in inflammation and new bone formation in the sacroiliitis pathogonomonic for patients with ankylosing spondylitis (AS) we performed biopsies of the sacroiliac joints assisted by computed tomography. We examined 5 AS patients with a mean disease duration of 4.5±2.1 years and a radiographic stage II-III. Immunohistology was performed with the APAAP technique and cytokine mRNA was detected by in situ hybridisation.

Dense cellular infiltrates with varying amounts of CD3+ (48.4% ± 23.6%), CD4+ (29.7% ± 17.6%), CD8+ (15.8% ± 11.4%), CD14+ (23.6% ± 16.9%), CD45RO+ (53.3% ± 24.1%), and CD45RA+ (4.5% ± 2.9%) cells were found in the synovial part of the sacroiliac joints of all 5 patients. In these infiltrates a high amount of TNF-α mRNA and, close to the site of new bone formation, a lower amount of TGF-β mRNA was detected while no message for IL-1 was found in the 3 patients examined with this technique. In 2 patients examined a high HLA B27 expression was observed in the inflamed sacroiliac joint.

In conclusion, this study shows T cells and macrophages in cellular infiltrates in the sacroiliac joints of 5 patients with active AS. The demonstration of abundant TNF-α message in these joints could have immunotherapeutic implications for AS patients. TGF-β might be involved in new bone formation in AS. Dr. Braun and Dr. Sieper are supported by grants from Stifterverband der deutschen Wissenschaften and from Bundesministerium für Forschung und Technologie, Germany.

III.2. Presence of bacterial flora derived antigen in synovial tissue macrophages and dendritic cells.

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Rheumatoid arthritis (RA) is considered as a T cell mediated reaction against an unknown indigenous or exogenous antigen on antigen presenting cells in the synovial tissues. We hypothesize that bacteria or bacterial products from the normal resident flora are the antigens involved in the immune reaction of chronic human polyarthritis of unknown etiology like RA. A prerequisite for proof of this hypothesis is that bacterial antigens from the normal resident flora are present in synovial tissues. 2E9, a monoclonal antibody we produced against intestinal peptidoglycan-polsaccharides was used in a histochecmical study in rats and stained macrophages in the spleen red pulp. Human synovial tissues from 10 RA and 20 non-RA patients were stained with 2E9. We found that 8 out of 10 RA patients had 2E9 positive macrophages and dendritic cells in their synovia. A significant difference (p=0.003) was observed with the control group in which 7 out of 20 were positive. Serial sections stained with α-CD3 and 2E9, showed that in T cell infiltrations antigen presenting cells are present which are positive for peptidoglycan-polsaccharides. No positive cells or staining of the matrix were found in the cartilage of 6 RA patients and 5 osteo-arthritis patients. These results show that exogenous bacterial antigens are present in synovial tissue macrophages and dendritic cells. It was concluded that the unknown antigen in the immune reaction in RA is not necessarily endogenous, but could be peptidoglycan from the intestinal flora.

We purified the peptidoglycan degrading enzyme N-acetylmuramyl-L-alanine amidase from human serum. This is the only human enzyme capable of removing the interpeptide bridge from the peptidoglycan sugarchain. Monoclonal antibodies we produced to this enzyme were able to localize amidase positive cells in human spleen, tonsils, liver and in synovial tissue of RA patients. Staining of synovial tissue of healthy subjects is under current investigation. N-acetylmuramyl-L-alanine amidase renders peptidoglycan from Eubacterium aerofaciens, a resident of the intestinal flora, non-arthropatic in Lewis rats and less capable of inducing production of TNF-α, IL-1 and IL-6 by human monocytes from healthy subjects.

III.3. Detection of antibodies to klebsiella capsular antigens in ankylosing spondylitis and other rheumatic diseases.

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Klebsiella species have been suggested to be involved in the pathogenesis of ankylosing spondylitis (AS). However, inconsistent data gave rise to much controversy in this field. No study considered the epidemiology and pathogenicity of the 77 known Klebsiella serotypes (K1-K77). In the present study, sera from healthy controls (n=85) and patients with AS (n=54), rheumatoid arthritis (RA, n=24), psoriatic arthritis (PA, n=24), systemic lupus erythematosus (SLE, n=24) and reactive arthropathies after Yersinia enterocolitica infection (ReA, n=20) were
analyzed. Antibodies to all known Klebsiella serotypes were detected by ELISA (1). ELISA specificity was shown by the lack of reactivity with non capsule Klebsiella mutants and other Enterobacteriaceae. Significantly elevated frequencies and titers of antibodies to the capsular polysaccharides K26 (32%), K36 (51%), and K50 (54%) were detected in the sera obtained from the AS HLA-B27+ patients (p < 0.01). HLA-B27- AS, RA, PA, SLE, ReA, and the control group (HLA-B27 + or -) showed a lower incidence of antibodies (mean of K26: 10.8%; K36: 16.2%; K50: 22.7%). Antibodies to the remaining 74 Klebsiella serotypes were absent or detectable in <10% of all serum samples. In addition, a risk group for the development of AS, patients with uveitis anterior (UA) was investigated. In K36: 16.2%; K50: 22.7%). Antibodies to the remaining 74 Klebsiella serotypes were absent or detectable in <10% of all serum samples. In addition, a risk group for the development of AS, patients with uveitis anterior (UA) was investigated. In HLA-B27+ UA, antibodies to K26, K36 or K50 were found in 85% (n=20). These results suggest a specific association between Klebsiella capsular types and HLA-B27+ AS. The high incidence of Klebsiella antibodies in UA might support the idea of a pathogenic role for this gram-negative microorganism in the development of AS. (1) Sahly et al., Arthritis Rheum 1994, 37, 754.

III.4. Salmonella monocyte interaction in reactive arthritis.
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Reactive arthritis develops as a complication of certain infections of the gastrointestinal or genitourinary tract (eg Salmonella). Development of this complication is strongly associated with HLA B27. Pathogenesis of reactive arthritis is unknown, but it is characteristic that bacterial structures, especially LPS, have been found in peripheral blood cells for long periods and are also found in synovial fluid cells. However, it is not known how bacterial structures get into joints from the original site of inflammation, but monocytes as longliving and highly mobile cells are in particular interest in this context. CD14 is a high affinity LPS-receptor expressed on the surfaces of monocytes. CD14 has also been shown to indirectly mediate the adhesion of monocytes to endothelium via β3-integrins by cross-linking the receptor. In the present work we followed the expression of CD14 and β3-integrins on peripheral blood monocytes during seven days of bacterial processing after feeding with live Salmonella enteritidis. We noticed an interesting two-step regulation of CD14: first, expression of CD14 decreased, then strongly increased. There was also a strong correlation between CD14 and β3-integrin expression. This might be due to same regulatory and activation process of these molecules suggesting a potential role for the modulation of monocyte function in the pathogenesis of the infection. When β3-integrins were studied separately, interesting observation was that the HLA B27 positive monocytes showed a marked up-regulation of VLA-4 (α4/β1-integrin). This molecule has earlier shown to be important in adhesion of monocytes to synovial endothelium in rheumatoid arthritis. These findings suggest that VLA-4 contributes to transfer of cells containing bacterial structures into joints in patients developing reactive arthritis after Salmonella infection.

III.5. Conserved hydropathy profiles in CDR3 regions of HLA-B27-restricted TCR β chains of autoreactive or Yersinia-specific CTL indicate characteristic differences of bacterial and autologous antigens.
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Reactive Arthritis (ReA) and other spondylarthropathies are highly associated with the MHC class I allele HLA-B27 and previous infection with enterobacteria such as Yersinia and Salmonella. It was proposed that bacteria-derived peptides presented on B27 could act as the causative agents due to their putative high similarity to self peptides also bound to B27. Autotimmunity mediated by crossrecognition CD8+ T lymphocytes (CTL) could thus be induced. The occurrence of HLA-B27-restricted CTL with specificity for bacterial or autologous antigens in the synovial fluid (SF) of affected patients has previously been demonstrated (Lancet 342, 646, 1993). We have now sequenced the TCR-β chains of 25 HLA-B27 restricted CTL clones and 2-clonal lines and 14 non-HLA-B27 restricted controls. All cells were derived from the SF of 3 HLA-B27+ patients with ReA or from PBL of one B27+ healthy donor. We have isolated total mRNA from each clone, performed RT-PCR using primers for 24 different TCR-Vβ-families and carried out solid phase DNA sequencing. The examination of genomic segment rearrangement revealed limited variance (Immunobiology 1994, 191, 2-3). Due to the absence of information about the recognized peptide structures we spent special effort on the properties of the most likely peptide contacting CDR3 regions. Using both CDR3 amino acid sequences and known B27-binding self-peptides we generated multiple overlay-hydrophobicity plots according to the algorithm of Hopp and Woods. Results: Although there is no or only very limited formal homology of the studied sequences within their CDR3 regions, hydrophobicity plots of CDR3 of both 14 autoreactive and 6 clones specific for Yersinia-antigens revealed conspicuously conserved profiles. None of the CDR3 from autoreactive clones contained any charged amino acid at three positions (P95, 98 and 99). In contrast, sequences from Yersinia-specific clones did not avoid using charged residues at these positions but totally excluded strongly hydrophobic residues at P99, 100 and 102, positions which are heterogenic in autoreactive CTL. Control clones showed random distribution of hydrophilic and hydrophobic amino acids allow the sequence. With respect to the identical HLA-restriction, conserved differences of physico-chemical patterns within the CDR3 could indicate contact-mediating residues and might implicate conserved differences of corresponding antigenic peptides. Accordingly, overlay plot shape of 11 published sequences of B27-binding self nonapeptides shows striking similarity to the profile existing in autoreactive cells.

III.6. Molecular mechanisms in the inflammatory disease of HLA-B27 transgenic rats.
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Inbred rats transgenic (tg) for HLA-B27 and human b2m develop a spontaneous multisystem disease which mimics hu-
man spondarthrophy (Sp), with arthritis, inflammatory bowel disease, and psoriiform skin lesions. Lesional tissues have a lymphocytic infiltrate, and the disease can be transferred with T cells. The B27 molecule may present a pathogenic peptide, and particular B27 residues have been implicated. We sought evidence for a specific response by examining T cell antigen receptor beta chain variable region (TcR Vb) families and cytokines, and the effect of HLA-B27 mutants.

RNA was obtained from animals with preclinical or established disease and from non-tg littermate controls, and reverse transcribed to cDNA. Specific primers amplified transcripts for 22 TcR Vb and 11 cytokine cDNA's using PCR, with quantitation by dot-blot hybridisation. All TcR Vb families were present in lesional synovium, gut and draining nodes, with no oligoclonality or preferential TcR Vb usage in comparison to healthy non-tg animals or to non-lesional tg tissue. In preclinical disease significant elevations in IFNg, IL-2, IL-6 and IL-10 mRNA were found in distal colon, and the increase in IFNg was particularly disproportionate to the elevation in total TcR message. In established disease, mRNA transcripts were also identified in lesional colon for IL-1a, IL-1b, IL-5, TNF, and for putative IL-8 and IL-12 p40 products. By contrast, IFNg was not prominent in synovium from acutely arthritic joints. In preliminary studies, rats tg for HLA-B27 with the codon for cysteine at position 67 mutated to serine (67C-S) develop inflammatory disease similar to the wild-type.

The increase in IFNg and IL-2 mRNA in established disease is consistent with a Th1 response, and further investigation of synovia may reveal clues to the initiating events. Similarly, study of more synovia or of earlier preclinical disease may yet reveal TcR specificity and therefore evidence for an antigen-specific response. The occurrence of disease in the 67C-S mutants argues against a unique importance for this site in Sp pathogenesis. Further B27-mutant tg lines will be studied. The role of peptide presentation by B27 to specific T cells remains unproven.

III.7. Immunological criteria of differential diagnoses of reactive arthritis and rheumatoid arthritis.

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The differential diagnostics of reactive arthritis and rheumatoid arthritis is an urgent task of practical rheumatology since the tactics of the above diseases treatment differ.

The aim of the present study was to develop distinctive immunological characteristics of rheumatoid arthritis.

Two groups of patients have been examined: 18 patients with reactive arthritis and 26 with rheumatoid arthritis.

In peripheral blood CD4+ , CD8+ and CD25+ cells were detected by means of indirect immunofluorescence, in synovial liquid concentration of immunoglobulines was determined by the method of radial immunodiffusion by Manchini and immune complexes were determined by the method of precipitation by polyethylene glycol with further determination of their classes by Manchini.

In the group of patients with rheumatoid arthritis the content of CD8+ cells was reduced or within normal limits; the content of CD25+ cells increased; in synovial liquid the increased content of IgM and immune complexes of IgM class was detected.

In the group of patients with reactive arthritis the content of CD8+ cells remained within normal limits or increased. The content of CD25+ cells remained within normal limits or slightly increased. In synovial liquid concentration of IgM and IgM complexes was considerably lower than that for rheumatoid arthritis.

The results of the study suggest that the studied parameters are important for differential diagnoses of reactive and rheumatoid arthritis.

III.8. The measurement of IgA coating of anaerobic bacteria in human faeces and the detection of IgG and IgM antibodies against faecal bacteria in patient serum.

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Antigens in the gastro-intestinal tract might play a role in the pathogenesis of rheumatological disease. The intention of this study is to test the hypothesis that serum of patients contains more antibodies against faecal bacteria than the serum of their healthy partners.

We developed a method for the measurement of IgG, A or M coating of faecal bacteria. A suspension of bacteria isolated from faeces is incubated with fluorescent (FITC labeled) antibodies against Ig and FITC fluorescence of bacteria is analyzed with flowcytometry. After incubation with anti IgA-FITC the bacteria showed fluorescence, but not after the incubation with anti IgG-FITC or anti IgM-FITC. After a preincubation of the bacterial suspension with serum an increase in fluorescence was found when anti IgG-FITC or anti IgM-FITC was used. We concluded that the endogenous IgA coating of faecal bacteria can be measured and that serum IgG and IgM against faecal bacteria can be analyzed with flowcytometry.

For the determination of the intra-individual variation of serum Ig against faecal bacteria 4 healthy volunteers have collected 6 samples of their faeces during a period of three weeks. Blood was collected at the start and end of this period. The variation of the bacterial fluorescence after serum preincubation and incubation with IgG-FITC was 15-29% (CV, n=6) in these individuals.

At this moment faeces and serum of patients with ankylosing spondylitis and patients with rheumatoid arthritis are collected. A pilot study with 8 patients with rheumatoid arthritis is finished. Patients have a significant higher concentration of IgG antibodies against faecal bacteria than their partners.

III.9. Activating effects of Borrelia burgdorferi on human monocyes.

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In Lyme borreliosis, the paucity Borrelia (B.) burgdorferi spirochetes present at lesional sites contrasts to the severity of disease manifestations. Therefore, there must be pathogenetic mechanisms that amplify the inflammatory response like the chemotactic attraction of immune cells. In a previous study we
had demonstrated that human monocytes react to the present of *B. burgdorferi* in vitro with the formation of homotypic cell clusters. To further address this effect an in vitro assay was established. Monocytes were prepared from peripheral venous blood of healthy human donors and incubated with *B. burgdorferi* using various conditions. The reactive aggregation of the monocytes was determined via number of cell clusters and the total cluster area.

The results showed that the presence of *B. burgdorferi* significantly induced aggregation of monocytes, but not of neutrophils or T lymphocytes. This was paralleled by the release of the pro-inflammatory monokines IL-1β and TNF-α. Stimulation of monocytes with other bacteria or PMA or LPS yielded much weaker effects. The aggregation of monocytes was most pronounced in the presence of viable or heat-killed spirochetes, whereas borreliae killed by UV-light, freeze-thawing, glutaraldehyde or enzyme treatment borreliae induced less clustering. The effect of supernatants from *B. burgdorferi* cultures or of OsPA preparations differed among monocytes from different donors. Blockade of β2-integrins or L-selectin on monocytes, or addition of anti-inflammatory drugs to the incubation medium reduced the aggregation effect. It is concluded that both surface-bound as well as soluble compounds of *B. burgdorferi* provide activating signals to human monocytes which, in turn, amplify the effects by their own cell reactions. These results may shed light onto the in vivo situation in Lyme arthritis, where the synovitis is characterized by dense infiltration with mononuclear cells and mast cells, while the synovial fluid is abundant in neutrophils. Most likely, this hyperplasia is induced by the presence of viable microbes as well as spirochetal antigens.

### III.10. Ureaplasma urealyticum and reactive arthritis.

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The role of *Ureaplasma urealyticum* in triggering reactive arthritis has been discussed in a few reports, but as yet no definitive conclusion has been drawn. We report 5 cases of arthritis following a genital infection due to *Ureaplasma urealyticum*. Four 48, 50, 62 and 67-year-old men with urethritis and a 37-year-old woman with vaginitis and cervicitis suddenly developed 3 to 4 weeks after the genital infection an asymetric oligoarthritis associated with diarhoea in two cases and a conjunctivitis in one. Two had fever. One had a sausage finger and heel pain. Another patient had a spondyloarthropathy inactive since 30 years. X-rays were normal in all patients. Laboratory data were as follows: high erythrocyte sedimentation rate in all cases, HLA-B27 positive in three cases, rheumatoid factor and antinuclear antibodies negative in all cases, no hypoglobulinemia, HIV, *Chlamydia trachomatis* and gut bacteria serologies negative. Synovial fluid analysis showed a high leucocyte count, and the cultures were negative for mycoplasma and bacteria. Culture of urethral and endocervical samples were positive only for *Ureaplasma urealyticum* at significant levels (> 10^4/ml). Immunofluorescence technique and polymerase chain reaction (PCR) for *Chlamydia trachomatis* were negative on these genital samples. All patients had a 2 month antibiotic therapy with doxycycline 200 mg/day, and non-steroidal anti-inflammatory drugs. In 4 cases, the protracted course led to a successful administration of intramuscular gold. The link with the genital infection seems to be certain in these 5 cases as the onset of the arthritis occurred 3 weeks after a genital infection. The chronology of symptoms and the negativity of synovial fluid cultures are highly suggestive of reactive arthritis. The absence of other bacteria (*Chlamydia trachomatis*, *Neisseria gonorrhoea*) and the high titer of the cultures of *Ureaplasma urealyticum* in the genital tract are strongly suggestive of the responsibility of this agent in the triggering of the disease.

### III.11. Reactivity of sera from patients with Chlamydia-induced reactive arthritis with Chlamydia-specific lipopolysaccharide.

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Immunoblot studies of the humoral response to Chlamydia trachomatis in patients with complicated chlamydial infection like reactive arthritis have shown reactivity of a considerable number of sera with a 10 KDa antigen most probably representing chlamydial lipopolysaccharide (LPS). In addition, chlamydial LPS was demonstrated by immunofluorescence in synovial fluid from a patient with Chlamydia-induced arthritis (CIA), suggesting that chlamydial LPS might possibly play a role in the immunopathogenesis of CIA. In order to characterize the humoral immune response to LPS of *C. trachomatis*, we determined the reactivity of 27 sera from CIA-patients with a synthetic glycoconjugate containing chemically defined carbohydrate ligands of recombinant Chlamydia-specific LPS in an enzyme-immunoassay (medac, Hamburg, Germany). Antibodies of the IgG-class reacting with the synthetic Chlamydia-specific LPS-antigen were present in 24 sera (89%) with geometric mean titer (GMT) of 1:1.059 (range 0 to 1:6,400). Nineteen patients (70%) had IgA-class LPS-antibodies (GMT 1:69, range 0 to 1:200), and IgM-class antibodies were present in only one patient (4%). All LPS-antibody positive patients also reacted with Chlamydia-infected fibroblasts in a class-specific enzyme-immunoassay (Savyon diagnostics, Beer Sheva, Israel) and showed a 10 KDa-band in western immunoblot using purified elementary bodies of *C. trachomatis* as antigen source. Our study demonstrates that antibodies to Chlamydia-specific LPS are consistently produced by patients with Chlamydia-induced reactive arthritis. So far it remains unknown whether this humoral immune response reflects a pathogenetic role of LPS in the etiology of reactive arthritis.

### Session IV: Adhesion molecules and apoptosis/monocytes

**IV.1. Expression of the Fas/Apo-1 receptor and its ligand in rheumatoid arthritis patients.**

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The Fas/Apo-1 membrane protein is thought to be involved in the regulation of T-cell activation and in the control of autoreactive lymphocytes. One of its functions is the transmission of
apoptotic signals in previously activated lymphocytes. Rheumatoid arthritis is an autoimmune disease which is caused and maintained by autoreactive T cells that infiltrate joints and start an autoimmune response against joint antigens. To investigate possible control mechanisms, we analyzed Fas/Apo-1 expression in T cells of rheumatoid arthritis patients by comparing synovial and peripheral T cell subsets. All synovial T cells express the Fas/Apo-1 receptor and many of these cells are sensitive to apoptosis induced by Fas crosslinking. To analyze if synovial cells also express the natural ligand of the Fas/Apo-1 receptor, we screened a cDNA library of activated human peripheral blood cells and isolated the cDNA encoding for the human Fas/Apo-1 ligand (FasL). Preliminary expression studies revealed FasL expression in peripheral blood T cells, monocytes and in T cell clones isolated from the synovial fluid of rheumatoid arthritis patients.

IV.2. 80 kDa interleukin-6 soluble receptors strongly increase the production of acute-phase protein by human hepatoma cells but exerts only minimal effects on human primary hepatocytes.

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The 80 kDa soluble interleukin-6 receptor (sIL-6R) can combine IL-6 and induce cellular responses by association with gp130. In a previous study on the production of acute-phase proteins (APPs), we have found that the hepatoma cells PLC/PRF/5 have lost most of their responsiveness to IL-6 in comparison with human primary hepatocytes. In addition, evidence indicates that high levels of circulating 80 kDa sIL-6R might play a role in the modulation of IL-6 effects on certain cells. We therefore investigated the effects of sIL-6R on the response of hepatoma cells and primary hepatocytes to IL-6. Liver surgical biopsies were obtained from patients undergoing partial hepatectomy. Hepatocytes were isolated after collagenase perfusion. PLC/PRF/5 cells and hepatocytes were incubated with different concentrations of IL-6, IL-1, and sIL-6-R. The supernatants were tested for their content in C-reactive protein (CRP) and fibrinogen (Fb).

Results show that sIL-6R alone is able to induce the production of APPs in PLC in a dose-dependent manner. The presence of 100 ng sIL-6R alone is able to induce a higher production of CRP and Fb than 10 ng/ml IL-6 alone (3.4 ± 0.4 ng/ml vs 1.6 ± 0.07 and 9.1 ± 0.3 µg/ml vs 7.7 ± 0.5); respectively, the addition of 100 ng sIL-6R to 10 ng IL-6 enhances its effects (5.05 ± 0.35 ng/ml and 11.8 ± 2.1 µg/ml). Furthermore, IL-6-R increases significantly the effects of IL-1 on the production of CRP and Fb (6.3 ± 0.5 ng/ml vs 0.75 ± 0.07 and 9.0 ± 0.03 µg/ml vs 3.4 ± 0.3). In contrast, 100 ng IL-6-R has only a slight effect on the production of CRP and Fb by primary hepatocytes in comparison with 100 pg IL-6 alone (36 ± 6.3 ng/ml vs 78 ± 5.7 and 517 ± 80 ng/ml vs 685 ± 147). The addition of sIL-6R to IL-6 or IL-1 do not alter significantly the effects of these cytokines alone.

In conclusion, our results show that the loss of responsiveness of hepatoma cells to IL-6 is partially reversed by the addition of 80 kDa sIL-6R suggest a defective expression of IL-6R in hepatoma cells. On the other hand, it appears that IL-6R expression by primary hepatocytes is sufficient and that circulating sIL-6R is unlikely to play significant role in the modulation of IL-6 effects. These data also support that hepatoma cell cultures in presence of sIL-6R might represent a useful model for studies on APPs regulation.

IV.3. ICAM-1/LFA-1 enhanced presence/function on murine premorbid SLE-prone cells, is inducible by UVA/UVB.

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Sunlight is known to induce generalized manifestations in systemic lupus erythematosus (SLE) patients, but the underlying mechanism of photosensitivity in this disease remains obscure. Recently, we demonstrated enhanced membrane binding of SLE-related autoantibodies to cultured keratinocytes of SLE patients following UVB/UV-A irradiation. We have now investigated whether UVB/UV-A-irradiation may affect the expression/function of proinflammatory adhesion molecules on cell membrane in premorbid murine SLE derived cells in vitro. Monolayers of SLE-prone MRL/1, BWF1 and BALB/c or C57BL/6 (controls) derived dermal fibroblasts were exposed to UVB (0-200 J/m²) and the expression of ICAM-1 on cell membrane of living cells was analyzed (by immunofluorescence microscopy and stained by FACS) 20 and 40 h post-irradiation. The data shows enhanced and dose-dependent ICAM-1 expression in SLE derived cells in comparison to normal cells. In addition, we observed that, though LFA-1 expression on cell membrane in single cell suspensions of lymph node cells, is equally represented (by α-LFA-1 mAb in FACS analysis) in MRL/1 and BALB/c, its functional activity is 4-fold increased in MRL/1 cells (measured 16 h post incubation in flat-bottomed microplates, 3×10⁶ cells/well + 4 ng/well PMA, by a cell aggregate assay). Although a suboptimal concentration of PMA (0.2 mg/well) affected equally both non-irradiated MRL/1 and control cells, a low dose (50 J/m²) UVB-irradiation further increased 3-fold LFA-1 activity in MRL/1 cells (but not that of controls), an increase that could be blocked by α-LFA-1 co-treatment. Furthermore, irradiation with UVA (320-400nm) or UV-A1 (340-400nm) at doses of 20-40 KJ/m² of PMA treated cells resulted in 2-3 fold increased LFA-1 functional activity in the two SLE-prone strains derived cells (but not in controls). This increase also could be abolished following α-LFA-1 co-treatment. We are currently investigating whether cytokines are mediators of these events.

IV.4. Inhibition of human mucosal lymphocytes migration in rheumatoid synovium engrafted in SCID mice by antibodies against LFA-1 and αβ7.

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Human lymphocytes circulate 6 months and remain functiona-
suggesting a retention of mucosal lymphocytes by synovial tissue. We studied in vivo the migration of human mucosal lymphocyte in human rheumatoid synovium engrafted in SCID mice and the effect of murine antibodies (mAb) against LFA-1 (CD11a) and αEβ7 (HML-1).

Methods: We engrafted subcutaneously 20 SCID CB17 mice with synovial tissue obtained after surgery of 5 RA patients. Human tonsil lymphocytes (expressing αEβ7) and peripheral blood lymphocytes were isolated on Facoll. Three weeks after engraftment, 20 × 10^6 lymphocytes were labelled with HMPAO-Tc99m and injected intraperitoneally after one hour incubation with either mAb HML-1 or CD11a. Total body mice scintigraphy was performed 20 hours post-injection. A region of interest (ROI) of 200 pixels was determined in the area of the human graft. We assessed the activity within the ROI of graft and within a ROI including the total mouse. Immunohistology was obtained from the engrafted synovial tissue and the mouse spleen.

Results: After injection of tonsil lymphocytes we observed a retention of the cells in the synovial graft, reflected by an increased signal on the graft area. The mean activity ROI (synovial graft)/total activity was 3.43 ± 1.37%, and was not different from the results obtained after PBL injection (4.69 ± 3.17%). Tonsil lymphocytes retention by the graft decreased to 2.76 ± 1.7% after pre-incubation with mAb CD11a and to 2.27 ± 0.6% after pre-incubation with mAb HML1.

Conclusion: In vivo, labelled mucosal lymphocytes migrate to engrafted human rheumatoid synovium. Cell retention is inhibited by antibodies against LFA1 and αEβ7. This suggests the implication of these adhesion molecules in RA.

IV.5. Investigation of a role for nitric oxide in pathogenesis of inflammatory and degenerative arthropathies.

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Nitric oxide (NO) has emerged as an important mediator in several animal models of inflammation. Rodent macrophages and synoviocytes, and human and rodent osteoblasts and chondrocytes produce NO in vitro. We therefore sought to elucidate the role of NO in rheumatoid (RA) and osteoarthritis (OA).

Collagen type II arthritis (CIA) develops from day 28 after collagen priming in DBA/1 mice and histologically resembles RA. Treatment of mice for 10 days (day 1-10) after priming with collagen with the NO synthase (NOS) inhibitor, L-NMMA (5 mg/day), significantly reduced the incidence of CIA up to day 42 (p < 0.05). However, treatment with L-NMMA for 10 days of established CIA did not improve paw swelling or articular index, although progression of the latter was significantly reduced compared to controls (p < 0.05). Thus NO appears implicated in development of CIA, but at this dose, may only ameliorate rather than reverse established disease.

We next sought confirmation of NO production by human synovial tissue. Ex vivo human synovial cultures were prepared from 12 RA and 6 OA arthroplasty specimens. 9/12 RA and 4/6 OA cultures produced nitrite over 72 hours with medium alone. Addition of staphylococcal enterotoxin B and lipopolysaccharide induced higher levels of nitrite production in 11/12 RA and 5/6 OA cultures. Inducible NOS was detected immunohistochemically on cytopsin preparations of RA synovial cells using a monoclonal anti-iNOS (murine macrophage) with streptavidin-biotin-fast red detection.

Our data demonstrate inducible NO production from human synovial tissue derived from both RA and OA patients. The results obtained with the CIA model suggest that such NO production may have a significant pathological role in inflammatory joint disease.

IV.6. Greatly reduced lymphoproliferation in Lpr mice lacking MHC class I.

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Mice homozygous for the lpr gene have a defect in fas (CD95), a cell surface receptor which belongs to the TNF-receptor family and which mediates apoptosis. This genetic abnormality results in a syndrome characterized by lymphoproliferation, mostly of αβ+ CD4/8- T cells (DN), autoantibody production and strain dependent end-organ disease. Our previous results with C57BL/6-lpr/lpr (B6/lpr) mice showed a similarity between DN and CD8+ T cell receptor (TCR) VB repertoires, suggesting that DN cells were derived from CD8+ subset and selected on MHC class I. To test further the role of MHC class I in DN development, we bred B6/lpr mice that are deficient for the β2-microglobulin gene (β2 mIpr) and that have no detectable MHC class I expression. At 6 mo of age, compared to non-knockout B6/lpr littermates, these mice showed a decrease in total lymph node (LN) CD8+ T cells (mean ± S.E. β2 mIpr vs. controls: 5.2 ± 10^6 vs 75 ± 20x10^6, p = 0.005), greatly reduced lymphadenopathy and total LN cell numbers (LN weight: 0.36 ± 0.06 g, n = 12, vs. 1.09 ± 0.18g, n = 10, p = 0.002; LN cell #: 300 ± 43 x 10^6 vs. 1197 ± 250 x 10^6, p = 0.01). The reduction in total cellularity was mostly due to a dramatic decrease in the number of DN cells (45 ± 8 x 10^6 vs. 660 ± 228 x 10^6, p < 0.0001). There was no significant difference in CD4+ B220- or CD4+ B220+ T-cells, but γδ+ DN T cells were increased (22.4 ± 1.4 x 10^6 vs. 11.1 ± 3.3 x 10^6, p < 0.005). TCR VB repertoire analysis of the remaining DN T cells in β2 mIpr mice showed a shift to a CD4+ -like repertoire from a CD8+ -like repertoire in B6/lpr DN (particularly in VB5 and 14), suggesting that the β2 mIpr state unmasked a minor MHC class II selected DN population. In addition, compared to littermate controls, production of IgG autoantibodies (α-chromatin and α-ssDNA), total IgG and IgG2a, but not total IgM or IgM RF, was significantly reduced in the β2 mIpr mice.

This work suggests that over 90% of DN T cells in lpr mice are selected on class I, but that a minor subset is selected on class II. Class I also influences the production of IgG autoantibodies.
IV.7. Adhesion molecule endothelial expression in PMR synovial biopsies.
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Expression of cell adhesion molecules on vascular endothelium plays a fundamental role in the onset and perpetuation of immune mediated inflammatory disease. In a previous study we found high levels of the soluble form of ICAM-1 in the serum of patients with PMR (P. Macchioni et al. J. Rheumatol 1994, 21, 1860-1864). In this report we describe the expression of adhesion molecules in synovial vessels. Synovial biopsies were obtained during arthroscopic examination of the shoulder from 12 untreated PMR patients (9 females, age range 60-83 yrs) and 8 corticosteroid treated ones (7 females, age range 64-80 yrs). The specimens were snap frozen in liquid nitrogen and stored at -70°C until analysis. Immunohistochemical staining was performed using MoAbs to: ICAM-1 (CD54), VCAM-1 (CD106), E-selectin (CD62E), P-selectin (CD62P). Strong expression of ICAM-1, E-selectin and P-selectin was found in 11 out of 12 untreated patients. Intense molecule expression was present in > 30% of biopsy vessels in untreated cases. VCAM-1 was not expressed by endothelial cells in 10 patients; this adhesion molecule was strongly expressed by lining layer cells. Six out of 8 treated patients showed no expression of adhesion molecules; in the 2 remaining patients the number of positive vessels was less than 30%. The intense adhesion molecule expression on endothelial cells in untreated PMR cases strongly suggests a cytokine dependent activation of these cells. Thus an immune mediated synovitis of the shoulder is present in PMR. Steroid treatment is associated with a marked decrease of adhesion molecule expression.

IV.8. Cytokines regulate the cell adhesion apparatus of synovial cells.
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A significant pathologic process in rheumatoid arthritis is the proliferation of the synovial membrane. The normal synovial membrane consists of two different types of cells, type A macrophage like cells and type B fibroblastic cells. We have analysed the integrin pattern expressed by two synovial fibroblast cell lines corresponding to the type B cells in vitro and a cell line (THP), which resembles type A cells and is inducible to adherent macrophages. Treatment of fibroblast cells with IL 1, TNF α, INF γ and TGFβ induces these cells to express α2 type integrin shown by immunoprecipitation. Cytokines also modulate the α2 and β5 integrin subunit expression of fibroblasts. Nonadherent THP cells do not express α6 or β5 subunit, whereas when they are induced to adherent cells, these integrin subunits are observed. TGFβ strongly enhances mRNA and protein expression of the α6 integrin in adherent macrophages, while the expression of α6 protein is downregulated by TNFα.

We have previously shown that the rheumatoid synovial lining does not express α6 or β5 as compared to the noninflammatory lining cell layer. We show here that the integrin pattern is regulated in vitro by cytokines and this could be the mechanism how the integrin pattern is also regulated in vivo in rheumatoid arthritis.

IV.9. The role of macrophages in the onset of rheumatoid arthritis.
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The role of macrophages in tissue destruction in RA is increasingly recognized. Many reports and observations point to this cell type as a likely candidate for the onset and maintenance of inflammation in RA. For example, one of the early signs of RA is hyperplasia (activation) of the resident synovial lining cells. The observation that RA is mainly localized in tissues containing large numbers of resident macrophages like the synovium, the tendon sheaths, the bursa, the pleura, the pericard and the lungs also substantiate the role of macrophages. With our study we intend to find differences in gene expression patterns between (activated) monocytes/macrophages of healthy persons and (activated) monocytes/macrophages of rheumatoid arthritis patients. Although a large number of differences in expression have been found between synovial lining cells from RA patients and controls, the initial events leading to RA were most probably obscured because inflamed tissue was used. To circumvent these problems we have used highly purified, non-activated peripheral blood monocytes (the precursor cells of the resident macrophages). These monocytes were then cultured in vitro to differentiate into macrophages. Part of these macrophages were stimulated with LPS and γ-IFN. The expression patterns of the monocytes and (activated) macrophages of RA patients and controls were compared by preparing cDNA libraries of these cell types and performing subtractive hybridizations. So far we were able to identify several gene products which are overexpressed in either the RA monocytes or RA macrophages. Interestingly, some of these sequences represent unknown proteins. In the near future we will further analyze these differentially expressed clones and develop a quantitative assay to determine whether the observed differences in expression can be observed in large numbers of RA patients and controls. Depending on the specificity of the differentially expressed gene products, new diagnostic and therapeutic strategies directed at macrophages and their products can be developed.

IV.10. Oncoprotein expression in synovial tissue of patients with different types of arthritis.
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Based on the fact that synovial lining cells have some properties of transformed-appearing cells and that the pannus formation resembles tumor-like growth, we have examined the ex...
pression of Myc, Myb, Fos, Jun and Ras oncproteins in synovial tissues from patients with different types of arthritis. Formalin fixed and paraffin embedded sections of synovial tissue from 12 patients with rheumatoid arthritis (RA), 14 with reactive arthritis (ReA), 9 with other seronegative arthritis (OSA), 7 with bacterial arthritis (BA), 8 with probable bacterial arthritis (PBA) and 8 with osteoarthritis (OA) were studied using immunoperoxidase staining. The oncproteins studied were expressed both in synovial lining cell layer and in sublining layer, consisting of lymphocytes, other inflammatory cells and blood vessels. The oncproteins expression seem to be typical for each group of patients, those with RA and OA forming own entities whereas the results obtained for ReA and OSA, and on the other hand for BA and PBA, closely resemble each other. The expression of Myc, Myb, Fos and Jun was significantly correlated to the degree of cellular proliferation and inflammation. For Ras such a correlation could not be seen.

We conclude that quantitative and qualitative differences which exist between RA and other types of arthritis rather reflect different modes of cellular proliferation and inflammatory cell infiltration than primary events of etiopathogenesis.

IV.12. Chronic processes and late destruction distant from the operated knee in hu/mu SCID-Arthritis.

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Synovitis and cartilage erosion are highly complex processes requiring multicellular interactions which are hard to study in vitro. Therefore we have developed a model in which small pieces of human tissues are implanted in knee joints of immunodeficient (SCID) mice (J. Rheumatol. 21 (1994) 10-6). We could demonstrate that specific features of RA synovial tissue were retained in this model. Most characteristic for this human/murine (hu/mu) SCID arthritis was formation of a mixed pannus tissue primarily composed of human and murine macrophages. Control implants induced a transient exsudative joint inflammation while RA-synovial membrane initiated a characteristic arthritis with pannus tissue of high cellular density, erosion, multinuclear giant cells, lining cell hyperplasia, fibroblast-like cell layers, chondroideal metaplasia, and fibrin deposits. Mice were monitored by non-invasive methods, i.e. by serological parameters (human IgM, IgG, and IL-6) and Tc-99m-scintigraphy. Significantly elevated levels of human immunoglobulin and characteristic signs of chronic inflammation persisted for more than four weeks. Tc-99m-DPD scanning joint scintigraphy revealed an accumulation of Tc-99m in the joint affected by transplantation. The accumulation of Tc-99m-DPD inside the operated knee joint correlated with histological features of cartilage erosion (n = 19, p = 0.05). Most importantly, beginning at the third week after implantation of RA-SM, the contralateral knee joint and hips become involved and developed a severe destructive arthritis in most of the animals. No remarkable infiltration of destructed joints by mononuclear cells could be observed.

We conclude, that this model shows some similarities to human RA. Migration of the disease process to distant non-implanted joint may help to elucidate interesting pathogenetic features on systemic spreading of the disease.

|         | sICAM-1 (ng/ml) | sVCAM-1 (ng/ml) | sE-Selectin (ng/ml) | TNFα (pg/ml) | TNFαR55 (ng/ml) | TNFαR75 (ng/ml) |
|---------|----------------|-----------------|---------------------|---------------|-----------------|-----------------|
| SLE (n=25) | 326±194        | 1022±689        | 59±47               | 53±24         | 5.2±7.3         | 13.3±9.7        |
| Vasc. (n=23) | 417±241        | 1017±557        | 52±27               | 39±26         | 6.0±5.2         | 10.7±5.7        |
| Cont. (n=16) | 229±78         | 561±184         | 35±12               | 27±2          | 1.8±0.2         | 2.9±0.3         |

When all samples were taken together, a positive correlation was observed between sVCAM-1 and both TNFαR levels, but no correlation between sICAM-1 and TNFαR, nor with sE-Selectin and TNFαR. There is also a slight correlation between TNFα and sVCAM-1. These results suggest that sVCAM-1 generation is differentially regulated from sICAM-1 and sE-Selectin. They also suggest that there is a relationship between TNFαR55 and TNFαR75, which act as TNF inhibitors, and soluble forms of adhesion molecules which may also play a protective role in inflammatory processes.
IV.13. Anti-CD40 and IL-4 reduce apoptosis of lymphocytes of patients with systemic lupus erythematosus (SLE).

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An increased rate of apoptosis of peripheral blood mononuclear cells (PBMC) has been reported in SLE. Since stimulation of cells via CD40 has been shown to prevent apoptosis and we have observed a diminished capacity of SLE T cells to express the CD40 ligand, the present study was performed in order to investigate whether the stimulation with an antibody to CD40 can reduce programmed cell death in SLE. For that purpose, PBMC as well as B cells from SLE patients were incubated with anti-CD40 and IL-4, which as a T and B cell growth factor can increase the cell life span. Apoptosis was measured after an incubation period of 24, 48 or 72 hours. It was evaluated by staining with propidium iodine as well as by DNA ladder formation according the standard protocols. Normal blood donors and patients with rheumatoid arthritis served as control groups. A significantly elevated rate of apoptosis was observed in SLE, but not in patients with rheumatoid arthritis or normal blood donors. The incubation of the cells with a combination of anti-CD40 and IL-4 led to a markedly reduces apoptosis in SLE cells. This effect was less pronounced in normal blood donors, whereas anti-CD40 and IL-4 rather increased programmed cell death in patients with rheumatoid arthritis. We conclude from these data that a deficient CD40/CD40 ligand interaction may contribute to increased apoptosis in SLE.

IV.14. Effect of endothelin-1 (ET-1) on the production of IL-6 by human umbilical endothelial cells (HUVEC).

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Endothelin-1 has been known to possess various biological activities. ET-1 has a strong and longlasting in vivo effect on vasoconstriction. In addition, it has a mitogenic effect on mesangial cells, dermal fibroblasts, synovial fibroblasts and vascular smooth muscle cells. The cytokine IL-6 exhibits also multiple activities, especially the induction of acute phase response. In the present study we investigated the role of ET-1 on the production of IL-6 by HUVECs. Material and methods: HUVECs were isolated and cultured under standard conditions. Cultured HUVECs were treated with different ET-1 concentrations (1-100 p/ml) in the presence of absence of C1306, a specific endothelin-A-receptor antagonist. IL-6 production was determined by ELISA technique. Results: The production of IL-6 by HUVECs was significantly downregulated after the incubation with Et-1 with a maximum effect after 24 h at 100 pg/ml Et-1. In contrary TNF-α and γ-IFN showed a significant increase in the cytokine production. The coincubation with C1306 had no influence on the Et-1 effect. Conclusions: In contrast to the yet well-known mitogenic effects of Et-1 we could demonstrate in the present study that Et-1 downregulates the IL-6 production by HUVECs. The importance of these data may be a negative control of Et-1 of the IL-6 mediated induction of acute phase responses.

IV.15. The LFA-1 Phenotype is Predominantly Expressed in Acute Inflammatory Joint Lesions of Collagen-Induced Arthritis in Rats.

M.F. Seidel, R. Keck, C. Viebahn, A. Sachinidis and H. Vetter.

Collagen-induced arthritis in rats shares many similarities with rheumatoid arthritis in humans. However, the phenotype expression of joint inflammation is mostly unknown in this model. Therefore, we examined the lymphoid infiltration for cell and tissue determinants in this rat model that can also be detected in humans. Cryostat sections of proximal hind paws were assayed by immunocytochemistry for CD4-T-helper cells, B-cells, macrophages, for intercellular adhesion molecule-1 (ICAM-1) and leukocyte-function associated antigen-1 (LFA-1) expression. In control animals B-cells and the LFA-1 expression could not be detected in the synovium. Occasional staining of the synovium was detected for the CD4 marker and prominent staining for ICAM-1 of the vascular endothelium. In animals immunized with bovine collagen type II, all markers were detected at sites of acute inflammation which were frequently associated with cartilage erosion and bone destruction. ICAM-1 and CD4 expression was most prominent followed by macrophage and LFA-1 staining. B-cells were less frequent. CD4 + lymphocytes, macrophages and ICAM-1 + blood vessels were also detected in peripheral areas of joint swelling. LFA-1 staining was prominent and concentrated in proximity to joint cartilage or bone destruction suggesting that LFA-1 + cells accumulate selectively at the site of acute inflammation. The results parallel findings in human rheumatoid synovium and provide further evidence that collagen-induced arthritis in rats can be regarded as a similar disease. Thus, future analyses of joint inflammation in this model may also be relevant to human disease.

IV.16. Cytokine profile dependent on the type of arthritis: Dominant role of IL-1 in cartilage destruction.

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Cytokines are believed to play an important role in rheumatoid arthritis. First experiments with humanized anti-TNF antibodies in RA patients look promising and the focus of therapy on TNF is based on the observed cascade of TNF - IL-1 in the rheumatoid synovium. We now investigated the role of TNFα and IL-1 in various murine arthritis models, including Antigen induced arthritis (AIA), Immune complex arthritis, Collagen arthritis (CIA) and Streptococcal cell wall arthritis. We used neutralizing antibodies and IL-1ra (Synergen, Boulder) in vivo to investigate the involvement of TNF and IL-1. Joint inflammation and cartilage destruction were analyzed on whole joint sections and chondrocyte function was measured with 35S-Proteoglycan labelling in patellae. IL-1 production is evident in synovial tissue and prolonged message expression is found in CIA and SCW arthritis. TNF expression seems more transient and varies between models, with the highest expression in SCW arthritis. Anti-TNF treatment was clearly antiinflammatory in early, but not late stages of CIA and highly suppressive in SCW arthritis, but protection of cartilage damage was limited. In contrast, anti-IL-1 suppressed arthritis both in early and late stages of CIA, but was hardly anti-inflammatory.
in AIA and SCW arthritis, yet markedly reduced cartilage destruction and fully prevented chondrocyte proteoglycan synthesis inhibition in all models. Our data indicate that the cytokine profile is stimulus/process dependent and that IL-1 is a prime target in prevention of cartilage destruction.

IV.17. Phagocytic lining cells are involved in cell influx in the flare-up of chronic arthritis.

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We investigated the role of phagocytic lining cells in the reactivation (flare-up) of chronic antigen (mBSA)-induced arthritis. Phagocytic lining cells were selectively depleted prior to induction of flare. For this purpose we injected clodronate (CL-MDP) containing liposomes in the chronic arthritis knee joint 14 days after arthritis induction (7 days prior to induction of flare-up). Most phagocytic lining cells disappeared within one week after injection. 24 hrs after reactivation of inflammation (flare), either by giving the antigen systemically (350 μg) or locally (2 μg) into the knee joint, the total amount of cell influx in the synovial layer was significantly lower in the lining depleted knee joints if compared to control (PBS treated) arthritis joints. In addition to quantitative differences also qualitative changes were noted. In control flares, most cells accumulated at the lining layer and their numbers gradually decreased in the deeper layers. In contrast, in lining depleted knee joints, most cells accumulated in the deeper layers around the blood vessels of the synovium, whereas less cells were found in the superficial synovial layers. This altered cell migration pattern is probably due to a different antigen handling. Autoradiographs show that 125I-radiolabeled mBSA normally accumulates in the lining layer, probably giving rise to local release of chemotactic peptides and distinct cell accumulation. In contrast, in lining depleted joints antigen was mainly found in deep synovial layers. Our study indicates that phagocytic lining cells seem involved in handling of the antigen and the subsequent influx of inflammatory cells in the flare-up of chronic arthritis.

Session V: Genetic aspects of auto-immune rheumatic diseases.

V.1. A new TNFα gene variant: a susceptibility factor for rheumatoid arthritis?

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Tumor necrosis factor alpha (TNFα) is a central mediator of the inflammatory response and has been implicated in the pathogenesis of a number of MHC-associated diseases. Laboratory studies have suggested a significant role for TNFα in rheumatoid arthritis (RA). This suggestion is strongly supported by the results of a randomised double-blind placebo controlled trial in RA patients treated with anti-TNFα monoclonal antibodies. In healthy individuals, interindividual differences in TNFα production have been described and increased TNFα production has been reported to be associated with the HLA-DR4 and -DR3 alleles. The differences in TNF production may be explained by variation (polymorphism) within the regulatory regions of the TNFα gene. Until now, two polymorphisms within the 5' regulatory region of the TNFα gene have been described. Recently, McGuire et al. reported an association between a variant TNFα allele and an increased risk to contract cerebral complications during malaria infection (Nature 371, 508, 1994). This finding implicates a functional role for TNFα variants in disease. We identified a new polymorphism involving an insertion at position +70 of the TNFα gene. The frequency of the variant allele was found to be 1.7% in healthy individuals. No linkage was observed between the two known TNFα polymorphisms and the +70 polymorphism. The position of the new insertion polymorphism within the region that is transcribed into mRNA opens up new perspectives to discriminate between the relative level of allelic transcripts. Studies to determine whether the TNFα insertion polymorphism plays a role as a genetic marker for susceptibility or outcome of RA are currently being performed in a group of 200 RA patients.

V.2. Tsk2, a novel animal model of scleroderma: biochemical, histopathological and genetic characterization.

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The newly discovered Tsk2 mouse is a novel mutation which occurred in the 10l/H mouse strain after administration of ethylnitrosourea. The mutation is inherited as an autosomal dominant trait and has been mapped to chromosome 1 employing visible chromosomal markers. Mice carrying the Tsk2 mutation phenotypically appear similar to mice carrying the Tsk1 mutation, an animal model of Scleroderma (SSc). Tsk2 mice develop tightness and induration of the skin of the interscapular region which is detectable by 3-4 weeks of age. Histological examination of Tsk2 mouse skin, showed marked accumulation of collagen in the dermis and adipose tissue. Additionally, the presence of mononuclear inflammatory cells in the lower dermis and in the adipose tissue. Additionally, the presence of mononuclear inflammatory cells in the lower dermis and in the adipose tissue septa were a prominent feature. Biochemical studies of the skin showed increased collagen content and collagen biosynthesis which were accompanied by elevated levels of α1(I) procollagen mRNA. These changes are similar to those found in affected skin from patients with SSc. As a first step toward identifying the gene responsible for the Tsk2 mutation, Tsk2 was mapped with respect to three known microsatellite markers: D1Mit4, D1Mit5 and D1Mit7. The results map Tsk2 to the region between D1Mit4 and D1Mit5. There are several extracellular matrix proteins which map in or near this region, including the genes for fibronecin and for α1 (III) procollagen. The occurrence of a new mutation localized in a different chromosome than Tsk1 causing exaggerated production of connective tissue molecules will allow the identification of a distinct pathway involved in the regulation of collagen gene expression under normal and pathological conditions.
V3. Herpetic viruses in rheumatoid arthritis multicase families.
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ková, K. Seeman, K. Vojtěchovský. Institute of Rheumatology, Prague, Czech Republic Montreal General Hospital Research Institute, Canada.

Introduction: The etiology of rheumatoid arthritis (RA) is unknown, but likely involves a number of factors both genetic and environmental. The aim of this study is to investigate the rate and extent of infection by Epstein-Barr virus (EBV), cytomegalovirus (CMV) and herpes virus-6 (HHV-6), in families (affected and not affected members) with multiple cases of RA.

Methods: 28 members of RA multicase families – 17 affected and 11 not affected – and 4 members of healthy control family were investigated. All affected members were seropositive for EBV, RV, Prague, Czech Republic Montreal General Hospital Research Institute, Canada.

Results: Viral DNA, particularly EBV was detected in increased frequency in the family members with RA compared to their unaffected relatives, followed by HHV-6 and CMV. The saliva DNA was found at a 2-fold or higher frequency (in all cell compartments) in the affected individuals compared to the unaffected relatives, followed by HHV-6 and CMV. The saliva EBV DNA was found at a 2-fold or higher frequency (in all cell compartments) in the affected individuals compared to the RA patients. No difference was found for the frequency of CMV DNA.

Conclusions: We suggest that in RA multicase families, increased frequency of viral infection is likely a consequence of the disease state and/or due to gene(s) as yet unidentified.

V4. Functional and morphological abnormalities of the immune system in TNF LTA double deficient mice.
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In order to investigate the roles of tumor necrosis factor (TNF) and lymphotokin alpha (LTA) for the development and function of the immune system, the Tnf and Lta genes were simultaneously inactivated in mice by homologous recombination. These mice combine the phenotypes of TNF-RI knockout (resistance to endotoxin and increased susceptibility to Listeria monocytogenes infection) and LTA-knockout mice (absence of peripheral lymphoid system). In addition, the TNF-LTA double deficient mice have an unexpected, tissue specific upregulation of MHC class I and II antigen expression, immunoglobulin depositions in the real glomerulus and increased anti-DNA antibody titres in the presence of 6 fold increase of circulating B-lymphocytes. IgG2b, IgG3 and IgA serum levels are dramatically reduced and no IgA producing plasma cells are detectable in the gut mucosa. Preliminary data from experimental arthritis models will be discussed. These data suggest, that the TNF and LTA are required for the proper function of the immune system and immunopathology.

V5. TNF Nco-1 RFLP associated with increased TNFα production is not a risk factor in rheumatoid arthritis.
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Rheumatoid arthritis is characterised by the presence of the DR4 MHC Class II antigen which constitutes the main genetic risk for severity of this disease. Cytokine expression in the rheumatoid joint is dominated by TNFα, so a genetic predisposition to overproduction could be an important risk factor in RA. Of several polymorphic sites at the TNF locus in the class III region of chromosome 6, one (defined by Nco-1 RFLP) has been associated with high or low TNFα production (B*2 and B*1 respectively) in SLE and IDDM. We therefore examined this locus in RA patients and controls, and expressed results as population percentage in which the allele or combination is found. (see Table)

These data show that the enhanced TNFα production related to the TNFB*2 allele is not reflected in the genetic make-up of RA patients.

V6. Frequency of HLA-B27 subtypes in patients with rheumatic disease and/or uveitis: Molecular typing of PCR and subtyping by SSO.
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Aim of study: The aim of the study was to investigate if HLA-B27 typing by polymerase chain reaction (PCR) can improve the typing results compared to the commonly used serologic typing (lymphototoxicity assay [LCA]). Furthermore, all HLA-B27 positive patients with rheumatic disease and/or uveitis should be subtyped by sequence specific oligonucleotides (SSO).
Methods: 185 consecutive patients with rheumatic disease and 97 patients with uveitis were HLA-B27 typed by LCA and PCR. Amplified sequences (exon 2 and 3) of HLA-B27 positive patients were then tested with eight SSO to distinguish all HLA-B27 subtypes.

Results: In patients with rheumatic disease 35/185 patients tested HLA-B27 positive by LCA. Using PCR technique 37 patients tested HLA-B27 positive. Patients with a weak signal or possible cross-reaction (HLA-B7) in LCA testing (n=10) gave a positive PCR result in five cases. In patients with uveitis 23/97 patients tested positive for HLA-B27 (LCA/PCR). Subtyping in 43 HLA-B27 positive patients revealed 6× B2702, 5× B2704, and 30× B2705. In 2 patients even after sequencing, the HLA-B27 subtype could not be distinguished and may be characterized as new subtypes.

Conclusions: PCR technique can improve HLA-B27 typing as a reliable and reproducible method. Compared to LCA testing, PCR technique is able to classify uncertain results (weak positive LCA results, cross reaction with HLA-B7).

V.7. Immunogenetic characteristic of juvenile rheumatoid arthritis (JRA) in Latvia.
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So far in Latvia HLA antigens in chronic articulation diseases have not been studied. 107 JRA children were examined. HLA antigens - locus A, B were determined by Terasaki two-phase complement binding microlymphocytotoxic test. The obtained results suggest that risk antigen is HLA-B27 (RR 3.5), haplotype HLA-A2, B40, frequency of antigen HLA-B15 was believably decreased (p<0.01).

We have detected a combination of haplotype HLA-A2B5 and prognostically unfavourable type (25% to 0% in the opposite group).

Results of this research can be used for prognosis of illness and for separation of risk group, predisposed to JRA. This group comprised children of RA patients and siblings of JRA patients, particularly females. Genetic predisposition, additional diseases, antigens HLA-B27, HLA-B35, haplotype HLA-A2, B40, deviation of humoral immunity in healthy relatives increase the risk of the disease.

V.8. DRB1*01 association of elderly onset rheumatoid arthritis in Hungarians. 
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Thirty-eight older age onset rheumatoid arthritis (ORA) patients were DR1/DR4 positive and genotyped in order to define whether ORA's immunogenetic background differed from that of the adulthood RA.

ORA was defined as RA starting after the age of 60. RA was diagnosed according to the 1987 revised ARA criteria. HLA DR antigens were determined by serological typing and a molecular biological method (PCR-SSP). Our results were analyzed in comparison to data of European and Hungarian healthy and RA populations.

As well as in Europe, in the Hungarian population DR1 allele frequency is similar in RA and healthy controls, whereas DR4 allele frequency is 2× increased in RA (relative risk (RR) = 2.9 vs 2.4). Comparing our ORA results to the European and Hungarian data ORA seems to represent the healthy population. Nevertheless, when comparing to 37 age-matched Hungarian healthy controls, the frequency of DR1 seems to be 3× (RR = 3.3), whereas that of DR4 2× increased (RR = 2.1). In each of our DR1/DR4 positive patients DNA typing proved those subtypes that expressed the shared epitope of RA.

In conclusion, in Hungarians DRB1*01 association seems to be characteristic of the older age onset RA.

V.9. Detection of aminoacid polymorphism in fetally expressed immunoglobulin DP10 gene in patients with rheumatoid arthritis (RA).
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The repertoire of antibodies is determined by the use of different immunoglobulin variable region (IgV) genes. It has been suggested that some of the developmentally important genes may be missing in patients with RA.

Therefore we have studied the occurrence of frequently fetally expressed IgV gene DP10 from the V_{11} family. Forty seven patients with RA were compared to 59 healthy controls. Genes of the DP 10 locus were amplified from genomic DNA by polymerase chain reaction (PCR) using primers designed for an allelic germline variable gene segment hv 1263. Polymorphic variants of DP10 were distinguished by the sensitivity of various restriction enzymes to naturally or PCR induced polymorphic recognition sites.

Results showed: 1) only DP10 and hv 1263 polymorphic segments were amplified by designed oligonucleotides, 2) DP10 and hv 1263 are most probably alleles of the same locus, 3) one of the polymorphic variants of DP10 locus is always present in every patient and control, 4) new allele of the DP10 gene was found with glutamic acid in position 73 and isoleucin in position 57 and 5) there are more homozygotes for Lys/Lys (position 73) in RA patients (29.2%) than in controls (12.5%).

Conclusions: Allelic variants of DP10 gene are not deleted in the germline repertoire of healthy controls or in RA patients. Significantly more homozygotes of DP10 with lysine in position 73 in patients with RA may be biologically important as genes from this locus frequently encode for rheumatoid factors. The number of alleles in this locus is higher than previously described.

The methodological approach used in this study is applicable also to other important IgV germline genes from which Humv3005 gene is currently under our investigation.

V.10. Class I heterodimers as a risk factor in murine ankylosing enthesopathy.
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Ankylosing enthesopathy (ANKENT) is a murine joint disease similar to human B27-associated peripheral joint disease, at the level of genetics, age/sex distribution, and pathology.
AB27 transgene has been shown to increase the risk for ANKENT in C57Bl/10 mice with the H-2a or H-2b haplotype. The H-2 haplotype itself is also a relative risk factor. In order to gain more insight into the pathogenic role of class I genes and their products, we studied whether class I heterodimers, i.e. heavy chain plus β-2-microglobulin (β-2-m) are necessary for the development of ANKENT. We also are examining whether the B27 transgene is still a risk factor when it is not expressed as a heterodimer with β-2-m. To address these questions, we bred homozygous β-2-m knockout, C57Bl/10 mice and then crossed in transgenics for B27 and human β-2-m. This resulted in 4 phenotypes: mice devoid of class I heterodimers (with or without the B27 transgene) and mice with heterodimers on the basis of hu-β-2-m (with or without B27). Although larger numbers of each of these 4 groups of mice must undergo life-long observation to allow definitive comparisons, it is already apparent that ANKENT does occur among mice devoid of class I heterodimers. Preliminary data indicate that in the presence of class I heterodimers, the frequency of disease is increased. So far there is no indication that the B27 transgene is a relative risk factor, when its product cannot associate with β-2-m. These findings argue in favour of a pathogenic effect of the B27 transgene product, rather than a mutation caused by the insertion of transgenetic material. They also suggest that the pathogenic effect of B27 is only exerted when the molecule is associated with β-2-m.

V.11. The shared epitope and COMP as predictors of erosiveness in recent onset RA.

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It has been proposed that presence of the “shared epitope” in single or double dose is a useful predictor of disease severity in RA. The data supporting this are, however, mainly derived from retrospective cross sectional studies. We are following a cohort of 150 RA patients included within two years (mean 11.5 months) from disease onset (Eberhardt KB et al., Rheumatol Int 1990, 10, 135-142 Forslind K. et al., Br J Rheumatol 1992, 31, 593-598). A group of 9/150 patients was distinguished by requiring large joint replacement during follow up (2-3 yrs). The median time from disease onset to surgery was only 3 years. Small joint damage progressed from 7 to 46 over two years in Larsen’s index. This group was compared to an age and sex matched control group with no evidence of large joint destruction and with a more benign early course (Larsen index 2 to 4 over two years). There was no difference with regard to Waaler-Rose positivity, but ESR was higher in the aggressive group (median 54 vs 20, range 29-105 vs 4-48). Cartilage oligomeric matrix protein, COMP, was increased in 8/9 aggressive but in none of the non-aggressive patients in early serum samples. The table shows results of genomic typing for DRB1 alleles. DRB1*0401 was more prevalent in the aggressive group (p = 0.04) but there was no overall difference between the groups with regard to number of disease susceptibility related epitopes. Thus genetic typing was of limited use as a predictor of joint damage in this cohort of early RA. If confirmed, COMP may be a more useful discriminator between aggressive and more benign RA than “shared epitope” status.

| DRB1-typing in early rheumatoid arthritis. |
|-------------------------------------------|
| **Aggressive (n=9)**                     | **Non-aggressive (n=9)** |
| 1 0401/0405 | 1 0401/0401 |
| 2 0401/0401 | 2 0401/0401 |
| 3 0401/0401 | 3 0401/0401 |
| 4 0101/0401 | 4 0102/0401 |
| 5 0401/0404 | 5 0101/15  |
| 6 0408/13  | 6 0101/15  |
| 7 0401/13  | 7 0101/15  |
| 8 0401/15  | 8 11/15    |
| 9 Deceased | 9 Not available |

Underlined alleles carry the “shared epitope”.

Session VI. New immunotherapeutic strategies in autoimmune rheumatic diseases.

VI.1. IL-4 or IL-13 transfected xenogenic fibroblasts in the treatment of collagen-induced arthritis in mice.

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**Objectives:** To study the anti-inflammatory effect of interleukin-4 (IL-4) and interleukin-13 (IL-13) in collagen-induced arthritis (CIA) using a strategy of gene therapy.

**Methods:** CIA was induced in DBA/1 mice by immunization of native bovine type II collagen (CII). Chinese hamster ovary (CHO) fibroblasts transfected with the murine IL-4 or IL-13 gene were inoculated (7.10^5 cells s.c.) on days 10 and 25 post-priming. Mice were then monitored blindly for signs of arthritis. TNF-α expression in spleen cells was assessed by quantitative RT-PCR on days 28, 45 and 60 after immunization.

**Results:** Incidence and severity of CIA were significantly reduced in the groups treated with either IL-4 or IL-13 gene transfected cells, compared to control groups treated with untransfected cells. High levels of cytokines was detected in the sera of animals after inoculation of transfected cells. TNF-α expression was dramatically decreased in mice treated with CHO IL-13 on day 45 compared to control groups.

**Conclusions:** Administration of IL-4 and IL-13 by gene therapy using transfected cells is a powerful method for delivery of cytokines in vivo and blocking the progression of CIA. Moreover, we have shown that the anti-inflammatory effect of IL-13 is associated with an important decrease in TNF-α mRNA levels.

VI.2. Cell surface structures of leflunomide treated MRL/LPR mice induce a transferable disease ameliorating activity in autoimmune MRL/LPR mice?

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At an age of 12 to 14 weeks MRL/lpr mice develop a spontaneous and progressive autoimmune disease which resembles human systemic lupus erythematosus (SLE). Leflunomide, an
isoXazol derivative currently evaluated in clinical trials against rheumatoid arthritis, has proven to be very effective in inhibiting the symptoms of this murine SLE-like disorder. A daily treatment with leflunomide strongly reduces the severity of this disease in MRL/lpr mice. Even after treatment discontinuation, the mice remain nearly symptom free. In analogy to the fact that leflunomide induces transferable tolerance in allogenic skin grafted rats, we asked if this could be due to the induction of self tolerance. After a washout and observation period fixed or vital splenocytes from leflunomide treated or non-treated animals were transferred into 8 weeks old healthy MRL/lpr mice. We found that the animals receiving vital splenocytes from untreated mice developed an accelerated illness whereas the mice grafted with spleen cells from the treatment group showed nearly no symptom of the disease. Thus, cell transfer into naive mice of leflunomide treated animals resulted in rectification of the characteristic symptoms of the severe disease compared to the animals receiving cells from untreated mice. Fixation of the cells from leflunomide treated animals prior to the adoptive transfer exerted a similar protective effect as vital lymphocytes. Thus, these data show that leflunomide induces transferable disease ameliorating activity and indicate that this activity may be mediated through surface structures on the splenocytes of treated MRL/lpr mice.

VI.3. Construction and expression of a recombinant anti-rat-CD4 miniantibody in E.coli.
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Specific binding of anti-CD4 monoclonal antibodies (mAbs) to CD4 molecules present on T cells infiltrating the inflamed synovium in arthritis allows therapeutic intervention and immunoscintigraphic imaging of the disease. The use of a complete anti-CD4 mAb, however, may hamper its full potential efficacy due to the large size (150kD) of the molecule and to non-specific interactions of its Fc-part with Fc-receptors on inflammatory cells.

To achieve better penetration of the mAb while retaining its specificity and affinity, the development of a so-called miniantibody (50kD) of the anti-CD4 mAb W3/25 was attempted, consisting of 2 single-chain-Fv-fragments connected by a dimerization domain.

The light and heavy chain of the mAb W3/25 were cloned and sequenced after screening a cDNA bank. A single-chain-Fv-fragment of the variable domains of the mAb chains was constructed. The fragment was then cloned into expression vectors for production and purification of the recombinant antibody. The antibody constructs will now be tested for their therapeutic efficacy and imaging properties in experimental arthritis models.

VI.4. Human anti-chimeric antibody (HACA) response in rheumatoid arthritis (RA) patients treated with, cM-T412, a chimeric anti-CD4 monoclonal antibody.
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Monoclonal antibodies (mAb) have been used to treat RA in a number of recent trials. Previously experience with murine antibodies showed that almost all patients developed an anti-globulin response with increased risk of anaphylaxis and reduced efficacy on re-treatment. The development of chimeric and humanised mAb raised the possibility of reducing the antigenicity of mAb therapy. We have used the chimeric anti-CD4 mAb, cM-T412, in RA patients and assessed the HACA response after therapy.

Nineteen patients with ACR defined RA were recruited. They were treated with four different dosing regimens. In group I: a single dose of 50 mg of cM-T412, group II: 50 mg of cM-T412 weekly for 4 weeks, group III: 50 mg daily for 5 days followed by weekly 50 mg of cM-T412 for 5 weeks, group IV: 50 mg daily for 5 days followed by a repeated course after 5 weeks. The number of patients in each group and the incidence of significant HACA response (titre > 1/20) are shown in table.

| Group | I | II | III | IV |
|-------|---|----|----|----|
| Total no. of patients | 4 | 3 | 6 | 6 |
| No. HACA + ve | 2 | 2 | 4 | 5 |
| % of patient | 50% | 67% | 67% | 87% |

Time course of HACA was different between group III & IV. The former became positive at week 2 or 4 and peaked at week 8 and 10. This is probably due to continued weekly treatment with cM-T412. Conversely, group IV patients showed a positive response from week 2 which declined or only increased slightly but showed a suddenly transient increase after re-treatment at week 6. All HACA titre declined after cessation of treatment. No statistical significant difference was found in the incidence of HACA between the 4 groups. Only 1/4 HACA positive patients in group III had an urticarial skin rash, no serious anaphylactic response was seen on weekly treatment.

In conclusion, chimeric anti-CD4 mAb therapy led to the development of HACA response in many patients but was less than that seen after treatment with murine antibodies. This may be the advantage of using chimeric or humanised mAb or it may be due to the tolerogenic effect of anti-CD4 mAb. Since the HACA titre declines after cessation of therapy, repeated infrequent treatments may be feasible.

VI.5. Humanised anti-TNFα antibody (Ab) in rheumatoid arthritis (RA).
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Introduction: Tumour necrosis factor alpha (TNFα) is potent pro-inflammatory cytokine which is thought to have a major
role to play in the pathogenesis of RA. A placebo-controlled trial using a chimeric anti-TNFα Ab showed that it produced significant disease improvement in RA. However, since humanised Abs are thought to be less antigenic than chimeric Abs, we tested the efficacy of a humanised anti-TNFα Ab, CDP571, in a two phase study.

Method: The initial phase was a double-blind, placebo-controlled, dose escalation study using three doses of CDP571: 0.1, 1 and 10 mg/kg. The second phase was an open study in which patients were treated with either 1 or 10 mg/kg of CDP571. Thirty-six RA patients were recruited; 12 received placebo and 8 each dose of CDP571 during the first phase. Treatment was given as a single intravenous infusion. Disease activity was measured by the EULAR core data set for disease assessment in RA and performed before and at 1, 2, 4 and 8 weeks after treatment. Disease activity score (DAS) was used to summarise results.

Result: Treatment was well tolerated. In the 10 mg/kg group, statistically significant reductions were seen in the visual analogue scale of pain, tender joint count, ESR and C reactive protein (p < 0.05). The median reduction in the DAS was 0.45 (p < 0.05). Despite significant reduction in the acute phase reactants, there was no change in serum interleukin-6 (IL-6) levels. After a single dose of CDP571, no significant anti-globulin response was seen. Serum levels of CDP571 showed a clear dose kinetic. The serum half life of CDP571 was 1-2 weeks. Twenty patients completed the second phase of this study; repeated treatment was again effective in suppressing disease.

Conclusion: TNFα is an effective treatment target in RA. A 10 mg/kg dose of CDP571 produced significant disease improvement lasting 4-8 weeks. Despite reduction in ESR and CRP, there was no change in IL-6. The long term efficacy of CDP571 in RA remains to be determined.

VI.6. Validity of newly developed response criteria for rheumatoid arthritis (RA).

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Purpose: The study construct, criterion, and discriminant validity of newly developed response criteria for RA.

Patients and methods: Response was retrospectively assessed in a double-blind trial comparing hydroxychloroquine (HCQ) and sulphasalazine (SASP). Disease activity parameters and functional capacity were measured at weeks 0, 12, 24, 36, and 48. X-rays of hands and feet were taken at weeks 0, and 48. Three response criteria were studied: WHO/ILAR 1, ACR 2, and DAS. DAS response was determined to comprise both change from baseline and reached level of disease activity. Good response was defined as a significant decrease in DAS from baseline (>1.2) and a reached DAS ≤2.4. Non-response was defined as a decrease in DAS ≤0.6 (±1 SE), or a decrease >0.6 and ≤1.2 and a reached DAS > 3.7. The remaining patients were classified as moderate responders. For construct validity the association between response at weeks 12, 24, 36, and 48 with x-ray progression at week 48 was studied. For criterion validity the association between response with relative change in functional capacity (HAQ) at weeks 12, 24, 36, and 48 was studied. For discriminant validity the number of responders on SASP and HCQ were compared at weeks 12, 24, 36, and 48.

Results: Patients with good DAS response at weeks 12, 36, and 48 had significantly less progression in joint destruction. No significant associations were found with WHO/ILAR and ACR criteria. At week 12 all response criteria were associated with functional capacity. At week 36 only good DAS responders showed significantly more improvement in functional capacity. At week 48 both DAS and ACR response was associated with functional capacity. At week 36 patient treated with SASP showed significantly better response (DAS) than patients treated with HCQ. With WHO/ILAR and ACR criteria no differences between the groups were apparent.

Conclusion: Despite the component functional capacity in the ACR response criteria, no significant association with relative change in HAQ-score at weeks 24 and 36 was apparent. Apart from this component ACR and WHO/ILAR criteria were almost identical. The DAS response criteria showed better construct, criterion, and discriminant validity than the WHO/ILAR and ACR response criteria.

1. World Health Organisation and International League Against Rheumatism. Proposed definition for ‘decreased inflammatory synovitis’. Rheumatology in Europe 1994, 23, 20-21.
2. Felson D. American college of rheumatology (ACR) preliminary criteria for improvement in rheumatoid arthritis (RA) trials. Abstract. Arthritis Rheum 1994, 37, S 195.

VI.7. Treatment of severe systemic lupus erythematosus (SLE) with monoclonal anti-CD4 antibodies (MAX.16H5).

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Monoclonal anti-CD4 antibodies (mAb) have been introduced in the therapy of SLE as an instrument to selectively affect the immune regulation by CD4+ cells (Lancet, 338, 1529-1530, 1991). Up to now eight patients (5 women, 3 men) suffering on intractable SLE have been treated with the anti-CD4 mAb MAX.16H5 in an open trial. Each patient had an exacerbation of disease with signs of nephritis despite aggressive immunosuppressive treatment cycles. They received 0.3 mg/kg of the anti-CD4 mAb intravenously on 7 consecutive days. In one patient the anti-CD4 infusion was interrupted at the first day due to urticaria. Five of the remaining seven patients showed a response to anti-CD4 therapy. The clinical improvement was associated with a significant reduction of SLAM-Index, anti-dsDNA antibodies and proteinuria. There was no persistent decrease of CD4+ cells. Three of these patients displayed an initial flare up of the disease after 3-4 weeks. Therefore, treatment with methylprednisolone bolus was given. Surprisingly, this led to a complete long-lasting remission. Two patients did not respond to anti-CD4 therapy. Beside the interruption of anti-CD4 infusion due to urticaria side effects appeared in two further patients: one patient had an aggravation of arthritis during the infusion period and the other developed herpes zoster 1 week after treatment.

The data show that anti-CD4 therapy may be effective in intractable SLE. A single treatment cycle may not be sufficient to persistently suppress disease activity, but it appears to re-
store the sensitivity to a therapy with methylprednisolone or immunosuppressive drugs. In vivo findings and in vitro experiments suggested different immunomodulatory effects of the anti-CD4 therapy in SLE affecting the CD4⁺ cells and the release of proinflammatory cytokines.

VI.8. Diverging health status and joint damage in rheumatoid arthritis.

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The specialist management of rheumatoid arthritis (RA) involves a paradox. Long term studies show inevitable progression of joint damage and functional decline. These question the effectiveness of therapy. But clinicians believe therapy is advantageous and maintain a positive attitude towards anti-rheumatic therapy, even in late disease. One possible explanation is a divergence between health status and joint damage in late RA. We tested this concept in a cross-sectional study of 150 RA patients (age: 58.7 ± 13 years, M/F = 33:117) with diverse disease durations (mean: 10.5, range: 0.5-45 years) attending a specialist follow up unit. We used the following instruments to assess RA: the EULAR core data set for disease activity, Larsen's method for X-ray damage, health status was measured by the Health assessment questionnaire (HAQ), the Nottingham Health Profile (NHP) and SF-20. There were three main findings. (1) HAQ, the Larsen score and the functional components of the NHP and SF-20 all showed highly significant correlations with disease duration, and were worse in late disease. They also correlated with each other. (2) The other domains of health status (social, general, pain, mental health, emotional and sleep) showed no relationship to disease duration. They were not worse in late disease. (3) These other domains of health status all showed highly significant correlations with patient's global assessments of disease activity, acute phase response indicated by CRP, early morning stiffness, HAQ, all items of SF20 and NHP. These results show patients' general health does not deteriorate with disease duration, though progressive joint damage is inevitable and is feature of disease duration.

We suggest rheumatologists and patients focus on general health as treatment beneficially influences these at all stages. Radiological damage may be less relevant, explaining the paradox of disease duration. They also correlated with each other. (2) The other domains of health status (social, general, pain, mental health, emotional and sleep) showed no relationship to disease duration. These and other phenomena got us interested in how the context in which a potentially disease-inducing antigen was introduced into an individual could determine the consequences as to disease development. Two types of experiments will be described and discussed:

(i) Collagen II or Myelin was mixed either alone or together with a number of other protein molecules with FIA and used for immunization of DA rats. We observed a complete inhibition of arthritis development after co-immunization with all tested immunogenic molecules (BSA, Ovalbumin, heterologous immunoglobulin, heat shock protein etc.), but not with non-immunogenic molecules (such as rat serum albumin). The inhibition was specific (EAE could be induced in rats protected against CIA), the protection was long lasting (no CIA could be induced upon re-immunization with collagen II in FIA 2 months later). The protection was furthermore not simply due to antigenic competition since similar levels of anti-collagen II IgG levels were recorded in diseased as in protected collagen-immunized rats.

(ii) In a second series of experiments we have tested different immunization schedules and different adjuvants together with collagen II or myelin. A striking effect of different adjuvants were noted, and will be presented at the meeting.

VI.10. New immunization strategies for specific prevention of induced organ-specific autoimmune disease.

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Immunization of DA rats with Freund's incomplete adjuvant (FIA) only induces a distinct but self-limiting polyarthritis. Inclusion of collagen II in the FIA causes a chronic and destructive arthritis. Inclusion of myelin basic protein causes a chronic encephalitis, but no arthritis. These and other phenomena got us interested in how the context in which a potentially disease-inducing antigen was introduced into an individual could determine the consequences as to disease development. Two types of experiments will be described and discussed:

(i) Collagen II or Myelin was mixed either alone or together with a number of other protein molecules with FIA and used for immunization of DA rats. We observed a complete inhibition of arthritis development after co-immunization with all tested immunogenic molecules (BSA, Ovalbumin, heterologous immunoglobulin, heat shock protein etc.), but not with non-immunogenic molecules (such as rat serum albumin). The inhibition was specific (EAE could be induced in rats protected against CIA), the protection was long lasting (no CIA could be induced upon re-immunization with collagen II in FIA 2 months later). The protection was furthermore not simply due to antigenic competition since similar levels of anti-collagen II IgG levels were recorded in diseased as in protected collagen-immunized rats.

(ii) In a second series of experiments we have tested different immunization schedules and different adjuvants together with collagen II or myelin. A striking effect of different adjuvants were noted, and will be presented at the meeting.

In conclusion, we see from the current experiments that consequences of a given autoimmune reaction can be changed drastically by changing the context in which the immunization takes
VI.11. Infusion of an anti TNF-α mAB in patients with rheumatoid arthritis: short term effects on monocytes

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Rheumatoid Arthritis (RA) is a chronic autoimmune disease characterized by symmetric arthritis leading to destruction on the joint anatomy and disability. Treatment of RA is non-specific and often requires change of the therapeutic regime due to side effects or non-response. Recently, in a double blinded, placebo controlled, multicenter trial, in vivo blockade of TNF-α by a single infusion of the chimeric TNF-α mAb cA2 has been proven to be highly effective in treatment of RA. In parallel to this trial, we performed experiments testing the ex vivo and in vitro effects of cA2 infusion. In this paper we describe a decrease in peripheral monocyte counts, reaching significance compared to placebo after one week predominantly in the high dose (10 mg/kg cA2) group. Expression of MHC class II or CD25 molecules on monocytes did not change. In parallel to the decrease of monocytes we found diminished concentrations of monocyte activation markers (soluble CD14 and Neopterin) in sea of cA2 treated patients. However, the ratio of monocyte activation marker per monocyte cell count did not change significantly. In addition, cross over experiments incubating purified RA patient’s monocytes with autologous T-lymphocytes or T-lymphocytes of a normal donor (ND) and vice versa for ND monocytes revealed no consistent changes in the proliferative response to SEB, OKT-3, CD28 mAb/PMA, or PHA. In conclusion, focusing on the monocyte level, we found a significant decrease of cell numbers without functional impairment per cell, even after infusion of high doses of cA2.

VI.12. IFNα2a and IL-6 antibody binding activities after IVIG treatment in rheumatic diseases

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Seven patients receiving IVIG for rheumatic disorders were tested for the occurrence of IgG anti-IL-1a, anti-IL-6 and anti-IFNα2a before and after IVIG. Binding was detected by means of 125I-traced human recombinant cytokines, molecular size chromatography and absorption of IgG to protein G-Sepharose containing columns. More than 90% of the cytokine binding to sera was to IgG. Serum IL-1a and IL-6 were measured by ELISA and the IFN activity by antiviral bioassay.

Patients received 0,4 g IVIG/kg (Gammagard or Sandoglobin), given on three consecutive days up to four times with intervals of one month (n = 15 pairs of sera). The increased IgG anti-IL-1a activities in the sera immediately after infusion was linearly correlated to the anti-IL-1a activity of the IVIG and indicated full recovery of the infused anti-IL-1a. Recovered anti-IL-6 and anti-IFNα2a activities were about 25%. Serum levels of IL-6 in preinfusion sera were 1-70 pg/ml (median: 5 pg/ml), in postinfusion sera 2-55 pg/ml (median: 13 pg/ml, p > 0,05). Serum IFN activities were 0 - 2,5 IU/ml (median: 0) before infusion compared to 0 - 0,5 IU/ml (median: 0 p < 0,02) after infusion. IL-1a was not measurable in the sera (< 100 pg/ml).

The data suggest that the levels of anti-IL-6 and anti-IFNα2a infused, were significantly reduced shortly after infusion. Circulating IFN and IL-6 in the patients is likely to be responsible for the clinical efficacy of this therapy. IL-6 mRNA levels declined immediately after infusion and reached pre-treatment values 1-4 weeks after the first mAb infusion and increased again before the onset of clinical deterioration. The fact that serum IFN activities were not detectable after infusion suggests that the clinical efficacy of this therapy may be mediated by the IL-6 cytokine family and not by IFN-α.

VI.13. Cytokine mRNA levels in peripheral blood mononuclear cells of patients with rheumatoid arthritis: impact of treatment with a monoclonal antibody to intercellular adhesion molecule-1

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Intercellular adhesion molecule-1 (ICAM-1, CD54) contributes to leukocyte extravasation into inflamed tissue. Because of the potential anti-inflammatory effects of blocking ICAM-1-mediated interactions, monoclonal antibodies (mAb) to ICAM-1 were used to treat patients with rheumatoid arthritis (RA). The current studies were carried out to examine whether cytokine mRNA levels in peripheral blood mononuclear cells (PBMC) might be correlated with the anti-inflammatory effects of anti-ICAM-1 mAb therapy. To investigate this, a sensitive and reproducible PCR technique to assess changes in the cytokine mRNA content of PBMC was employed that minimized in vitro manipulation of the cells. The assays were quantitative and sensitive enough to detect one molecule of cDNA per 1 - 100 PBMC. Before treatment, individual RA-patients demonstrated abnormally increased mRNA levels for IL-1β (3/7), IL-2 (3/7), IL-2R α-chain (1/7), IL-4 (1/7), IL-6 (3/7), IL-10 (4/7), and IFN-γ (3/7), whereas all patients had significantly elevated levels of IL-8 mRNA (p < 0.0001). Patients with markedly elevated expression of IL-1β, IL-8 and TNF-α before treatment did not benefit from anti-ICAM-1 mAb therapy. Anti-ICAM-1 mAb administration was followed by a prompt and transient increase of monocyte (IL-1β, IL-8, TNF-α) and T cell (IL-4, IFN-γ) cytokine mRNA. Elevation of IFN-γ mRNA expression throughout the treatment period reflected a temporary increase in the number of circulating CD3+CD4+ T cells, suggestive of altered recruitment or retention of activated Th1 cells into sites of inflammation, and was related to clinical efficacy. IL-6 mRNA levels declined immediately after the first mAb infusion and reached pre-treatment values variably after the five day treatment course. IL-6 mRNA levels were significantly reduced in patients responding to therapy one month after treatment. The data indicate that the pattern of cytokine mRNA derangements predict outcome to therapy with anti-ICAM-1 mAb and that alterations of cytokine mRNA expression induced by anti-ICAM-1 mAb might in part account for the clinical efficacy of this therapy.
VI.14. Purine enzyme activities in early rheumatoid arthritis: preliminary results.

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Background: The interest in purine enzyme activities in patients with rheumatoid arthritis (RA) has been inspired by two thoughts: firstly, RA patients may react very different to treatment with azathioprine (AZA) which is metabolised by purine enzymes while on the other hand it is known that abnormal purine enzyme activities may influence the outcome of AZA treatment (hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) deficiency (Lesch-Nyhan syndrome), 5'-nucleotidase (5'NT) deficiency) and secondly, RA can be characterised as a dysregulation of the immune-system while other diseases of the immune-system (severe combined immune deficiency syndrome, adenosine deaminase deficiency; T-lymphocyte malfunction, purine nucleoside phosphorylase (PNP) deficiency) are known to be related to deficiencies of purine enzymes. World literature counts only two reports on this issue showing that cross-sectionally studied, heterogeneous groups of RA patients and healthy controls differ in their purine enzymatic make-up. To rule out several confounders we have studied a homogeneous group of early RA patients in comparison with healthy controls.

Patients & Methods: We studied 23 patients and 28 control persons cross-sectionally (δ/2 ratio 13/10 and 11/17 and mean age 54 (23-73) and 44 (26-63) years respectively). Early RA was defined by a diagnosis of rheumatoid factor positive RA of less than one year and no previous treatment with disease modifying antirheumatic drugs. Only the use of NSAIDs and paracetamol was allowed. All patients met the 1987 ARA criteria for RA. Healthy control persons had to be free of joint complaints as well as their first degree relatives. Clinical assessments included a visual analogue scale of pain and general health, the Ritchie articular index and number of swollen joints and the ESR. The Disease Activity Score of 4 variables (DAS₄) was calculated. Laboratory assessments included routine blood analyses and determination in blood mononuclear cells of purine enzyme activities of 5'NT, PNP, HGPRT and TPMT. Activities of 5'NT should be adjusted for age and sex.

Results: 1) Concerning purine enzyme activities, adjusted for age and sex, no differences were found between patients and controls. 2) In both groups 5'NT activity significantly decreases with age (ρ=0.05 in the RA group and 0.009 in the control group). 3) Averaged over both groups 5'NT activity of males was significantly lower (ρ=0.007) compared to females. 4) Correlations coefficients between disease activity parameters, including DAS₄ and enzyme activities or age or sex were only very weak to weak and never significant (highest correlation (Spearman) ρ=0.37 between the ESR and 5'NT activity).

Conclusion: Neither early rheumatoid arthritis itself nor the disease activity exert influence on the basic activities of the purine enzymes 5'NT, PNP, HGPRT and TPMT. Activities of 5'NT should be adjusted for age and sex.

VI.15. A new high-performance liquid chromatography procedure to measure purine enzyme activities in rheumatoid arthritis.

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Background: Abnormal purine enzyme activities may influence the outcome of azathioprine (AZA) treatment (hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) deficiency (Lesch-Nyhan syndrome), 5'-nucleotidase (5'NT) deficiency) and are also related to some diseases of the immune-system (severe combined immune deficiency syndrome, adenosine deaminase deficiency; T-lymphocyte malfunction, purine nucleoside phosphorylase (PNP) deficiency). For several reasons it is very interesting to learn more about purine-enzyme activities in patients with rheumatoid arthritis (RA). Firstly RA patients may react very different to treatment with AZA which is metabolised by purine enzymes; secondly, RA can be characterised as a dysregulation of the immune-system and at last the scarcely world literature reports differences in purine enzyme activities between RA patients and healthy controls. Purine enzymes are often measured by radiochemical procedures in combination with separation by Thin Layer Chromatography (TLC). We found some distinct disadvantages of these procedures. Firstly, the coefficient of variation of quadruplicate measurements was relatively large; secondly, time-stability of enzyme activities of the freeze-dried blood mononuclear cells (BMC) stored at –20°C was not as expected over a period of at least 4 weeks; thirdly, regarding environmental protection, radiation hygienics and general applicability, radiochemical assays are less preferable nowadays and finally the fact that these procedures are rather time-consuming.

Method: Partly based on the radiochemical procedure (1), a High-Performance Liquid Chromatography (HPLC) procedure with ultra violet detection of substrate and products was developed. To separate substrate and product(s) a C18, reversed phase HPLC column is used in combination with a gradually changing two-buffer system gradient. Complete separation is reached in 27 min. Apart from the separation itself the three main differences between the radiochemical TLC and the HPLC procedure are, firstly, the larger number of cells used in the HPLC procedure while it still remains a micro-method; secondly, after lyophilization of the BMC essential steps (storage, incubation and separation) have a different sequence in the HPLC procedure: and, thirdly, the incubation is terminated by protein denaturation instead of cooling on ice. Reproducibility and time-stability were tested with PNP and 5'NT assays using both procedures. HGPRT was only tested for time-stability with the HPLC procedure. Tests were done with blood samples from healthy volunteers.

Results & Conclusion: For the tested assays, the HPLC procedure shows a significantly higher (ρ=0.002) reproducibility and also a considerably better time-stability to a period of at least 10 weeks. So, in conclusion, the new HPLC procedure is preferable to the radiochemical TLC procedure also because it is non-radiochemical and less time-consuming.
VI.17. Longterm follow up of total lymphoid irradiation therapy.

1. van Laarhoven J., Spierenberg G., de Bruyn C. J Immunol Meth 1980, 39, 47-58.

VI.16. Comparative effects of anti-CD4 monoclonal antibodies directed against different CD4-epitopes in the prevention of rat adjuvant arthritis.

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Treatment with the anti-CD4 monoclonal antibodies (mabs) W3/25 (IgG1) and OX35 (IgG2a), recognizing different epitopes of the CD4 molecule, prevents adjuvant arthritis (AA) in the rat. Clinical efficacy may be related to induction of tolerance against the arthritogenic Mycobacterium tuberculosis (MT). The mab RIB52/2 (IgG2a), directed against yet another CD4 epitope, has already shown tolerogenic properties in rat kidney transplantation models, but has not yet been investigated in AA. Therefore, a comparison of the relative efficacies of these 3 anti-CD4 mabs in preventing AA and in affecting the T-cell response to the inducing arthritogen was undertaken.

Female Lewis rats were immunized with 0.5 mg MT in paraffin oil (day 0) and treated with 3 mg RIB52/2, 2 mg OX35 or 3 mg W3/25 i.p. on days 1, 0, 3 and 6 before/after induction of AA. The effects of treatment were measured by monitoring arthritic score, paw volume, and body weight. On day 13, a DTH reaction against MT and purified protein derivative (PPD) of MT was performed in the ear of mab or vehicle-treated AA rats. In addition, the 3H-thymidine uptake of purified CD4⁺ spleen T-cells from AA rats (day 13) in response to stimulation with Con A in the presence of dendritic cells was assessed in vitro.

W3/25 and OX35 suppressed AA from the early phase. RIB52/2, in contrast, first accelerated the onset of clinical signs and then significantly ameliorated the late phase of AA (from day 19). Unexpectedly, the DTH reaction of MT at day 13 of AA was not decreased upon treatment with any of the anti-CD4 mabs; in the case of OX35 the DTH was even significantly higher than in vehicle-treated AA rats. Furthermore, the in vitro proliferation of purified CD4⁺ spleen T-cells in response to ConA was increased in all anti-CD4 mab-treated groups showing clinical benefit interestingly, the increased in vitro proliferation rate of CD4⁺ T-cells significantly correlated with the clinical amelioration of the treated animals in vivo.

The therapeutic efficacy of anti-CD4 mabs in AA may be based on T-cell dependent, regulative mechanism, and not on tolerance to the inducing antigen.

VI.17. Longterm follow up of total lymphoid irradiation therapy.

R. Westhovens, J. Verwilghen, M. Vanderschueren, M. Vandeputte, J. Dequeker. University Hospitals K.U. Leuven, Belgium.

Total lymphoid irradiation (TLI) was used in the early '80s in an attempt to control disease activity in severe rheumatoid arthritis. Short-term side effects and efficacy were acceptable at that time (1). However, the use of this mode of non-specific T-cell directed therapy was discontinued with the advancement of more specific anti-lymphocyte treatments.

At our center, 20 RA patients were treated in a randomized study with TLI versus cytotoxic drug treatment daily [cyclophosphamide (1 mg/kg) + azathioprine (1 mg/kg)] between January 1983 and December 1985 (2). We studied the long-term effects of these treatments by retrospective analysis of the hospital charts. One patient was lost in follow up in the cytotoxic drug group. Morbidity and mortality were much higher in the TLI group. Ten years after TLI treatment, 70% of the patients had died, whereas mortality was only 22% in the cytotoxic drug group. The number of hospital admissions, as well as the number of necessary orthopedic interventions, was higher in the TLI-treated group (x = 5.2; x = 2.1 respectively) when compared to the cytotoxic group (x = 4.0; x = 1.3). In addition, in the TLI-treated group 2 patients developed multiple myeloma and 1 patient developed amyloidosis, however, no hematological malignancies were seen in the control group.

Conclusion: TLI has severe long-term side effects compared to the combined cytotoxic drug treatment group.

1. Westhovens R. et al. Rhumatologie 1988, 40, 141-145.
2. Westhovens R. et al. Clin Exp Rheumatol 1987, 5, 330.

Session VII. Pathogenetic mechanisms in vasculitis syndromes + autoimmunity.

VII.1. Antibodies to proteinase 3 (C-ANCA) modulate the function of human renal tubular epithelial cells (TEC).

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Circulating autoantibodies directed against neutrophil cytoplasmic antigens (ANCA), especially those with specificity for proteinase 3 (PR-3) are specific markers for systemic vasculitides such as Wegener's granulomatosis (WG) and microscopic polyarteritis. The renal lesions of these ANCA-related diseases are characterized by an accumulation of cells in the extracapillary space of glomeruli and tubuli including monocytes/macrophages, tubular epithelial cells (TEC) and T-lymphocytes. Renal biopsy studies reported the presence of ICAM-1 and VCAM-1 expression on renal TEC. In our study we investigated the role of anti-PR3 antibodies on the expression of adhesion molecules and on the production of IL-6 by TEC. Material and methods: TEC were isolated and cultured from kidney cortex obtained from the normal pole of kidneys removed at our center. The renal lesions of these ANCA-related diseases are characterized by an accumulation of cells in the extracapillary space of glomeruli and tubuli including monocytes/macrophages, tubular epithelial cells (TEC) and T-lymphocytes. Renal biopsy studies reported the presence of ICAM-1 and VCAM-1 expression on renal TEC. In our study we investigated the role of anti-PR3 antibodies on the expression of adhesion molecules and on the production of IL-6 by TEC. Material and methods: TEC were isolated and cultured from kidney cortex obtained from the normal pole of kidneys removed for renal carcinoma. The TEC were characterized as tubular cells by immunofluorescence technique: positive staining for ulex-lectin and cytokeratine, negative staining for factor VIII-related antigen and CD14. Cultured TEC were incubated with affinity purified anti-PR3 antibodies. The expression of VCAM-1 and ICAM-1 was determined by cyto-ELISA technique. The production of IL-6 by TEC was measured by ELISA technique. Results: ICAM-1 and VCAM1 were constitutively expressed on cultured TEC. After incubation with anti-PR3 antibodies VCAM-1 expression increased significantly with a maximum after 4 hours. The expression of ICAM-1 showed a slight in-
Electrophoretic mobility shift assays were performed with syn-
that showed > 2-fold increase in COL1A1 mRNA levels in
broblasts from patients with early diffuse cutaneous disease.

VII.3. Transcriptional regulation of cO(I) procollagen
vovirus B19 specific oligonucleotide probes. Strict measures
were taken to avoid false positive results. In contrast to the ini-
tial case, no evidence for recent parvovirus B19 infection was
found in any of the 47 patients studied.

VII.4. Induction of a lupus-like autoimmune syndrome in
ormal mice by intraperitoneal injection of pristane.
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Intraperitoneal (i.p.) injection of pristane is used to obtain
mAb-enriched ascitic fluid. After 6 mo, pristane also induces
plasmacytomas and chronic arthritis resembling rheumatoid ar-
thritis in susceptible mice. Female BALB/c ByJ mice were in-
jected once i.p. with 0.5 ml pristane, and sera were analyzed
monthly for autoantibodies by immunoprecipitation (IPP), western
blot, and ELISA. Unexpectedly, anti-Su antibodies were detected in 3/11 mice
by IPP as early as 1-2 mo, followed by anti-U1RNP antibodies
(3/11 at 3 mo). At 6 mo, 45% had anti-Su, 45% had anti-
U1RNP, and 82% had anti-U1RNP, U5RNP and/or anti-Su.
Sera from pristane primed mice reacted with the 70K and A
protein patterns or intensities of protein - DNA complexes with any of
the oligonucleotides tested, thus indicating that other regulatory
sequences are involved in the upregulated COL1A1 gene
expression in SSc. We then transfected these fibroblasts with
constructs containing - 804 bp from COL1A1 promoter ligat-
ed to the CAT reporter gene, or with constructs containing in
addition to the promoter a portion of the first intron (+380 to
+1440 bp). The results showed 2 to 4 fold increases in CAT
activity driven by constructs containing the intronic sequences in
SSc fibroblasts in comparison to normal cells. These data
suggest that sequences located in the first intron of COL1A1
gene may play an important role in the upregulation of its ex-
pression in SSc.

VII.2. Wegener's granulomatosis; no conclusive evidence for
parvovirus B19 etiology.
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ens Serumisstitute, Copenhagen, Denmark.

An etiologic relation between parvovirus B19 infection and sys-
temic necrotising vasculitis has recently been suggested in sev-
eral reports. Particularly in association with Wegener's granu-
logmatosis the symptoms in the early disease resemble those of
acute parvovirus B19 infection (upper respiratory tract symp-
toms, arthralgia/arthritis, skin rash). After our initial case of a
12-year old boy with Wegener's granulomatosis and chronic parvovirus B19 infection (Arthritis Rheum 37, 1707, 1994), we have
analysed paired sera from 10 patients with recent onset Wegener's granulomatosis (6 with generalised disease and 4 with limited
disease). In addition, 37 sera from patients with different systemic vasculitides were studied. Parvovirus B19
specific circulating IgM and IgG antibodies were determined using an ELISA with a commercial recombinant antigen. Par-
vovirus B19 specific DNA sequences were amplified from two dif-
ferent segements of the parvovirus B19 genome; the prod-
ucts were further studied by Southern hybridisation using par-
vovirus B19 specific oligonucleotide probes. Strict measures
were taken to avoid false positive results. In contrast to the ini-
tial case, no evidence for recent parvovirus B19 infection was
found in any of the 47 patients studied.

The mechanisms involved in the regulation of collagen gene
transcription during physiologic and pathologic processes are not
completely understood. Transforming growth factor β1 (TGFB1)
has been implicated in the pathogenesis of various diseases characterized by fibrosis including SSc. We previous-
ly identified a TGFB1-responsive element located between
-174bp and -84bp from the transcription start site of the hu-
man COL1A1 promoter. Here we investigated the role of se-
quences located in this region which are potential binding sites
for the transcription factors Sp1, NF-1, and AP-1 in the regu-
lation of COL1A1 gene expression in SSc fibroblasts. SSc fi-
broblasts from patients with early diffuse cutaneous disease that showed > 2-fold increase in COL1A1 mRNA levels in
comparison to normal fibroblasts were used for these studies. Electrophoretic mobility shift assays were performed with syn-
thetic oligonucleotides corresponding to regions with homol-
ogy to Sp1 (-164bp/-142bp and -93bp/-77bp), AP-1 (-175bp/
160bp) and NF-1 (-102bp/-85bp) binding elements. No differ-
ences were found between nuclear extracts obtained from cul-
tured fibroblasts from 3 normal and 6 SSc individuals in the
patterns or intensities of protein - DNA complexes with any of
the oligonucleotides tested, thus indicating that other regulatory
sequences are involved in the upregulated COL1A1 gene
expression in SSc. We then transfected these fibroblasts with
constructs containing - 804 bp from COL1A1 promoter ligat-
ed to the CAT reporter gene, or with constructs containing in
addition to the promoter a portion of the first intron (+380 to
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comparison to normal fibroblasts were used for these studies. Electrophoretic mobility shift assays were performed with syn-
VII.5. Mast cells in salivary glands of Sjögren's syndrome.
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Salivary gland is the main target tissue of the histopathologic lesion in Sjögren's syndrome (SS). Tissue mast cells are multifunctional immune cells and have been implicated in inflammatory reactions. Different subtypes of mast cells in several tissues and in a variety of inflammatory conditions have been demonstrated. In order to recognize the putative role of these cells in the histopathologic lesion of SS, mast cells were studied in labial salivary glands obtained from 18 patients with primary SS. Eight patients with systemic lupus erythematosus (SLE), one patient with rheumatoid arthritis (RA), one patient with sarcoidosis, one patient with Hodgkin's disease, one patient with urticaria and 10 individuals with normal salivary gland biopsy. There was no correlation between the intensity of focal lymphocytic infiltration and the morphometrically analyzed number of mast cells stained positive with toluidine blue (p < 0.16). In contrast, a significant correlation between the number of mast cells and the degree of fibrosis (p < 0.0001) as well as the fatty cells (p < 0.0004) was observed. The mast cells were distributed mainly in the interlobular space where fatty cells and fibrosis were present. Chymase, c-kit (CD117) and carboxypeptidase stained with mAb1257 (clone B7), VBSB8 and 10C3 and mAb a-carboxypeptidase-α respectively showed almost the same positive cells than staining with toluidine blue. Tryptase staining with mAb 1222 (clone G3) and AA1 showed significantly fewer positive cells than staining with toluidine blue in all tissues (range of tryptase positive cells/toluidine positive cells: 0.06-0.5). In contrast in 5 skin biopsies from scleroderma patients tryptase positive mast cells were almost equal to those staining positive with toluidine blue. Mast cells in the salivary gland were not degranulated except in the patients with urticaria. In conclusion, the majority of mast cells in salivary gland tissue do not produce tryptase. It seems that they strongly collaterate with the fatty cells and the fibrotic process in this tissue independently of degree of inflammation in Sjögren's syndrome exocrine glands.

VII.6. Antibodies to proteinase-3 stimulate the endothelin-1 production by human renal tubular epithelial cells (TEC) and human umbilical vein endothelial cells (HUVEC).
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Endothelin-1 (Et-1) has been initially identified as a potent vasoconstrictor peptide with 21 amino acid residues, produced by cultured endothelial cells. In the mean time, it is well-known that Et-1 has a mitogenic effect on mesangial cells, dermal fibroblast, fibroblast-like synovial cells and vascular smooth muscle cells. Increased serum levels of Et-1 are found in patients with acute renal failure, sepsis and ARDS. Circulating autoantibodies directed against neutrophil cytoplasmic antigens (ANCA), especially those with specificity for proteinase 3 (PR-3) are specific markers for systemic vasculitides such as Wegener's granulomatosis (WG) and microscopic polyarteritis. In our study we investigated the role of anti-PR3 antibodies on the production of Et-1 by TEC and HUVEC. Material and methods: TEC were isolated and cultured from kidney cortex obtained from the normal pole of kidneys removed for renal carcinoma. The TEC were characterized as tubular cells by immunofluorescence technique: positive staining for ulex-lectin and cytokeratine, negative staining for factor VIII-related antigen and CD14. HUVECs were isolated and cultured under standard conditions. Cultured TECs and HUVECs were incubated with affinity purified anti-PR3 antibodies. The production of Et-1 was measured by radioimmunoassay-technique. Results: After incubation with anti-PR3 antibodies Et-1 production increased significantly by HUVECs as well as by TECs. Interestingly, the Et-1 production by TECs (250 pg/ml) was nearly five times as high as the Et-1 production by HUVECs (52 pg/ml). Normal serum levels range up to 5 pg/ml. Conclusions: The c-ANCA mediated increased production of Et-1 by HUVECs and by TECs may explain systemic complications like glomerulonephritis and hypertension in patients with Wegener's granulomatosis.

VII.7. In vitro suppression of serum anti-proteinase 3 (PR3) antibodies binding to PR3 by anti-idiotypic antibody 5/7 in Wegener's Granulomatosis.
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The idiotype Id-5/7 was originally defined on the human monoclonal anti-PR3 antibody, designated WGH1. In a previous study we demonstrated that the anti-idiotypic monoclonal antibody 5/7 inhibits the anti-PR3 activity (approx. 90%) of WGH1. The possibility that this idiotype 5/7 may be present on autoantibodies of different specificities was explored using sera from 87 WG patients with anti-PR3 antibodies, 43 SLE patients with anti-DNA antibodies and 101 healthy donors. Serum levels of idiotype marker (5/7 Id) were higher than normal in 43 of 87 patients (49%) with SLE, compared with only 8 of 43 patients (18%) with SLE. Levels of the 5/7 idiotype tended to correlate with levels of antibodies to PR3, but in some cases the clinical status was reflected better by the levels of anti-PR3 antibodies than by levels of the idiotype 5/7. Furthermore we investigated the modulation of autoantibody activity by means of anti-idiotypic antibody 5/7. We demonstrated that the binding of anti-PR3 Abs to PR3 can be blocked by the anti-Id 5/7 between 30-50% in 11 from 19 patients. These in vitro findings provide further evidence for the presence of an idiotypic network in WG. Studies are in progress to investigate possible therapeutic application of the anti-Id 5/7.

VII.8. Sjögren's syndrome: comparison of the European Community Study Group criteria with the immunohistologic criteria. Preliminary results.
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Objective: To compare the preliminary diagnostic criteria for primary Sjögren's syndrome (SS) as proposed by the European Community Study Group (ECSG) 1 with the quantitative im-
munohistologic (QIH) criterium (%IgA containing plasma cells < 70) as set up by Bodeutsch et al.²

**Patients and Methods:** The records of 149 patients that underwent a sublabial salivary gland biopsy from January 1988 to May 1994 were examined with special attention focused on clinical and serological findings contributing to the ECSG criteria. Quantitative immunohistologic examination was performed on all sublabial salivary glands.

**Results:** Eighty patients could be classified as either definite (4 out of 6 criteria positive) or probable (3 out of six criteria positive) SS according to the ECSG criteria. Only 40% of these patients fulfilled the QIH criterium for SS. Most of the patients that did not fulfill the QIH criterium were classified as probable SS by the ECSG criteria. Eight patients that met the QIH criterium could not be classified as definite or probable SS by the ECSG criteria. Further analysis of these patients showed serologic findings (SS-A and/or SS-B autoantibodies) suggestive for SS.

**Conclusion:** These preliminary results suggest a higher specificity for the QIH criterium than the ECSG criteria. Further prospective comparative studies are needed.

1. Arthritis and Rheumatism 36, 340-347, 1993
2. Arthritis and Rheumatism 35, 1075-1087, 1992.

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**VII.9. The MxA Protein: a new marker for disease activity in systemic lupus erythematosus.**

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The MxA protein is an intracellular 78 kDa protein, which is specifically inducible in human leukocytes by type I interferons. Over a period of two years 62 patients with a systemic lupus erythematosus diagnosed according to the revised ARA-criteria were screened for the presence of MxA in their leukocytes using a specific ELISA. The disease activity of these patients was determined by employing the scoring system SLE-DAI.

The measured MxA concentrations in whole blood correlated with the disease activity of all 62 patients (p < 0.01). They also showed a significant association with anti-dsDNA antibodies (p < 0.03) and CH 50-levels (p < 0.02). In particular, all eight patients with a completely inactive disease (clinical activity score 0) showed MxA values below 3 mU/1000 l. In contrast, all 16 patients with active disease possessed MxA concentrations higher than 8 mU/1000 l. Upon treatment with prednisolone 14 of these 16 patients responded to treatment and showed a drastic MxA level reduction. These data suggest that the MxA protein is a useful marker to discriminate between patients with active and inactive and between patients with prednisolone-sensitive and insensitive disease. Furthermore, these results indicate a pathophysiological role of the activated IFN-α-system in SLE patients.