THU0051 **PROLIFERATION AND STABILITY OF CYTOKINE EXPRESSION OF IL-4 OR IL-10 SECRETING T HELPER CELLS**

¹AH Radbruch, ¹M Loehning, ¹A Richter, ¹T Stamm, ¹O Soezeri, ¹HD Chang, ¹L Tykocinski, ²M Assenmacher. ¹Cell Biology, Deutsches Rheuma-Forschungszentrum Berlin (DRFZ), Berlin, Germany; ²Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

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Background The cytokines IL-4 and IL-10 have been implicated in the regulation of T cell proliferation and differentiation. IL-4 drives the development of Th2 cells from naive precursors and it is supposed to enhance T cell proliferation, whereas IL-10 has been suggested to support the differentiation of regulatory Th cells and to negatively affect T cell proliferation.

Objectives To analyse the proliferation and stability of cytokine expression of individual cytokine-producing T cells.

Methods We established the cellular affinity matrix technology for the immunofluorescent identification and isolation of IL-4- or IL-10-secreting cells. With this technology we purified IL-4- or IL-10-secreting and non-secreting Th cells, labelled them with the fluorescent dye CFSE to track their proliferation after antigen-specific or polyclonal restimulation, and analysed their cytokine expression upon later restimulation.

Results Previously, we have shown that naive Th cells have to enter the initial S phase of the first cell cycle to respond to an IL-4 signal by production of IL-4 or IL-10 upon restimulation. By direct counterstaining of cytokines and DNA content we now tested whether in general cytokine production is restricted to certain cell cycle phases, which we found not to be the case. The isolated IL-4+ and IL-4- T cells showed the same proliferation behaviour over 6 days, and the sorted IL-10+ cells were not inhibited in their proliferation compared with the IL-10- T cells. Thus, neither IL-4 nor IL-10 production by an individual T cell directly influences the proliferation of the same cell. When we analysed the stability of cytokine memory of the sorted T cells in later restimulations, a substantial fraction of the purified cytokine-producing cells did not reexpress the cytokine that it had expressed in an earlier stimulation.

Conclusion Thus, there is a certain degree of instability in the cytokine memory of Th cells, the molecular basis of which we are currently analysing.

THU0052 **HEPARIN AFFIN REGULATORY PEPTIDE (HARP) IN ACUTE AND CHRONIC ARTICULAR DISEASES**

¹M Hérault, ²F Lioté, ²R Champy, ¹D Barritault, ¹J Courty. ¹Laboratoire de Recherche Sur La Croissance Cellulaire, La Réparation Et La Régénération Tissulaires (CRRET), UPRES-a CNRS 7053, Université Paris XII-Val de Marne, Creteil, France; ²Laboratoire de Radiologie Expérimentale Et de Physiopathologie Articulaires (JE2149) (Centre Viggo Petersen), Université Paris VII et Hôpital Lariboisière (Assistance Publique-Hôpitaux de Paris [AP-HP]), Paris, France

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Background Heparin affin regulatory peptide (HARP), also known as pleiotrophin (PTN) is a potent mitogen for various cell types and an angiogenic polypeptide implicated in tumour development.¹⁻⁴

Objectives Since the progression of the pannus is associated with angiogenic processes and synovial membrane hypertrophy,^{5,6} the involvement of HARP in comparison with two other growth factors including MK and VEGF in rheumatoid pathologies was investigated.

Methods Presence of HARP in human synovial fluids and sera from patients was investigated by immunoassay. The distribution of HARP mRNA and protein was studied by *in situ* hybridization and immunohistochemistry respectively.

Results Comparative analysis showed that HARP as well as MK concentrations were significantly higher ($p < 0.001$) in synovial fluids from patients with inflammatory arthritis including rheumatoid arthritis ($n = 14$), ankylosing spondylitis ($n = 8$), chondrocalcinosis ($n = 7$) and gout ($n = 11$) than from patients with osteoarthritis ($n = 29$). In contrast, no significant difference was observed between inflammatory and non inflammatory diseases for VEGF. In addition, serum HARP and MK concentrations were significantly higher in patients with arthritis ($n = 37$) than in normal subjects ($n = 18$) ($p < 0.001$). Immunohistological studies demonstrated HARP within the synovial lining cells, endothelial cells of new capillaries, and macrophages of inflammatory synovial tissue. *In situ* hybridization studies revealed that synovial lining cells and vascular endothelial cells expressed high levels of HARP mRNA.

Conclusion These results suggest that HARP, in concert with other cytokines and growth factors, may play a role in the pathophysiology of acute and chronic synovitis in conditions such as crystal-induced arthritis and rheumatoid arthritis, respectively.

REFERENCES

- Li YS, Milner PG, Chauhan AK, Watson MA, Hoffman RM, Kodner CM, Milbrandt J, Deuel TF. *Science* 1990;**250**:1690-4
- Courty J, Dauchel MC, Caruelle D, Pederiset M, Barritault D. *Biochem Biophys Res Commun*. 1991;**180**:145-51
- Bernard-Pierrot I, Delbé J, Caruelle D, Barritault D, Courty J. *J Biol Chem*. in press
- Choudhuri R, Zhang HT, Donnini S, Ziche M, Bicknell R. *Cancer Res*. 1997;**57**:1814-19
- Peacock DJ, Banquerigo ML, Brahn E. *J Exp Med*. 1992;**175**:1135-8
- Koch AE. *Ann Rheu Dis*. 2000;**59**(Suppl 1):165-71

THU0053 **INHIBITION OF ARACHIDONIC ACID RELEASE FROM HUMAN PERIPHERAL MONONUCLEAR CELLS BY HEAT SHOCK TREATMENT AND GELDANAMYCIN**

S Szanto, P Csermely, I Kovács, J Csongor, GY Szegedi, S Sipka. *Third Department of Medicine, University of Debrecen, Debrecen, Hungary*

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Background Arachidonic acid and its derivatives play an important role in inflammation including arthritis.

Objectives To investigate the effects of heat shock (HS) treatment and geldanamycin (GM) on the release of arachidonic acid (AA) from human peripheral blood human mononuclear cells (PBMCs) (monocytes and lymphocytes).

Methods Mononuclear cells prepared from blood of healthy subjects were preincubated with 3H-AA. The release of 3H-AA incorporated into the membrane was studied after pretreatment of cells by HS (43°C, 1 h), GM and prednisolone sodium succinate (PRED). The activation of AA producing enzymes took place by the addition of phorbol 12-myristate 13-acetate (PMA) or by the combination of PMA and calcium ionophore A 23187.

Results The release of AA by mononuclear cells was dose dependently inhibited by GM similarly to PRED. The treatment of cells by HS also inhibited the release of AA. An additivity was found in the inhibitions induced by GM, PRED and HS.

Conclusion HS treatment can decrease the release of AA from human mononuclear cells. GM also results in a dose dependent inhibition of AA release. In the mechanism of additivity In the mechanism of additivity found when HS and GM were applied

concurrently, the increased blocking of mitogen-activated kinase cascade could be involved. In the additivity induced by HS + PRED and GM + PRED combinations different pathways, the MAPK (HS, GM) and some transcription factors (PRED) simultaneously could play a role. The inhibitory effect of GM on the AA release from mononuclear cells can be a new element in its antiinflammatory actions.

THU0054 REDUCTION OF IL-18 SERUM LEVELS IN RHEUMATOID ARTHRITIS DURING SHORT TERM-TREATMENT WITH INFLIXIMAB

M Bombardieri, V Pittoni, F Conti, FR Spinelli, A Spadaro, V Riccieri, C Alessandri, R Scrivo, G Valesini. *Divisione Di Reumatologia, Università "La Sapienza", Roma, Italy*

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Background IL-18 is a recently identified cytokine thought to play a role in many autoimmune diseases including RA. Initially known as INF γ -inducing factor, IL-18 was shown to be able to exert its inflammatory actions also upregulating, at least *in vitro*, other cytokines such as TNF α . It has also been shown that TNF α induces the expression of IL-18 in synovial tissues, an observation which raises the possibility of a positive feedback between these two cytokines. It has recently been demonstrated that TNF α -blocking treatments in RA patients result in the down-regulation of serum levels of other proinflammatory cytokines, thus providing an additional anti-inflammatory effect.

Objectives The aim of this study was to measure serum IL-18 levels in RA patients treated with monoclonal anti-TNF α antibody (Infliximab) and to evaluate the main clinical and laboratory parameters.

Methods Ten patients with RA, non responding to DMARDs, were enrolled. Infliximab was administered i.v. at a dose of 3 mg/Kg at baseline, after 2, 6 and 14 weeks. Sera from all patients were collected before each infusion and stored at $\pm 20^{\circ}\text{C}$ until tested using an enzyme-linked IL-18 assay. Statistical analysis was performed using non parametric methods for paired samples.

Results Serum IL-18 levels were significantly reduced from baseline value (median/25 $^{\circ}$ -75 $^{\circ}$ percentile 1008/465-1676 pg/ml) after 2 weeks (185/0-1241 pg/ml, $p < 0.005$), 6 weeks (175/0-644, $p < 0.01$) and 14 weeks (59/0-437, $p < 0.02$). No significant decrease was observed between IL-18 levels at 2, 6 and 14 weeks. After 2 weeks ESR ($p < 0.005$) and CRP ($p < 0.02$) values were significantly reduced from baseline, while we observed a significant increase in ESR ($p < 0.02$) and CRP ($p < 0.03$) values at 6 weeks as against 2 weeks, without any significant change at 14 weeks. There was a significant decrease from baseline in both the number of tender joints ($p < 0.02$) and the patient global evaluation of disease activity ($p < 0.02$) at 6 weeks, while no correlation between serum IL-18 levels and clinical/laboratory parameter was found at any time-point.

Conclusion Our results demonstrate that anti-TNF α therapy downregulates serum IL-18 levels already after 2 weeks of treatment and maintains them significantly lower than the baseline at 6 and 14 weeks. Thus, modulation of IL-18 could contribute to the anti-inflammatory actions of TNF α -blocking treatments.

Health services, economics and outcome research

AB0231 PHARMACOECONOMIC ANALYSIS OF THE TREATMENT OF RHEUMATOID ARTHRITIS WITH LEFLUNOMIDE IN COMPARISON WITH THE COMBINATION OF INFLIXIMAB AND METHOTREXATE

¹C Rubio-Terrés, ¹F Romero, ²A Domínguez-Gil. ¹Pharmacoeconomics Unit Scientific Department, Aventis Pharma, S. A., Madrid, Spain; ²Pharmacy Department, Hospital Universitario de Salamanca, Salamanca, Spain

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Background

Objectives To carry out a pharmacoeconomic analysis to compare the efficiency of two rheumatoid arthritis treatments in Spain.

Methods The study consisted of a systematic review of efficacy and toxicity as well as a cost-minimization analysis, carried out using a pharmacoeconomic model, comparing the treatment with leflunomide and the combination of infliximab and methotrexate during one year.

Results Clinical trials directly comparing both treatments are not available. The response rate ACR20 combined, after one year, was 53.0% (CI95%: 49.2%-56.4%) with Leflunomide and 42.0% (CI95%: 31.2%-52.5%) with the combination of Infliximab and Methotrexate ($P = 0.051$). There were no statistically significant differences in the ACR50 response (27.0 vs 21.0, respectively; $P = 0.19$). There were fewer infections with Leflunomide than with the combination, both respiratory (15.0% and 34.0%, respectively; $P = 0.0003$) as well as urinary (0.0% and 3.0%, respectively; $P = 0.10$). In the basic case, the cost per patient of a yearly treatment with Leflunomide or with Infliximab and Methotrexate is estimated to be 315,023 Ptas (Spanish pesetas) (1,893 euros, ?) and 2,596,286 Ptas (15,604 ?), respectively. Therefore, the incremental cost of the combined treatment would be 2,281,263 Ptas (13,711 ?). The sensitivity analysis was carried out using the minimum and maximum costs given by the standard deviations of the unit costs and by modifying other variables, as no significant differences compared to the basic case were found.

Conclusion The cost per patient after one year of treatment is higher with the combination of Infliximab and Methotrexate compared to Leflunomide, this is basically due to the higher acquisition cost of Infliximab.

AB0232 RELIABILITY AND RESPONSIVENESS OF THE RADAI, A SELF-ASSESSED RHEUMATOID ARTHRITIS DISEASE ACTIVITY INDEX

J Fransen, A Forster, D Uebelhart, BA Michel. *Department of Rheumatology and Institute of Physical Medicine, University Hospital Zurich, Zurich, Switzerland*

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Background The Rheumatoid Arthritis Disease Activity Index (RADAI) is a self-administered questionnaire on signs and symptoms of rheumatoid arthritis (RA). The main goal of the RADAI is to evaluate the course of RA disease activity over time. The RADAI consists of 5 items and produces a single index from 0-10; higher scores are indicating higher levels of disease activity. The RADAI has shown to be cross-sectionally valid¹ and responsive to clinical important worsening of disease activity.²

Objectives The study objectives are: 1) to determine the test-retest reliability of the RADAI, and 2) to determine if the RADAI is responsive to clinical important improvements in disease