THU0115 NOCTURNAL MELATONIN SERUM LEVEL RHYTHM IN RHEUMATOID ARTHRITIS

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Background The pineal hormone melatonin (MLT) exerts a variety of effects on the immune system. MLT activates Th1 lymphocytes and monocytes; moreover MLT enhances the production of inflammatory cytokines, like IL-1, IL-6, IL-12 and TNF α , as well as nitric oxide production.

Objectives Several symptoms of rheumatoid arthritis (RA), i.e. morning gelling, stiffness and swelling, are more evident in the early morning and might be related to the neuroimmunomodulatory effects exerted by MLT on synovitis. On the other hand, it is well known that MLT reaches its physiological serum peak level during the first hours of the morning.

Methods Nocturnal MLT serum levels (8 pm, 12 pm, 2 am, 4 am, 6 am, 8 am o'clock) were evaluated in ten RA patients (pts) (mean age 57 ± 13 SD ys) and in six healthy controls (cnts) (mean age 50 ± 18 SD ys). RA pts were taking only nonsteroidal antiinflammatory drugs and the mean disease duration was 4 ± 2 SD ys. MLT assay was performed by RIA and statistical analysis was carry out by nonparametric tests.

Results MLT serum levels at 8 pm and 8 am o'clock resulted higher in RA pts than in cnts ($p < 0.05$). The differences were more evident in the older RA pts (age > 60 ys) when compared to the younger ones (age < 60 ys). In both RA pts and cnts, MLT progressively increased from 8 o'clock pm to the first hours of the morning, reaching the peak level that gradually decreased ($p < 0.02$). However, MLT serum level reached the peak at least three hours before in RA pts than in cnts ($p < 0.05$); subsequently, in RA pts, MLT concentration showed a plateau lasting two-three hours. After 2 am o'clock MLT levels decreased similarly in both RA pts and cnts. MLT levels progressively decreased with ageing in all subjects; nevertheless, MLT serum levels resulted significantly lower only in RA pts at 4 am and 6 am o'clock ($p < 0.05$).

Conclusion The results of this study confirm the existence of a nocturnal rhythm of MLT also in RA pts, with the peak level in the early morning. However, RA pts showed higher mean MLT serum levels, that reached earlier the peak of concentration, and maintained the high levels for longer time when compared to cnts. The imbalance between cortisol serum levels (notably lower in RA pts) and MLT serum levels (higher in RA pts) might explain several clinical aspects related to the neuroimmunomodulatory effects on RA synovitis, such as morning gelling, stiffness and swelling.

THU0116 AUTOANTIBODIES TO VASOACTIVE PEPTIDES AND ANGIOTENSIN-CONVERTING ENZYME IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background Low levels of autoantibodies (AAb) are in fact a normal feature of a healthy immune response. The distinction between pathological and natural AAb is not well understand.

Objectives To study the levels AAb specifically reacting with angiotensin-converting enzyme (ACE) and endogenous vasoactive mediators in sera patients with rheumatoid arthritis (RA) and healthy control group.

Methods We tested sera from 19 patients with RA, 12 pts had extra-articular manifestations, 7 pts had only joint damage. The patients were compared with 10 healthy donors. Solid-phase enzyme immunoassay was used to examine the levels of IgM AAb to ACE prepared from human kidney tissue and AAb to low-molecular peptides – bradykinin (BK), angiotensin II (AII) and vasopressin (VP).

Results Analysis of IgM AAb to vasoactive peptides showed a significant increase account AAb to bradykinin (BK) in sera from RA patients compared to donors ($p = 0.0074$). The highest value of AAb to BK were observed in RA patients without extra-articular features. Level of AAb to BK correlated with CIC, joint score ($p < 0.05$). Significant discrepancies between levels AAb to AII and VP in RA pts and donors have not been revealed. Level of AAb to AII correlated negative with ERS ($r = -0.464$, $p < 0.05$). Amount AAb to ACE was increasing in RA patients, but the differences were not significant.

Conclusion The biological effects of AAb to vasoactive peptides and ACE are unknown. The possible role of AAb to BK in the pathophysiology of joint inflammation requires careful consideration.

THU0117 THE BIAS FOR TH1 DIFFERENTIATION OF RHEUMATOID ARTHRITIS T CELLS IS CHARACTERISTIC OF MEMORY BUT NOT OF NAIVE T CELLS

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Background Rheumatoid arthritis (RA) is likely to be driven by activated Th1 cells. In contrast, Th2 cells and their cytokines, in particular IL-4, are rarely found in RA.

Objectives To determine whether memory or naive CD4^{pos} T cells in RA manifest an intrinsic bias for Th1 differentiation.

Methods Th differentiation was assessed in an *ex vivo* cell culture system in CD4^{pos}, CD45RA^{pos} (naive) and CD45RO^{pos} (memory) T cells isolated from the peripheral blood from patients with active, early RA (mean disease duration < 7 months) who had never been treated with disease modifying anti rheumatic drugs or corticosteroids. The phenotype of freshly isolated and effector cells was determined by flow cytometrical analysis of cytoplasmic cytokines.

Results Th2 effectors could be induced from memory T cells in all healthy controls by stimulation with anti-CD28 and IL-4 but in only one third of the RA patients. Priming conditions that were optimal for the differentiation of Th2 effectors from memory T cells, e.g. priming with anti-CD28 and IL-4 in the absence of TCR-ligation, did not result in Th2 differentiation from resting naive T cells in any individual. In contrast to memory T cells, however, Th2 cells could be induced from naive T cells in all healthy donors and in all RA patients by priming with anti-CD3 in the presence of anti-CD28.

Conclusion CD4^{pos} memory T cells from the majority of patients with early RA manifest a deficiency in the capacity to

differentiate into Th2 effectors. In contrast, naive T cells are capable of differentiating into Th2 cells with appropriate stimulation suggesting that the bias in Th1 differentiation of most RA patients is acquired during T cell maturation presumably in response to antigenic stimulation.

THU0118 SEQUENTIAL EP1 AND EP4 REGULATION IN A RABBIT MODEL OF ANTIGEN-INDUCED ARTHRITIS

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Background Prostaglandin E2 (PGE2) exerts its actions through the binding of the high affinity receptors EP. There are 4 isoforms of EP receptors with extracellular domains highly conserved, each coupling different intracellular transduction systems. Distribution of EP subtypes in the target tissues is therefore central to the pathways elicited by PGE2.

Objectives The aim of this work was to study the regulation of EP1 and EP4 receptors in the inflamed synovial tissue.

Methods We have studied different time periods in a rabbit model of antigen-induced arthritis: 24, 48 and 72 h (acute phase) and 1, 2 and 3 weeks (chronic phase). Knee synovial fluid (SF) volume and white cell count were measured. Total RNA was extracted from the synovial membranes and expression of EP1 and EP4 receptors was studied by RT-PCR techniques. A fraction of synovial tissue was fixed and embedded in paraffin for morphological studies.

Results At the acute stages of the disease the animals depicted a synovial effusion that peaked at 48 h. The highest white cell infiltration was found at 24 h and was dominated by neutrophils; at 72 h neutrophils and mononuclear cells showed similar concentrations in the SF. At longer time-periods only half of the cases showed a synovial effusion, of smaller proportion. The acute histopathologic lesions consisted of leakage of plasma elements with prominent neutrophil invasion. At 72 h lining hyperplasia developed and there was a switch to mononuclear cell infiltration at the subintima. At further stages, fibrotic changes and increases vascularisation were also found. EP1 receptor expression was up-regulated at every point of study, when compared to controls. EP1 expression increased in a time dependent fashion, peaking at 2 weeks (14-fold vs. control). On the other hand, healthy controls expressed moderate EP4 levels, which were dramatically reduced at the acute stage of the disease (90% reduction at 72 h). At the latest periods studied, there was a normalisation and even slight up-regulation of EP4 expression.

Conclusion Our data may suggest that EP1 receptor mediates some of the PGE2 actions in experimental arthritis. This actions do not only include plasma leakage and activation of early inflammatory mediators, as EP1 up-regulation continues to increase when these typical features of acute arthritis are no longer evident. EP4 down-regulation at the early stages of the disease may suggest that this molecule do not participate in oedema and neutrophil activation induced by PGE2 in this experimental model. Restoring of the balance between PGE2 receptors in arthritis could be of help for the control of the inflammatory reaction.

THU0119 RHEUMATOID ARTHRITIS MYELOID CELLS EXPRESS AN INTRINSIC ABNORMAL CAPACITY FOR ACTIVATION

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Background Evidence suggests that monocyte precursor cells in rheumatoid arthritis (RA) might become partially activated in the bone marrow or the peripheral blood before they enter the rheumatoid synovium. However, whether this is the result of pro-inflammatory cytokines leaking from the inflamed synovium into the circulation or rather a functional abnormality of myeloid cells has not yet been conclusively delineated.

Objectives To test the hypothesis that monocytes in RA might express an intrinsic abnormality for activation, we investigated the function and the phenotype of peripheral blood monocytes from patients with RA and healthy controls *in vitro*.

Methods Monocytes were isolated by negative selection from patients with active, early RA and from patients with long standing RA under effective anti-inflammatory therapy with disease modifying antirheumatic drugs (DMARDs). A cell culture system was employed that permitted the differentiation of monocytes to macrophages. Expression of surface molecules indicative of activation and/or differentiation was assessed by flow cytometry.

Results No differences were detected for the expression of HLA-DR, CD14, CD40, CD68, CD80 and CD86 on freshly isolated monocytes between patients and controls. During differentiation to macrophages, the expression of HLA-DR, CD14 and CD80 was increased in RA monocytes. Interestingly, patients with untreated, active early disease manifested an even higher propensity to upregulate HLA-DR during differentiation compared to patients with long standing disease under effective anti-inflammatory therapy. In contrast, the expression of CD40, CD68 and CD86 was reduced in myeloid cells from RA patients during differentiation. This feature was more pronounced in patients with long standing disease, but was not correlated with clinical activity.

Conclusion The data indicate that monocytes from RA patients might have an intrinsically increased ability for activation during differentiation. This ability might contribute to clinical disease activity and emphasises the systemic nature of the disease. The data further suggest that long standing disease might alter the functional phenotype of circulating monocytes. However, the contribution of continuous treatment with DMARDs to this phenomenon remains to be elucidated.

THU0120 POLYMORPHONUCLEAR NEUTROPHIL NADPH OXIDASE PRIMING AND ACTIVATION IN RHEUMATOID JOINTS: CORRELATION WITH DISEASE ACTIVITY AND SYNOVIAL CONCENTRATIONS OF TNF, IL-8 AND GM-CSF

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Background Superoxide production by polymorphonuclear neutrophil (PMN) participates in the rheumatoid joint lesions and mainly depends on NADPH oxidase activation. Tumour necrosis factor (TNF), interleukin-8 (IL-8) and granulocyte/macrophage colony stimulating factor (GM-CSF) have a priming effect on NADPH oxidase, depending on the phosphorylation of p47phox, a major cytosolic component of NADPH oxidase.