**P23**

**Mice deficient in leptin (ob/ob) or in leptin receptor (db/db) have a milder form of antigen-induced arthritis**

N Busso, A So, V Péclat and C Gabay*

Service de Rhumatologie, CHUV, 1100 Lausanne, Switzerland; *Division de Rhumatologie, HUG, 1211 Geneve 14, Switzerland.

Leptin, the protein of the ob gene, is synthesized exclusively by adipocytes to regulate the body weight in a central manner through its interaction with long isoform of leptin receptor ob-Rb. However, Ob-Rb is also expressed in lymphoid tissues and leptin has been shown to play an important role in cell-mediated immunity. We therefore decided to examine the role of leptin in vivo by analyzing the phenotype of mice deficient in leptin (ob/ob) or in ob-Rb (db/db) during antigen-induced arthritis (AIA). Arthritis was induced by an intraarticular injection of methylated bovine serum albumin (mBSA) in the knees of previously immunized ob/ob, db/db, control littermates and wild-type mice, all in C57BL/6 background. The severity of arthritis was determined by 99m Technetium (99m Tc) uptake. In addition, the degree of articular inflammation was also determined after sacrifice by histology scoring. Levels of circulating immunoglobulins and antibodies against mBSA were measured by ELISA. The responses of isolated lymph node cells to mBSA were also examined. The results showed that joint inflammation, as measured by 99m Tc uptake, was significantly reduced in ob/ob mice as compared with control littermates and wild-type mice (on day 1 of arthritis: \( P < 0.002 \) and \( P < 0.001 \), respectively; on day 3: \( P < 0.03 \) and \( P < 0.02 \), respectively). In addition, histology studies showed that ob/ob mice had markedly less synovial inflammation than lean controls (\( P < 0.04 \)). In contrast, there was no difference in proteoglycan content within the articular cartilage as assessed by Safranin-O staining. The in vivo production of antibodies against mBSA was significantly decreased in ob/ob mice as compared with controls (\( P < 0.03 \)). Circulating levels of IgG2a were also significantly lower in ob/ob mice than in controls, whereas levels of IgM were not different. In vitro lymph node cell proliferation in response to mBSA was significantly reduced in ob/ob mice as compared with controls. In addition, production of interferon-\( \gamma \) by cultured lymph node cells was significantly lower in ob/ob than in control mice, whereas opposite results were observed for IL-10. Experiments performed in db/db mice confirmed the findings in leptin-deficient mice. In conclusion, leptin appears to regulate both the cellular and humoral components of the immune response against mBSA and to contribute to the mechanisms of joint inflammation in AIA. In addition, these results demonstrate that the effects of leptin on the immune system are mediated through its interaction with ob-Rb.

**P24**

**Temporal expression of cytokines and chemokines in rat adjuvant-induced arthritis**

Z Szekanecz, MM Halloran, JM Woods, MV Volin, GK Haines and AE Koch

Third Department of Medicine, University of Debrecen, Hungary and Northwestern University, Chicago, Illinois, USA

Adjuvant-induced arthritis (AIA) in rats is a relevant model for human rheumatoid arthritis (RA). In this study, the expression of the cytokines TNF-alpha, IL-1 and IL-6, as well as the chemokines MIP-1-alpha, MCP-1 and ENA-78 in the sera and joint homogenates of AIA and control, sham-injected rats was studied over a 47-day period. All of these cytokines and chemokines showed increased production in AIA. In addition, TNF-alpha, IL-1, ENA-78 and MIP-1-alpha could be termed as "early" mediators, as their production increased in the first 14-21 days and it correlated with early events in synovitis, such as neutrophil ingress, joint swelling and general symptoms. TNF-alpha may have mostly systemic, while IL-1 mainly local synovial effects. IL-6 and MCP-1 were found to be "late" inflammatory mediators, as their secretion was up-regulated after 2 weeks post-adjuvant injection and remained high during the observation period. Also, significant correlation was found between the production of TNF-alpha and that of chemokines. In conclusion, the differential expression of "early" and "late" cytokines and chemokines may account for various events underlying synovitis in AIA.

**P25**

**Dynamics of early synovial cytokine expression in rodent collagen-induced arthritis: a therapeutic study**

K Palmblad*, H Erlendsson Harris*, KJ Tracey† and U Andersson*

*Rheumatology research unit, Karolinska Hospital, CMM L8:04, 171 76 Stockholm, Sweden; †North Shore University Hospital, Manhasset, NY, USA

This study was performed to elucidate pathophysiologic events prior and during the course of collagen-induced arthritis (CIA) in DA rats. Kinetic studies of local cytokine responses were determined using immunohistochemistry and computer-aided image analysis. We also investigated the effect of the macrophage-pacifying compound CNI-1493 on proinflammatory cytokine expressions. Synovial cryosections were analysed at various time points for the presence of IL-1\( \beta \), TNF and TGF-\( \beta \). Unexpectedly, an early simultaneous TNF and IL-1\( \beta \) expression was detected in resident cells in the lining layer, preceding disease onset by more than one week. The predominant cytokine synthesis by synovial (ED-1+) macrophages coincided with clinical disease. TNF-production greatly exceeded that of IL-1\( \beta \). CNI-1493 treatment did not affect the early TNF and IL-1\( \beta \) synthesis, while disease-associated TNF and IL-1\( \beta \) production was greatly reduced. Furthermore, CNI-1493 significantly up-regulated synthesis of the anti-inflammatory cytokine TGF-\( \beta \) and thereby shifted the balance of pro-inflammatory and anti-inflammatory cytokines in the arthritic joint in a beneficial way.

**P26**

**Comparison of arthritogenic and nonarthritogenic Eubacterium aerofaciens cell walls**

X Zhang, M Rimpiläinen, E Simelyte and P Toivanen

Department of Medical Microbiology, Turku Immunology Center, University of Turku, Turku, Finland

We have recently reported that cell walls (CWs) of two closely related *E. aerofaciens* strains appear arthritogenic or nonarthritogenic when injected i.p. into the rats (Zhang et al. Rheumatology 2000). These strains have different structures of the CW peptidoglycan (PG). To further define what determines the arthritogenicity of these human intestinal bacteria, the tissue distribution of their CWs was compared. Muramic acid (MurNAC), a component of PG, was selected as a marker for bacterial CW as it is not synthesized by eukaryotic cells. Gas chromatography-mass spectrometry was applied to identify and quantify MurNAC. The results obtained indicate that the amount of MurNAC was much higher in the spleen and liver after injection of the arthritogenic CW than after injection of the nonarthritogenic CW. MurNAC was detected in synovial tissues and fluids from day 1 to day 28 after injection of the arthritogenic CW, but not after injection of the nonarthritogenic CW. This is probably due to the resistance of the arthritogenic CW against biodegradation; lysozyme and mutanolysin degraded the

Available online http://arthritis-research.com/supplements/3/SA