layer and predominantly recognize a 60 kD antigen in whole ovarian lysates. Initial antigen identification strategies and results are presented. Antigen identification in AOD will further our understanding of disease pathogenesis and enable development of improved diagnostic testing for a significant cause of human ovarian failure and infertility.

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F.131. DKK-1, a Master Regulator of Bone Remodelling, is Abundantly Expressed in Peripheral Inflammatory Arthritis

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Different inflammatory joint diseases have distinct patterns of bone damage with pronounced erosions in rheumatoid arthritis (RA), a combination of bone destruction and formation in psoriatic arthritis (PsA), and dominant new bone formation in spondyloarthritis (SpA). Although the underlying molecular mechanisms remain poorly understood, blocking of Dickkopf-1 (DKK-1), an inhibitor of the Wnt pathway, reverses the bone-destructive pattern to a bone-forming pattern in experimental arthritis. Therefore, we analyzed DKK-1 expression in the inflamed peripheral joint of different types of inflammatory arthritis *ex-vivo* and in fibroblast-like synoviocyte (FLS) cultures *in vitro*. There was no statistical difference in SF DKK-1 levels, but a striking variability within each cohort. As TNF alpha stimulates DKK-1 production, we assessed SF pro-inflammatory cytokines levels. Despite the similar DKK-1 levels, both TNF alpha and IL-1 beta levels but not IL-6 levels were significantly higher in RA than in SpA. Accordingly, TNF alpha and IL-1 beta levels were not correlated with DKK-1 levels. In contrast, there was a striking inverse correlation between DKK-1 and IL-6 in both RA ($r=-0.37$; $p=0.013$) and SpA ($r=-0.35$; $p=0.034$). In agreement with the SF studies, the DKK-1 level in FLS from RA patients was strongly elevated by TNF alpha (but not IL-1 beta) and suppressed by IL-6. DKK-1 is abundantly expressed in the inflamed joint of both destructive and remodelling forms of arthritis. The differential regulation of DKK-1 by TNF alpha and IL-6 is under further investigation as it may determine the pattern of inflammation-induced tissue remodelling in arthritic joints.

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F.132. Characteristics of Patients with Refractory Cutaneous Lupus Erythematosus: A Database Analysis of 152 Patients Seen at an Outpatient Dermatology Clinic

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Interest in cutaneous lupus erythematosus (CLE) by biotechnology companies has increased and in preparation for future clinical trials of new therapies for CLE, it is crucial that the prevalence, clinical severity, and characteristics of refractory cases be measured and evaluated. This prospective study was designed to assess disease severity and treatment responsiveness by using clinical outcome measures and establishing a web-based database of patients with skin manifestations of LE. We sequentially enrolled and followed 152 patients who presented to our outpatient clinic from January 2007 to December 2008 and met the criteria for having CLE or lupus-nonspecific disease. Nine patients (5.9%) presented with acute CLE, 38 (25%) with subacute CLE, 92 (60.5%) with chronic CLE, 12 (7.9%) with SLE and LE nonspecific skin lesions, and 1 (0.7%) with LE-nonspecific skin disease only. The disease in 12 of our patients (7.9%) was considered refractory to conventional therapies. Most of the refractory cases were in women ($p=0.37$, refractory vs. non-refractory). The distribution of subtypes in the refractory group was significantly different from the distribution in the non-refractory group, with generalized DLE patients composing 58% of the refractory group, localized DLE composing 17%, and SCLE composing 8% ($p=0.013$). Significantly more patients in the refractory group than the non-refractory group had a current ($p=0.003$) or previous ($p=0.044$) history of smoking. This web-based database is the first systematic multicenter epidemiologic study of CLE in the United States, and should allow collection of data related to disease activity and response to therapy at multiple centers.

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F.133. Antibody-producing Monoclonal B Cell Lines from Multiple Sclerosis Patients Obtained by B Cell Immortalization

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B cells and oligoclonal antibodies are present in the cerebrospinal fluid (CSF) of MS patients but their target antigens remain unknown. The focus of this study was to produce and characterize autoantibodies in MS based on B cell immortalization. Antibodies were produced from CSF and peripheral blood mononuclear cells (PBMC) of 7 MS patients and 6 control patients with non-inflammatory or other inflammatory neurological disease (NIND/OIND). PBMC or CSF cells were cultured in the presence of irradiated allogeneic PBMC, T cell inhibitory and B cell stimulating factors to activate B cells. Epstein-Barr virus (EBV) was then added to transform B cells which was verified by screening the culture supernatant for the presence of immunoglobulin G. Positive cultures were further cloned and clonality was verified. We obtained 37 immortalized B cell lines from 7 MS patients, 7 originating from CSF of 1 MS patient and 30 from PBMC of 6 MS patients. From 5 NIND and 1 OIND patients 9 B

cell lines have been isolated, 2 derived from CSF cells of 1 patient and 7 from PBMC of 5 other patients. B cell spectratyping analysis showed that most of the immortalized B cell lines were monoclonal. Preliminary screening demonstrated intracellular binding of antibodies obtained from 8 immortalized B cell lines to a human oligodendroglioma (HOG) cell line. B cell immortalization has proved to be a useful method for the production of antibodies. The obtained monoclonal antibodies will be further analyzed for autoreactivity by detecting antibody binding to healthy and EAE brain tissue from rat and rhesus monkey and to some viruses such as cytomegalovirus (CMV) and EBV.

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F.134. Regulation of Phenotype and Function of Myeloid Cells by PU.1 Expression Levels in the Human Intestinal Mucosa

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Human lamina propria macrophages (LPMO) unlike other tissue-specific macrophages lack certain pattern recognition-, Fc-, and complement-receptors. A common feature of these receptors is the regulation of their expression at the transcriptional level by PU.1. In this study, we demonstrate that compared to autologous peripheral blood monocytes (PBMO) resting LPMO show low gene and protein expression of PU.1, while SP1, another transcription factor involved in the expression of pattern recognition receptors (PRR) and complement receptors, is expressed equally in both cell populations. In situ analysis also confirms low expression of PU.1 in LPMO of healthy intestinal tissue. Expression of PU.1 target genes M- and GM-CSF-receptor is also down-regulated in LPMO. Furthermore, in response to tissue damage LPMO up-regulate PU.1 expression correlating with increased expression of PU.1 dependent surface receptors. Taken together, low intracellular concentrations of PU.1 in LPMO may represent a major mechanism underlying the low expression of PRR, complement-, Fc- and CSF- receptors in LPMO. Up-regulation of PU.1 expression levels and its target genes in "inflammatory" LPMO underlines the phenotypic plasticity of mucosal macrophages.

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F.135. Th1 Effector Cells Convert into Regulatory T Cells After Encountering Self-antigen *in vivo*

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The immune system has the remarkable ability to simultaneously recognize and eliminate an enormous diversity of foreign antigens while remaining unresponsive to self-antigens. In response to recognition of their antigens, CD4 T cells can differentiate into several subsets including pathogenic Th1 and Th17 effector cells and regulatory T cells (Tregs). Importantly, if Tregs are non-functional or absent, severe autoimmunity can occur. Our laboratory has developed a model of systemic inflammation where CD4 DO11 T-cells specific for chicken ovalbumin (Ova) are transferred into lymphopenic recipient mice expressing soluble Ova protein as self-antigen (sOva-Tg Rag-/- mice). After recognizing their self-antigen, these self reactive T cells develop into effector and regulatory populations with Th17 and Th1 effector cells being detectable prior to the appearance of Tregs, but it is unclear if Tregs arise de novo or from previously active effector subsets. To address this question, we have bred IFNγ-YFP reporter mice (YETI) onto the DO11 transgenic background and these DO11 CD4 cells were cultured under Th1 polarizing conditions to generate YFP+ Th1 effector cells for transfer into sOva-Rag-/- Tg mice. At 10 days post-transfer, T cells were harvested to assess inflammatory cytokine production and the expression of FoxP3, the transcription factor for Tregs. Surprisingly a subset of the transferred Th1 effectors became IFNγ-YFP- and expressed FoxP3. These data show that the phenotype of T cell subsets can have plasticity *in vivo* and support a novel concept where effector functions may attenuate their pathogenic function through conversion into protective regulatory T cells.

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F.136. Serum TNF- alpha, Soluble TNF-Receptor I and II levels in Patients with Brucellosis

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Similar to other intracellular bacterial pathogens, anti-brucellar host defence is mediated primarily by cellular immune system involving activated macrophages, T cells and their cytokines.The aim of the present study was to elucidate serum concentrations of tumor necrosis factor-α (TNF-α), soluble tumor necrosis factor-α type I and II receptors (sTNF-RI and sTNF-RI) in samples obtained from 21 patients with brucellosis and 10 healthy controls, by using enzyme-linked immunosorbent assay. Serum levels of TNF-α, sTNF-RI and sTNF-RI were significantly increased in patients with brucellosis compared with those in healthy controls ($P<0.01$). Significant correlation was found between these soluble factors and ESR and CRP values ($P<0.01$). The role of TNF and its receptors has been investigated in a variety of other bacterial infections. Despite a limited study population, increased levels of TNF-α and their receptors in serum may show that these soluble factors are important inflammatory mediators and should be used as a marker for disease activity.

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