854 Interaction of the Germinal Center B Cell-Specific M17 Promoter With Sp1/Sp3

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Antigen-stimulated B lymphocytes undergo additional genetic and phenotypic changes in the context of germinal centers, including affinity maturation of Ig, heavy chain isotype switching, and selection for appropriate Ig specificities. To address the identities of factors that control gene expression during humoral immune responses, we examined regulation of the *M17* gene, which is specifically expressed in germinal center B cells. Sequences that constitute a functional promoter in *M17* -expressing cells were determined. Using electromobility gel shift analysis, we have identified a protein(s) that specifically binds to the sequence CCTCCT at position -40 to -45 of the *M17* promoter. Competition with known DNA transcription factor binding sites identified the protein(s) as a member of the Sp1 transcription factor family. Antisera demonstrated that the proteins involved in the complex were Sp1 and Sp3. The *M17* promoter was transfected into *Drosophilia* Schneider cells which lack endogenous Sp1 transcription factors. Addition of a Sp1 or Sp3 expression vector modulates *M17* transcription. Together, these data suggest a role for Sp1/Sp3 as a modulator of *M17* transcription during B cell maturation in germinal centers.

855 Anti-Eosinophil Peroxidase: A New Marker for Churg-Strauss Syndrome

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Systemic vasculitis is often accompanied by anti-neutrophil cytoplasmic antibodies (ANCA). In Wegener's granulomatosis (WG), such antibodies produce a cytoplasmic staining pattern on ethanol-fixed neutrophils (c-ANCA) at indirect immunofluorescence (IIF), a pattern normally seen in conjunction with antibodies to proteinase-3 (PR3). In vasculitides other than WG (like microscopic polyangiitis) such antibodies produce a perinuclear staining pattern (p-ANCA) and in most cases those antibodies are directed against myeloperoxidase (MPO). Fifty percent of patients with Churg-Strauss syndrome (CSS), a systemic vasculitis accompanied with asthma and eosinophilia, demonstrate perinuclear staining by IIF, with target antigen defined by ELISA as MPO. In other half of patients there is no serologic laboratory marker. We were puzzled to find that some patients, tested in our laboratory, who had anti-MPO antibodies in ELISA, produced a classical c-ANCA rather than the expected p-ANCA pattern in IIF. We found that all these patients suffered from CSS. We speculated that the target antigen might be eosinophilic peroxidase (EPO), an enzyme produced and contained in eosinophilis, that shares 68% amino acid identity with MPO. We were able to demonstrate, by IIF and by ELISA binding and inhibition tests, that the autoantibodies in 4 CSS patients sera were specifically directed against EPO. Sera obtained from 3 MPO positive patients with microscopic polyangiits and from 31 healthy normal donors were all negative for anti EPO antibodies. Adsorption of the sera on EPO decreased the neutrophil cytoplasmic staining, suggesting that either EPO or EPO cross reactive molecules, are the cytoplasmic target for this antibodies. Five additional CSS patients in various stages of treatment and without ANCA by IIF, were also found to have anti EPO antibodies by ELISA. In summary: We suggest that anti-EPO antibodies might serve as a new diagnostic marker for CSS.

856 Modulation of Chondrocyte Function by Synovial Fluid and Peripheral Blood Immune Complexes of Rheumatoid Arthritis Patients

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INTRODUCTION: The destruction of cartilage is an important characteristic of rheumatoid arthritis (RA). Immune complexes (IC) are usually found in high amounts in RA synovial fluids (SF) and in the superficial layers of RA cartilage.

AIM OF THE STUDY: To investigate if immune complexes (IC) have an influence on chondrocyte growth, survival and production of nitric oxide (NO).

METHODS: Synovial fluid (SF) and peripheral blood (PB) of 14 rheumatoid arthritis (RA) patients and 5 osteoarthritis (OA) patients was obtained. In 5 healthy age and sex matched controls, only PB was collected. IgM-IC and IgG-IC were precipitated with polyethyleneglycol (PEG) and determined by ELISA and nephelometry respectively. Primary bovine chondrocytes were incubated with cytokines (huIL-α, bovIFN-γ, huTNF-α) and (IC containing) precipitates. After 48 hours, the morphology of the chondrocytes, including presence of apoptotic nuclei, was evaluated by a May-Grünwald-Giemsa staining. Growth was determined by incorporation of tritiated thymidine. NO production was evaluated using a spectophotometric assay, based upon the Griess reaction. The number of apoptotic chondrocytes was detected flow cytometrically with a TUNEL technique and annexinV/propidium iodide staining. Caspase-3 activity was determined, using polyclonal antibodies against activated caspase-3.

RESULTS: All RA PB and SF were positive for IgG-IC and/or IgM-IC, ranging between 61 and 314 μg/mL (normal value for IgG-IC ≤25 μg/mL, for IgM-IC ≤18 μg/mL). One PB of a patient with OA and one control PB was slightly positive for IgG IC (respectively 27 μg/mL and 35 μg/mL). The proliferation rates of chondrocytes, incubated with PB and SF IC of RA patients were significantly decreased compared to cells, incubated with PB and SF of OA patients and compared to PB of control persons (respectively p=0.001; p=0.003; p=0.001). The NO production by chondrocytes, incubated with SF IC of RA patients was enhanced, compared to OA SF (p=0.04). Staining with May-Grünwald-Giemsa after incubation with IC of RA PB and SF, showed a rise in apoptotic chondrocytes. Quantitative evaluation of apoptotic cells revealed a significant increase after incubation with PB and SF IC of RA patients (53% (26-77) (median (range) and 61% (26-87)), compared to control PB (22% (10-40)) and OA PB and SF (12% (2-16) and 28% (24-31)). In all TUNEL positive samples, active-caspase-3-positive cells were found.

CONCLUSION: These results support the hypothesis that immune complexes, present in serum and synovial fluid of rheumatoid arthritis patients, can induce in vitro caspase-3 dependent apoptosis in chondrocytes, which can be a mechanism of cartilage destruction in rheumatoid arthritis.

857 A New Approach to Investigate the Role of the Thymus in the Pathogenesis of Myasthenia Gravis

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Peripheral T cells migrate to the thymus in small numbers where they typically enter the medullary compartment. It is not known whether the rate or magnitude of T cell entry is increased by an inflammatory reaction in the thymus. Furthermore, it is not known whether self-reactive T cells in the immigrant pool, that have escaped central deletion, are activated to effector status following their engagement of specific autoantigen in this inflammatory mileu. These possibilities are particularly relevant to the autoimmune disease myasthenia gravis where a leading, albeit untested, hypothesis is that sensitization to the autoantigen, acetylcholine receptor (AChR), actually occurs in the thymus. This hypothesis is based on clinical, pathologic, and immunologic lines of evidence as well as the observation that the autoantigen, AChR, is expressed on potential antigen presenting cell populations in the thymic medulla. We sought to devise a model of intrathymic inflammation that provides an opportunity to address the above questions. Balb/c mice, immunized to β-galactosidase (β-gal), were injected intrathymically (i.t.) with a retroviral vector that encodes β-gal cDNA (LBSHG) or a control vector lacking β-gal cDNA (LXSHG). These vectors target the thymic medullary epithelium for expression of virally encoded genes. H + E stained sections of thymus obtained four days after i.t. injection of LBSHG, but not LXSHG, showed obliteration of the cortical/medullary architecture with