In 5 out of 68 cases we have observed an outgrowth of polyclonal B cells in coculture with fibroblasts. 85-99% of B cells in each population are positive for CD19 and CD20 indicating mature B cell lineage. CD23hi/CD10- markers (99%) and GIEMSA staining suggest that the cells are immunoblasts that also express activation markers CD69 (75%) and HLA-DR (98%). All cells express Epstein Barr Virus (EBV) encoded genes EBNA-1, EBNA-2, and LMP-1, characteristic of type III latency of viral infection. Two synovial B cell clones 4E (IgG, kappa) and 2B (IgG, lambda) were cocultured with either dermal or RA synovial fibroblast lines for 4 days. The preliminary analysis of changes in gene expression in both fibroblasts and B cells shows that B cells, possibly through the action of TNF-alpha, increase expression of growth factors by synovial fibroblasts that may promote B cell differentiation from activated immunoblasts into plasma cells.

763.19

Identification of the Ligand for CS-1, a novel member of the CD2 subset of immunoglobulin superfamily

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Members of the CD2 subset of receptors play a major role in lymphocyte function and immune regulation. We have recently identified a novel member of the CD2 subset, the CS1 molecule, which is localized to human chromosome 1. However, the biological function and the nature of the ligand of CS1 is not known. In this study, we examined the expression pattern and ligand specificity of CS1. Antibodies to CS1 were generated and FACS analyses showed that CS1 is mainly expressed on B cells and NK cells. We expressed CS1 as a soluble fusion protein with human IgG(Fc) portion. We found the soluble CS1-Fc protein bound to cells transfected with CS1 whereas untransfected cells did not. No interactions were observed with 2B4 or CD48 transfected cells. Thus, the data indicate that CS1 is a self-ligand. These studies contribute to the functional characterization of CS1 and will provide a better understanding of its role in the immune system. This study was supported by NIH grant CA85753.

763.20

Phenotypic Profiling of Blood Leukocytes using Microvolume Laser Scanning Cytometry for Autoimmune Disease.

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SurroMed applies proprietary phenotyping technologies to discover biological markers associated with disease and therapy. Our bioanalytical platform includes the SurroScanä microvolume laser scanning cytometer for high-throughput, multiparameter cellular analysis and uses fluorophore-labeled mAbs specific for cell surface antigens to identify and enumerate hundreds of cell populations from small volumes (<2mL) of unprocessed blood or biological fluid. SurroScans are used for clinical studies in many disease areas including autoimmune disease. Objectives are to apply differential phenotyping to develop disease-specific fingerprints and pharmacodynamic profiles. Studies present comparisons of allergic and arthritic cohorts. Assay panels included >80 unique Ags expressed on leukocytes and identified granulocyte, T, B, NK and myeloid cells in blood and disease-specific compartments. Biomarker profiles demonstrated expected biological changes, however, novel activation- and regulatory-specific markers were identified in each disease group. Measuring many biological markers simultaneously will aid in identifying markers related to disease prediction, control and prevention of adverse responses to therapy.

763.21

Restraint Stress Modulation of the Skin Immune Response is Independent of T cell Proliferation

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Previous reports suggest that restraint stress applied to sensitized mice before challenge enhances the mouse ear swelling response. To determine if this event was localized to the skin and/or the lymph nodes, we assessed Langerhans cell (LC) morphology by analyzing FITC-conjugated Ia stained cells in epidermal sheets and cell proliferation by the Local Lymph Node Assay. We determined that stress induced significant alterations in LC morphology and decreased DNFB-induced lymph node (LN) cell proliferation by 46%. To understand if these suppressive effects were T cell specific, we asked if restraint modulates LN cell surface expression of CD3, CD4, CD8 and CD62L. Using flow cytometry, we found that restraint had no effect on CD3, CD4 or CD8 expression. Although DNFB significantly decreased production and staining intensity of CD62L, restraint did not alter DNFB-decreased CD62L expression. We extended these studies to T-cell deficient nude mice and found that stress before challenge enhanced ear swelling in the absence of a specific immune response. These data suggest that stress applied prior to challenge modulates the skin response to chemical but not the specific T cell response in lymph nodes

CYTOKINES AND AUTOIMMUNE DISEASE (764.1-764.24)

764.1

Dendritic cells in NOD mice drive T cells toward a T helper-1 phenotype

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Type 1 diabetes (TID) is characterized by T cell-mediated destruction of pancreatic islets, events that are marked by skewing toward a T helper-1 (Th1) response. Since dendritic cells (DCs) are the first cells to infiltrate the islets and normally dictate the extent and nature of naïve T cell activation, we hypothesized that DCs might contribute to the immune dysregulation in TID. The non-obese diabetic (NOD) mouse model was used to characterize bone marrow-derived DCs in the context of their interactions with T cells using an in vitro DC-T cell co-culture system. Several aspects of the DC-T cell interplay in NOD were assessed by comparison to control strains. We analyzed costimulatory molecules on DCs, T cell proliferation, and cytokine production. The results collectively revealed that NOD DCs possess both higher T cell stimulatory activity and heightened Th1 driving capabilities compared to DCs from controls. These findings have implications for elucidating the role of DCs in initiation and propagation of autoimmune responses in TID.

Supported by Canadian Institutes of Health Research, Natural Sciences and Engineering Research Council of Canada, and Juvenile Diabetes Foundation International

764.2

Role of Th1 and Th2 cytokines in the induction of experimental autoimmune Graves disease in Balb/C mice

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Animal model for Graves disease (GD) shows predominantly an IgG1 response against TSHR, suggesting involvement of Th2 autoreactive cells. Th1 and Th2 subset of T cells have a critical role in determining qualitative nature of autoimmune responses. Therefore, modulating the balance between Th1 and Th2 cytokines might influence the development of an autoimmune disease. Flt3-Ligand and GM-CSF induce the development of dendritic cells and skew the immune response towards Th1 and Th2 type respectively. In the present study, we investigated the effects of treatment with Flt3-L and GM-CSF at the early stages of immune response to TSHR and its effects on the induction of GD in Balb/C mice. Mice treated with either Flt3-L or GM-CSF and then immunized with TSHR showed significantly lower and higher T cell responses respectively compared to the control group. While the mice treated with Flt3-L produced high IFN-gamma response, mice treated with GM-CSF produced high IL-4 response. The mice treated with Flt3-L produced low levels of anti-TSHR IgG1and IgG2a antibodies compared to the control mice. However, at the later stages of immune response, there was no significant difference in cytokine or antibody response to self-antigen or disease development among different groups of mice. These data suggest that the difference in Th1/Th2 cytokine balance induced by FLT3-L and GM-CSF at the early stage has no significant effect on GD development.

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Experimental Biology 2002®
New Orleans, Louisiana
April 20–24, 2002

ABSTRACTS
PART II

Abstracts 524.1–940.2

Official Publication of the Federation of American Societies for Experimental Biology
Volume 16, Number 5, March 22, 2002