278.3

Th1- and Th2-Type Cytokines Both Are Involved in Eosinophil-Rich Delayed-Type Hypersensitivity (DTH).

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We used gene knockout mice to analyze the roles of specific Th1- and Th2-type cytokines in DTH to the antigen keyhole limpet hemocyanin (KLH). Mice were immunized by *i.d.* injection with KLH in saline into abdominal skin without adjuvants and challenged *i.d.* in the ear skin. Macroscopic 24 hr DTH reactions were elicited beginning on day 3 after immunization. DTH was Ag-specific, and not elicited in animals lacking αβ T cells. The 24 hr ear infiltrates were eosinophil-rich, suggesting involvement of Th2-type cytokines. Consistent with this idea, ear swelling and eosinophil recruitment in ears on day 3, were decreased in IL-5$^{-/-}$, IL-4$^{-/-}$, or Stat-6$^{-/-}$ mice, but interestingly not in IL-13$^{-/-}$ mice. In contrast, DTH ear swelling at day 5 was not decreased in IL-4$^{-/-}$ or Stat-6$^{-/-}$ mice, but eosinophil infiltrates still were impaired. Surprisingly, both 24 hr ear swelling and eosinophil infiltrates were impaired in IFN-γ$^{-/-}$ mice, especially on day 5. Moreover, IFN-γ protein and the IFN-γ inducible chemokine IP-10 were detected by quantitative ELISA in 24 hr DTH ear extracts, and KLH immunized mice showed IFN-γ-dependent Ag-specific IgG_{2a} and IgG_{2b} responses, as well as IL-4-dependent IgG_1 antibody responses. We conclude that the early day 3 DTH to *i.d.* KLH requires both Th1-type and Th2-type cytokines, and rapidly evolves by day 5 to a Th1 predominant response. Remarkably, eosinophil recruitment in DTH depends on both types of cytokines.

278.4

TNF/TNFR2 Interactions Play an Important Role in T Cell Activation and Development of Colitis during MHC Class II Disparate B6 B6 X bm12 F1 GVHD.

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Background: TNF/TNFR2 interactions have been found to promote class II MHC stimulated alloresponses whereas TNF/TNFR1 interactions promote class I MHC stimulated alloresponses in MLC. In Class I+ Class II disparate GVHD models, absence of TNFR1 on either donor T cells or in recipient mice has been associated with diminished GVHD morbidity and mortality without effect on development of intestinal GVHD. The present studies were designed to evaluate whether TNF/TNFR2 interactions were involved in the in vivo generation of CD4+ T cell mediated enteropathy in the B6 B6 X B6.C-H-2^{bm12} (bm12) F1 GVHD model. **Methods:** 5 X 10^{6}splenic CD4+ T lymphocyte from B6 or B6.TNFR1-/- mice were transferred with 1-2 X 10^6 T cell depleted B6 BMC to irradiated class II MHC disparate B6 X bm12 F1 mice. In other experiments, B6 or B6.TNFR2-/- CD4+ T cells were transferred with B6 or B6.TNFR2-/- BMC, respectively to irradiated class II MHC disparate B6 X bm12 F1 mice. Weight loss and intestinal inflammation were evaluated over a 25 day interval. The surface expression of CD45RB (activation/memory marker) on intestinal and splenic lymphocytes was assessed by flow cytometry. IL-2 and IFN mRNA levels of splenic and intestinal lymphocytes were assessed by nuclease protection assays. **Results:** A significant reduction in weight loss and colitis was observed in recipients of the TNFR2-/- CD4+ spleen T cells. This was associated with a significant decrease in CD45RB^{low} (activated/memory) expression on intestinal and splenic T cells in recipients of the TNFR2-/- CD4+ spleen T cells. IL-2 and IFN γ mRNA levels 14 days after transplantation were also reduced in the spleen and the intestine in the recipients of B6.TNFR2-/- splenic T cells. **Conclusions:** These results indicate that TNF/TNFR2 interactions are important for colitis development and the activation/differentiation of alloreactive T cells in the intestine and spleen during class II MHC disparate GVHD.

278.5

Differential roles for IL-12 and TNF[alpha] in pathogenesis of experimental autoimmune uveitis in IFN-[gamma] deficient and wild type mice.

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Pathogenesis of experimental autoimmune uveitis (EAU) in the IFN-[gamma] deficient mouse (GKO) involves a deviant (Th2-like) effector response, in contrast to the typical Th1 profile exhibited by Wild Type (WT) mice. Suppression of EAU and cellular responses by anti-IL-12 treatment indicated that in both genotypes IL-12 is needed for priming and for disease expression. Lymphoid cells of mice protected by anti-IL-12 treatment produced less antigen-specific TNF[alpha] in culture. We therefore set out to determine if the role of TNF[alpha] in EAU becomes more central when IFN-[gamma] is absent. GKO and WT mice were immunized for induction of EAU with the retinal antigen IRBP and were treated every other day with mAb to murine TNF[alpha] or with control Ig during the afferem stage (d 1-7) or the efferent stage (d 8-15) of disease. Eyes collected on day 18 were evaluated for EAU by pathology and lymphoid cells were cultured for antigen specific cytokine responses. Results showed that GKO mice produced higher average baseline TNF[alpha] levels than WT mice (849 vs 345 pg/ml). Neutralization of TNF[alpha] in the afferent stage significantly downregulated EAU in both genotypes and reduced the number of antigen-responsive cells (by IL-2 ELISPOT assay). In contrast, neutralization of TNF[alpha] in the efferent stage decreased EAU scores and IL-2 producing cells only in the GKO, whereas WT mice developed higher EAU scores and more IL-2 producing cells. These data show that TNF[alpha] is needed during the priming phase in both GKO and WT, but only the GKO mouse uses TNF[alpha] to effect pathogenesis. We propose that in the GKO mouse IL-12, which in the WT promotes the pathogenic Th1 response, is needed to promote a TNF[alpha] response.

278.6

Coronary Artery Endothelial ICAM-1 and Neutrophil Adhesion in Endocarditis.

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There is evidence that neutrophil adhesion to endothelial cells activates ICAM-1 signaling pathways that may facilitate neutrophil migration. Few neutrophils are found in the vegetation of early streptococcal endocarditis in our animal models, and we have suggested that their low numbers contribute to disease progression. To clarify the role of adhesion molecule-neutrophil interaction, we measured adherence of activated, unstimulated neutrophils to cultured (4-7 passage) coronary artery endothelium (CAE) and to control endothelial cells from a venous site (HUVEC). ICAM-1 levels were measured on both types of cells by flow cytometry and by density of anti-ICAM-1 staining of cell homogenate blots. Activated, unstimulated neutrophils exhibited significantly less adherence to monolayers of CAE vs HUVEC cells at three neutrophil dilutions; 42.4± 20.5 cells per high-power field HUVEC vs 17.6 ± 6.2 CAE cells per hpf, p =0.001 Mann-Whitney test. ImageQuant-defined densities of three major cell homogenate bands reacting with anti-ICAM-1 were similar for both cell preparations, p=0.16. Cultured endothelial cells reacted with ICAM-1-FITC and evaluated by flow cytometry, however, show 31.2% CAE cells stained for ICAM-1 with only 2.2% HUVEC staining positive. We conclude that significantly decreased neutrophil adherence to coronary artery monolayers reflects the *in vivo* situation in endocarditis, but our experiments on ICAM-1 suggest that other adhesion factors are paramount.

278.7

Murine Bone-Marrow Derived Mast Cells Migrate in Response to IL-15

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We have previously reported that the stomachs of mice infected with *Helicobacter felis* have increased numbers of gastric mast cells and increased levels of Interleukin 15 (IL-15), compared to the stomachs of uninfected controls. IL-15 induces the proliferation of murine bone marrow-derived mast cells (mBMMC), but it is not known whether IL-15 also acts as a chemoattractant for these cells. We report that IL-15 causes directional movement of mBMMC. The chemotactic response was dose-dependent and significant migration was observed to levels of IL-15 as low as 10^{-10}M. True chemotaxis was confirmed by checkerboard analysis. The potency of IL-15 was of a similar magnitude to that of C5a, a well-established mast cell chemoattractant. IL-15 did not cause significant degranulation of mBMMC, as measured by beta hexaminodase release. These results suggest that locally produced IL-15 may contribute to mast cell recruitment during an inflammatory response.

278.8

INTERLEUKIN 6 PLAYS A CRITICAL ROLE IN CUTANEOUS WOUND HEALING

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It has been postulated that an inflammatory response following cutaneous wounding is a pre-requisite for healing, and inflammatory cytokines, such as interleukin 6 (IL-6), might be intimately involved in this process. Herein, we demonstrate that IL6 deficient transgenic mice (IL-6 KO) displayed significantly delayed cutaneous wound healing compared to wild type control animals, requiring up to three fold longer to heal. This was characterized by minimal epithelial bridge formation, decreased inflammation, and granulation tissue formation. Delayed wound healing in IL6 KO mice was reversed with a single dose of recombinant murine IL6, or intradermal injection of an expression plasmid containing the full length murine IL6 cDNA. We also demonstrate that dexamethasone significantly delays wound healing characterized by delayed re-epithelialization, granulation tissue formation and wound closure. Healing in the dexamethasone treated mice also can be augmented by administration of rmIL-6. In situ hybridization of wound tissue from wild type mice revealed IL-6 mRNA expression primarily in the epidermis at the leading edge of the wound. To further delineate the role of IL-6 in epidermal regeneration, we demonstrate that IL-6 mRNA is expressed and immunoreactive IL-6 is released from normal human epidermal keratinocytes (NHEKs) following in vitro wounding. We also show that IL-1 receptor antagonist will block wound induced IL-6 induction, and that constitutive keratinocyte-derived IL-1 is a major stimulus for IL-6 production in wounded epidermis. These results indicate the importance of IL-6 in wound healing, and a possible therapeutic application of this cytokine.

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