dence of 20 % among treated patients. Much of our work to elucidate the underlying mechanisms of this disease has been driven by the hypothesis that systemic autoimmunity results from a failure of self-tolerance during the generation of T cells in the thymus. The mouse model we developed was designed to test this notion and to determine its cellular and molecular basis. We previously demonstrated that two intrathymic injections of procainamide-hydroxylamine (PAHA, a reactive metabolite of procainamide) into B6D2 mice caused the appearance of chromatin-reactive T cells and anti-chromatin antibodies. We now determined how thymic PAHA exposure results in the release of autoreactive T cells. In reaggregate cultures of thymic epithelial cells with $CD4^+CD8^+$ double positive cells derived from TCR-AND mice, the emerging transgenic $CD4^+$ cells expressing a T cell receptor specific for pigeon cytochrome c (PCC) are normally unresponsive to PCC. However, if 20 μM PAHA was present during selection, the T cells subsequently exhibited a 40-fold increase in proliferation in response to PCC. In contrast, when single positive thymocytes were used in the reaggregate, PAHA presence did not result in PCC-responsive T cells. These observations suggest that PAHA affects double positive thymocytes undergoing selection. To determine whether PAHA interferes with negative selection, we measured deletion of $V_\beta 3$-expressing PCC-specific transgenic T cells as a result of exposure to PCC during selection. Presence of PAHA did not prevent the deletion of the transgenic T cell population. An effect on positive selection was tested using DPK cells, a double positive thymocyte cell line from a TCR-AND mouse, which mature to $CD4^+$ cells when exposed to fibroblasts presenting PCC. When selected on [PCC] < 2 μM, the emerging cells were unresponsive to PCC. Addition of 20 μM PAHA to this system resulted in a 4-fold increase in IL-2 production upon challenge with PCC. These studies suggest that PAHA induces autoimmunity by preventing acquisition of unresponsiveness to self peptides during positive selection of T cells in the thymus.

794 IMMUNOSTIMULATORY EFFECTS OF ANTI-DEPRESSANTS ON REPORTER ANTIGEN RESPONSE IN THE MODIFIED PLNA: ROLE OF SEROTONERGIC ACTIVITY.

R H H Pieters, M Tiesjema, A Van der Pijl, W Seinen and R Albers. *RITOX/Immunotoxicology, Utrecht University, The Netherlands.* Sponsor: M van den Berg.

The list of pharmaceuticals with autoimmunogenic potential is extensive, and includes drugs of the category of anti-depressants, in particular the serotonin-reuptake blockers. The immunomodulatory effect of these reuptake inhibitors may partly be caused by their capacity to act as hapten or induce neo-antigen formation generating non-cognate T cell help with subsequent determinant spreading to a (true) auto-antigen specific immune response. In addition, these compounds may cause inflammation and thus generate costimulatory signals that stimulate an immune response against an auto-antigen. The aim of this study was to discern whether anti-depressants were able to (non-)cognate help and to assess whether the serotinergic activity of these drugs might be involved in their immunostimulatory potential. To this end, we utilized the PLNA modified to read-out the characteristics of the response by using well defined reporter antigens. A number serotonin-reuptake blockers (clomipramine, imipramine, maprotiline, nortryptyline, zimeldine, paroxetine, fluvoxamine) and also serotonin itself were injected together with the T cell-independent TNP-Ficoll (requiring non-cognate T cell help) into the hind paw of a BALB/c mouse. In a next series of experiments, zimeldine, imipramine or serotonin were co-injected with the T cell-dependent antigen TNP-OVA (requiring cognate T cell help for IgG1). The immune reponse was monitored in the draining popliteal lymph node by using an TNP- and IgG1-specific ELISPOT assay. Results demonstrated that all drugs tested stimulated the IgG1-response against TNP-OVA and TNP-Ficoll whereas serotonin stimulated the response to TNP-OVA only. In addition, it was found that the 5HT2R-antagonist ketanserin caused partial decrease of the stimulatory effect of serotonin and zimeldine to TNP-OVA. Results indicate that the anti-depressive drugs can stimulate both cognate and non-cognate T cell help and that part of the cognate help may arise from their serotonergic activity.

795 POPLITEAL LYMPH NODE RESPONSE TO STREPTOZOTOCIN IS UNDER TYPE-1 CD8+ T-CELL CONTROL.

G Choquet-Kastylevsky[1,2], R Tedone[1,2], J Kehren[1], M-T Ducluzeau[1], J-F Nicolas[1] and J Descotes[1,2]. [1]*Unserm U98-X;* [2]*Dept. Pharmacology, Medical Toxicology and Environmental Medecine, Faculté de Médecine, Lyon-RTH Laennec, Lyon, France.*

The mechanism of chemical-induced autoimmune reactions is not known. The popliteal lymph node (PLN) assay has been proposed to predict the sensitizing potential of xenobiotics, but a better understanding of the mechanisms involved is absolutly needed to establish its value for preclinical safety evaluation. In this respect, the cytokine profile of PLNs was analyzed after the injection of streptozotocin (STZ). We used different groups of mice to assess the role of the CD4+ and CD8+ T-cells subtypes in the occurrence of positive responses: C57 BL/6 mice, CD4+ T-cells and CD8+ T-cells depleted mice, MHC-class I deficient mice and MHC-class II deficient mice. PLNs of 6-8 week-old mice were removed at day 7 after the injection of 0.25mg STZ (right footpad), or saline (left footpad) and the weight indices were calculated. After treatment for RNA extraction, the expression of mRNAs of interleukin (IL)-4, IL-6, IL-10, and gamma interferon (IFN-γ), was assessed in the PLNs by a quantitative RT-PCR method, using the coamplification of an internal plasmid. Naive or saline treated mice expressed few or no gamma-IFN, IL-4, IL-6, and IL-10. The prominent feature of PLNs from STZ treated C57 BL/6 mice was a high increase in IFN-γ mRNA (2900 copies), and in IL-6 mRNA (200 copies). No significant increases in IL-4, nor IL-10 mRNA were noted. CD8+ T-cells depleted mice and MHC-class I deficient mice, deficient in CD8+ T-cells, exhibited no positive PLN response, nor increase in the secretion of IL-6 or IFN-γ . On the contrary CD4+ T-cells depleted mice and MHC-class II deficient mice, deficient in CD4+ T-cells, exhibited a normal PLN response, with a cytokine profile equivalent to non-depleted mice.

These results suggest that positive responses to STZ are mediated by CD8+ T-cells restricted to MHC class-I, with a high production of IFN-γ .

796 TDI-SPECIFIC ANTIBODY GENERATION IS ALTERED IN TUMOR NECROSIS FACTOR R1/R2 DOUBLE KNOCKOUT MICE FOLLOWING EXPOSURE TO THE CHEMICAL.

R W Lange[1], J M Matheson[2], R Lemus[1], M I Luster[2], and M H Karol[1]. [1]*Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, USA;* [2]*Toxicology and Molecular Biology Branch, NIOSH, Morgantown, WV, USA.*

Toluene diisocyanate (TDI) is a widely used industrial chemical causing respiratory sensitization in some exposed individuals. In spite of intensive research, understanding of the mechanism of chemical allergy is unclear. B and T lymphocyte activation, T helper differentiation and tissue pathology, largely controlled by the release of cytokines, are required for an organism to mount an effective immune response. The pleitropic, potent proinflammatory cytokine TNF may contribute to the induction +/or illicitation phase of TDI sensitization. To address this hypothesis TNF R1/R2 -knockout mice (C57 black) were sensitized (sc, 20 μ l, day 1) boosted (sc, 5 μ l, days 4, 11) and repeatedly challenged (inhal., 100 ppb, days 21, 23 and 25) with TDI (80:20, 2,4:2,6 TDI). TDI sensitized and challenged wild type mice were included in the study as positive controls. Twenty-four hours following the last inhalation challenge the mice were sacrificed and their blood was collected for analysis of total IgE and TDI-specific IgG and IgE. Lungs were collected for RT-PCR measurement of IL-4, IL-5 and INF-γ mRNA expression and histopathologic evaluation of the airways. Total IgE was significantly increased in the knock-out mice compared with the wild type controls. In contrast, the titers of TDI-specific IgG and IgE were reduced in the TNF R1/R2 mice. Histopathology indicated differences in the trachea, nasal area and formation of germinal centers of regional lymph nodes. Similar results were obtained in normal mice receiving TNF neutralizing antibody prior to TDI exposure. The results of this study suggest that the proinflammatory cytokine TNF participates in the murine, immune response to TDI including TDI-specific antibody production and class switching. (Supported by NIEHS # 05651.)

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