PS 658 Cytochrome P450 2E1-Deficient Mice Show Attenuation of Trichloroethene-Mediated Autoimmune Response

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Environmental and occupational exposure to trichloroethene (TCE) has been linked to an autoimmune response. Mechanisms underlying the TCE-mediated autoimmunity remain unclear. Previous studies from our laboratory in MRL+/+ mice suggest that reactive TCE metabolites and oxidative stress contribute to TCE-mediated autoimmunity. The current study was undertaken to further assess the significance of TCE metabolism leading to oxidative stress and autoimmune response by using cytochrome P450 2E1 (CYP2E1)-null MRL+/+ mice. CYP2E1-null MRL+/+ mice were generated by backcrossing CYP2E1-null mice to MRL +/+ mice for 6 generations followed by intercrossing of N6 heterozygous mutants to obtain homozygous mutants. Female MRL+/+ and CYP2E1-null MRL+/+ mice were given TCE (10 mmol/kg, i.p., every 4th day) for 6 weeks; their respective controls received corn oil only. TCE treatment in MRL+/+ mice led to significant increases in serum malondiadelhyde (MDA)- and 4-hydroxynonenal (HNE)-protein adducts and their respective antibodies. TCE exposure was also associated with significant increases in serum anti-nuclear antibodies (ANA), anti-double stranded DNA antibodies (anti-dsDNA) and IL-17 levels. TCE treatment in CYP2E1-null MRL+/+ mice also led to increases in serum MDA-/HNE-adducts and their respective antibodies along with increases in ANA, anti-dsDNA and IL-17, but interestingly, the increases in the oxidative stress and autoimmunity markers in the CYP2E1-null MRL+/+ mice were significantly less pronounced compared to that in MRL+/+ mice. Attenuation of autoimmune response in CYP2E1-null MRL+/+ mice further supports the contribution of CYP2E1-mediated TCE metabolism in the induction of autoimmunity. Supported by NIH ES016302.

PS 659 Graphene Oxide Augments Airway Remodeling and Hyperresponsiveness in a Murine Model of Asthma

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Growing evidence indicates that local inflammatory responses following nanoparticle (NP) exposure result in modified innate immune responses, facilitating the development of respiratory diseases including asthma. Graphene oxide (GO) – having unique physico-chemical properties – is a promising carbonaceous nanomaterial with a multitude of medical and industrial applications. Here we investigated the modulation of allergic response in a murine model of ovalbumin (OVA)-induced asthma by pulmonary exposure to GO. The data showed that GO administration at the time of initial allergen sensitization augmented airway hyperresponsiveness and airway remodeling in the form of goblet cell hyperplasia and smooth muscle hypertrophy. The levels of IL-4, IL-5 and IL-13 were reduced in bronchoalveolar lavage (BAL) fluid and serum of GO-treated mice as compared to OVA-only group. Exposure to GO reduced eosinophil accumulation, but increased alveolar macrophages and lymphocytes in BAL fluid, as compared to those in OVA-only treated animals. Similarly, GO administration during sensitization stimulated the production of OVA-specific IgG2a and down-regulated the levels of IgE and IgG1. Moreover, exposure to GO increased the macrophage production of the mammalian chitinases, CHI3L1 and AMCase, whose expression is associated with asthma. Conclusively, while GO exposure reduces Th2 immune response in a murine model of asthma, it potentiates airway remodeling and hyperresponsiveness.

PS 660 Identification and Frequency of Naïve T Lymphocytes Specific for Penicillin: Implication in Drug-Allergy

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Allergic reactions to drugs are often unpredictable and may have many side effects such as anaphylaxis. According to the hapten hypothesis, small drug compounds bind to endogenous proteins to form immunogenic complexes. Antigen presenting cells, such as dendritic cells (DCs), recognize and internalize hapten-carrier complexes, and digest them into haptenized peptides, which are presented on HLA molecules to drug-specific T cells inducing an immune response. Knowing that drugs provoke IgE mediated hypersensitivity reactions in treated patients, naïve $CD4^+$ T-cell response to benzyl-penicillin (BP) was investigated. The aims of this study were to evaluate the frequency of naïve $CD4^+$ T-cells specific to BP and to identify BP-haptenized peptides (BP-P) from human albumin responsible for T-cell activation. Since BP is known to bind covalently to proteins, such as Human Serum Albumin (HSA), BP-HSA bioconjugates were synthesized, and BP binding-sites on HSA were identified using mass-spectrometry. Twelve BP-P of 15 mer long identified as potential T-cell epitopes were selected and synthetized. Naïve $CD4^+$ T-cells from non-allergic donors were stimulated once a week with autologous DCs loaded with BP-HSA or BP-P to amplify BP-HSA- or BP-P-specific T cells respectively. Activation of specific $CD4^+$ T cells were detected using interferon-γ EliSpot and their frequency was calculated using the Poisson distribution law. In this study, BP-HSA- and BP-P-specific $CD4^+$ T cells were detected in 13/14 and 7/8 of the tested donors respectively. Most donors responded to peptides with BP bound on lysines 159, 212 and 525. This study showed, for the first time, the capacity of BP-HSA to be recognized by naïve T-cells from healthy donors and the possibility to identify epitopes involved in the immunization to BP.

PS 661 Effects of Nickel Sulfate on Interleukin-12 Cytokine Family in Human Monocyte-Derived Dendritic Cells

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Allergic contact dermatitis (ACD) is a common skin disease that is caused by type IV delayed-type hypersensitivity responses to chemicals that come into contact with the skin. It has a high prevalence in Europe (15 to 20%). Among the 3000 known sensitizers, nickel is the most involved in ACD reactions. IL-12p70 (composed of IL-12p40 and IL-12p35) and IL-23 (composed of IL-12p40 and IL-23p19) are two cytokines involved in inflammation and adaptive immunity, from the IL-12 cytokine family and produced by dendritic cells. They are also involved in the amplification of ACD and play a major role in the generation of allergen-specific T cell responses. In this study, we address the question whether the sensitizer nickel sulfate ($NiSO_4$) can induce the secretion of IL-12p70 and IL-23 in human monocyte-derived dendritic cells (Mo-DC). In our model, $NiSO_4$ induced human Mo-DC maturation and enhanced the expression of cell surface markers (CD86, CD83). We found that IL-12p40 and IL-23 were produced by human Mo-DC in response to $NiSO_4$ stimulation. We also showed that $NiSO_4$ induced an early expression of *il-23p19* mRNA and *il-12p40* mRNA but mRNA levels of *il-12p35* weren't modified. Furthermore, we showed that $NiSO_4$ required the presence of IFN-γ to induce *il-12p35* mRNA expression. By contrast, this association induced a decrease in *il-23p19* mRNA expression. On the other hand, we found that p38MAPK was involved in the expression of *il-23p19* and *il-12p40* mRNA induced by $NiSO_4$. Finally, our results contribute to the understanding of the mechanisms of nickel-induced ACD and describe a novel effect of nickel on IL-12 cytokine family in human Mo-DC.

PS 662 Effect of Protein Aggregates from Therapeutic Proteins on Dendritic Cells Maturation: Implication for Immunogenicity

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Background: A major drawback in therapeutic biological products (BP) use is the development of anti-drug antibodies (ADA) in patients. Among other factors, BP aggregates has been demonstrated to promote immunogenicity. Moreover, the presence of ADA suggests a CD4 T-cell dependent adaptive immune response and therefore a pivotal role for antigen presenting cells, such as dendritic dells (DC). Aim and methods: We wanted to determine if BP aggregates participate to DC activation, with two protein aggregates models: human Growth Hormone (GH) and monoclonal antibodies (unformulated IgG1, and Rituximab). Native proteins were submitted to a shaking stress generating aggregates. After aggregate characterization (dynamic light scattering, fluorescence spectroscopy and circular dichroïsm analysis), the maturation status of human monocyte-derived DC upon exposure to BP or aggregates was evaluated *in vitro* (phenotypic marker expression and pro-inflammatory cytokines/ chemokines production). Results: Whereas native BP didn't modifiy DC maturation state, GH and IgG1 aggregates induced surface markers increase, mainly CD83 and CD86, and augmentation of IL-6, IL-8, IL-12p40 and MIP-1α and β production. Rituximab aggregates induced lower DC activation rates, although significant compared to the native protein. Finally, we showed that

The Toxicologist

Supplement to *Toxicological Sciences*

54th Annual Meeting and ToxExpo™

March 22–26, 2015 • San Diego, California

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 144, Issue 1
March 2015

www.toxsci.oxfordjournals.org

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