140.17

A RADIATION HYBRID MAP OF A SEVERITY QTL FOR COLLAGEN-INDUCED ARTHRITIS LOCATED ON RAT CHROMOSOME 10. David E. Dobbins*, Bina Joe, Elaine F. Remmers and Ronald L. Wilder. NIAMS, NIH, Bethesda, MD and Dept. of Physiology, USUHS, 4301 Jones Bridge Rd., Bethesda, MD 20814-4799*.

Collagen-induced arthritis is an animal model for human rheumatoid arthritis, a chronic and debilitating autoimmune disease. We have previously reported that the severity of collagen-induced arthritis in DA rats is controlled by genes located on various chromosomes across the rat genome. Analysis of arthritis severity following injection of native Type II collagen in DA (susceptible) x BN or F344 (resistant) F_2 rats indicates that the QTL designated as *Cia5* appears to be an important non-MHC locus regulating severity of arthritis in this model. In this study we report a radiation hybrid map of the *Cia5* region. Using the rat T55 radiation hybrid panel, we mapped numerous previously unresolved microsatellite markers, several genes not previously mapped by radiation hybrid techniques and several novel genes that have not yet been mapped in the rat. Included in the genes mapped are several candidate genes which might significantly influence the biochemical pathways involved in this disease process. These include genes involved in pro-inflammatory cytokine function, growth factor function, signal transduction and gene expression. Further analysis of the genes located in this region may yield important insights into the mechanisms of autoimmune disease pathways and indicate potential therapeutic targets for improved clinical management of rheumatoid arthritis and other autoimmune diseases.

140.18

THERAPEUTIC EFFECTS OF HUMAN CHORIONIC GONADOTROPIN ON STREPTOCOCCAL CELL WALL-INDUCED ARTHRITIS. X-y. Song, L. Zeng, W. W. Jin, C. M. Pilo, M. E. Frank, and S. M. Wahl. Oral Infection & Immunity Branch, NIDCR, NIH, Bethesda, MD 20892.

In autoimmune diseases such as rheumatoid arthritis, there is a marked difference in age and sex related incidence. Moreover, a dramatic amelioration of symptoms may occur during pregnancy, followed by an exacerbation postpartum. One of the hormones that increase sharply in early pregnancy and decline rapidly after parturition is human chorionic gonadotropin (hCG). In this study, we assessed the effect of systemic administration of clinical grade hCG on the development of arthritis in an experimental animal model also characterized by age and sex linkages. Administration of hCG, initiated two days prior to an arthritogenic dose of streptococcal cell wall (SCW) in nonpregnant rats, resulted in a dose-dependent reduction in the clinical arthritic index. Consistent with the clinical amelioration of the disease, hCG significantly reduced the inflammatory cell infiltration, pannus formation, and bone and cartilage degradation. Mechanistically, hCG was found to suppress the overzealous production of TNFα, IL-6, IL-1β and nitric oxide which contribute to synovial pathology in SCW arthritic animals. Moreover, the amount of iNOS protein was greatly reduced within the inflamed joints, and circulating TGF-β levels were upregulated by the hCG, all of which suggest monocyte/macrophages as a potential target. Our findings indicate that hCG exerts a protective effect in an experimental arthritis model through modulation of inflammatory mediators.

ENDOTOXINS (141.1-141.4)

141.1

HOST CELL RESPONSES TO MENINGOCOCCAL LIPOOLIGOSACCHARIDE. Peter C. Giardina,[1*] Theresa Gioannini,[2] Ben Buscher,[1] Anthony Zaleski,[1] Lynn Stoll,[3] De-Sheng Zhang,[2] Jerrold Weiss,[2] and Michael Apicella.[1] Departments of [1]Microbiology, [2]Biochemistry, and [3]Internal Medicine, Div. of Infectious Diseases, Univ. of Iowa, Iowa City, IA 52242.

Bacterial lipooligosaccharides (LOS) are believed to play a vital role in the pathophysiology of invasive meningococcal disease. Direct assays of host-LOS interactions have been hampered by the inability to utilize native LOS at relevant concentrations (≤ 10 ng/ml). We have constructed an acetate auxotroph (NMBACE1) of *Neisseria meningitidis* strain NMB that permits high specific radiolabeling of fatty acids (FA) of LOS by incorporation of [^{14}C]-acetate during bacterial growth. Acetate auxotrophy was accomplished by disrupting the gene encoding pyruvate dehydrogenase, *pdhA*. Incorporation of [^{14}C]-acetate into LOS was increased nearly 10-fold in comparison to wild-type NMB, as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), radioautography of purified LOS, and FA analysis. Purified [^{14}C]-LOS contained nearly 1,000 cpm/ng. Gel sieving through Sephacryl S-500 resin resolved [^{14}C]-LOS into 2 species of est. M_r of 20 x 10^6 kDa (peak A) and 1 x 10^6 kDa (peak B). The aggregate of higher M_r was disaggregated by the host endotoxin-binding proteins, LBP and sCD14, and potently stimulated synthesis and secretion of IL-8 by cultured endothelial cells. In contrast, the lower M_r aggregate was more active in stimulating oxidative responses of neutrophils. The ability to generate radiolabeled LOS of high specific radioactivity has permitted the examination of physical determinants of host-LOS interactions.

141.2

STUDIES ON THE ROLE OF IRON IN ENDOTOXIN-INDUCED PEROXIDATIVE DAMAGE. W. Ibrahim and C.K. Chow. Univ. Kentucky, Lexington, KY 40506-0054.

The toxic effect of endotoxin is partly attributable to increased oxidative damage resulting from increased generation of reactive oxygen species. The purpose of this study was to investigate the possible role of iron in endotoxin-induced peroxidative damage to membrane lipids. Male B6C3 mice were treated with endotoxin intraperitoneally with 0 or 20 mg/kg BW for 24 hours. Fresh liver homogenate was incubated with either EDTA, GSH, glucose, H_2O_2, AAPH, ascorbic acid (AA), $FeCl_3$, $FeCl_3$ plus AA, or AA plus EDTA in phosphate-buffered saline (PBS), pH 7.0, at 37°C for 60 min. The levels of malondialdehyde (MDA) were significantly higher in the liver of endotoxin-treated mice. MDA levels were increased significantly following incubation, especially in the endotoxin-treated group. Compared to PBS, EDTA was more effective than GSH in inhibiting the increase of MDA resulting from incubation. Glucose, AAPH or $FeCl_3$ had little effect, while H_2O_2 or AA significantly increased MDA formation. The greatest increase of MDA was found when AA and $FeCl_3$ were added together. EDTA also prevented the peroxidative effect of AA added subsequently, but was only partially effective when AA was added first. The results obtained suggested that iron plays an important role in endotoxin-induced peroxidative damage to membrane lipids.

141.3

EFFECTS OF THYROID STATUS ON LUNG DAMAGE AND ACTIVITIES OF LUNG PHAGOCYTES UNDER BASAL CONDITIONS AND FOLLOWING LPS EXPOSURE. L.J. Huffman, D.J. Judy, K.M.K. Rao, D.G. Frazer and W.T. Goldsmith. NIOSH, Morgantown, WV 26505.

The objectives of this investigation were to study the effects of hypo- and hyperthyroidism on lung cell damage and the activity of lung phagocytes under basal conditions and during inflammatory responses induced by exposure to lipopolysaccharide (LPS). Thyroid status in adult male rats was altered by thyroidectomy or thyroxine injections for 15 days. Rats were studied 18 h after inhalation exposures to air or to LPS (100 μg/ml; 3 h). In a hyperthyroid state alone, there was a greater degree of lung cell damage and alveolar-capillary barrier disruption, an increase in the number of phagocytes which could be harvested from the lungs by bronchoalveolar lavage, and an increase in nitric oxide production by lung phagocytes. On the other hand, hypothyroidism alone was associated with opposite effects. Similar effects of thyroid status on lung cell damage and lung phagocytes were also observed following inhalation exposures to LPS. These results suggest that thyroid status can modulate the overall state of oxidant balance in mature lungs and that increased thyroid hormone levels are associated with pro-oxidant pulmonary effects. As a consequence of this, lung inflammatory reactions to LPS appear to be relatively more pronounced in a hyperthyroid state and less pronounced in a hypothyroid state.

141.4

CARDIAC MYOCYTES ISOLATED FROM RATS WITH PERITONITIS INCREASE SURFACE LEVELS OF ICAM-1 ON RAT ENDOTHELIAL CELLS. W.S. Madorin, K. Wilkins, G. Cepinskas, P.R. Kvietys. Dept. of Physiology, UWO, and Vascular Cell Biology, LHSC-Research Inc., London, ON, Canada N6A 4G5

Cardiac myocytes isolated from rats 6 hrs after cecal ligation and perforation (CLP) increase the migration of naive rat polymorphonuclear leukocytes (PMNs) across rat endothelial cell monolayers. The myocyte-induced PMN transendothelial migration was abrogated when myocytes were isolated from CLP rats pretreated with LPS 24 hr earlier (LPS tolerance). In order to assess the molecular determinants of this LPS tolerance, we assessed the ability of cardiac myocytes to increase endothelial ICAM-1 surface levels during the development of LPS tolerance. Cardiac myocytes were isolated 4 hours after CLP or sham laparotomy in naive or LPS-pretreated rats. Myocytes were suspended in buffer for 90 min and the supernatants used in experiments. Confluent monolayers of rat skeletal muscle endothelial cells (rSMEC) were exposed to these supernatants for 4 hours and then fixed. ICAM-1 expression was measured by ELISA using a monoclonal antibody (1A29) to rat ICAM-1. Myocytes isolated from CLP-rats induced a dose-dependent increase in ICAM-1 expression on rSMEC. In contrast, myocytes isolated from LPS-pretreated rats produced only minimal changes in endothelial ICAM-1 expression. These results indicate that 1) endothelial cell ICAM-1 expression can be increased by cardiac myocytes from CLP rats and 2) LPS pretreatment can attenuate this response. (MT-13940).

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