139.1

Impaired Cutaneous Wound Healing in Interleukin-6 Deficient Mice

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It is well known that the inflammatory response following cutaneous wounding is necessary for healing, and it has been postulated that inflammatory cytokines, such as IL6, might be intimately involved in the healing process. However, studies concerning the exact roles of this cytokine during wound healing are unclear. When subject to full thickness cutaneous wounds, IL6 deficient mice displayed significantly delayed healing compared to control animals. Histology of wounds from IL6 deficient mice displayed no epithelial bridge formation, minimal granulation tissue formation and little inflammation. IL6 mRNA was expressed in the epidermis at the leading edge of the wound, in dermal fibroblasts, and macrophages in wildtype mice, but not in IL6 deficient mice. Mobility shift assays of skin samples from wildtype and IL6 deficient mice showed decreased AP-1 induction 16 hours post wounding. When IL6 deficient mice were treated with a single dose of recombinant IL6 they displayed healing virtually indistinguishable from wildtype mice. Gene replacement treatment, utilizing a plasmid construct containing the murine IL6 gene, of IL6 deficient mice produced results similar to treatment with recombinant IL6.

139.2

EFFECT OF RECOMBINANT SOLUBLE BOVINE CD14 ON CYTOKINE PROFILES OF WHOLE BLOOD STIMULATED WITH LIPOPOLYSACCHARIDE.

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Lipopolysaccharides (LPS) in the outer membrane of Gram-negative bacteria are responsible for most disease symptoms caused by these organisms. Soluble CD14 (sCD14) neutralizes LPS and prevents their binding to and activation of monocytes and macrophages. In the present study, two C-terminal truncated recombinant bovine CD14 (Δ1rbsCD14 and Δ2rbsCD14), each containing a six-histidine tag at the C-terminal end, were cloned into a baculovirus expression system. Neither rbsCD14 was detected on the surface of sf-9 cells infected with recombinant virus, but were present in the culture supernatants. Both ΔrbsCD14 and Δ2rbsCD14 reached their highest concentration (4 mg/l) approximately 72 and 96 hr after infection, respectively. The LPS-free rbsCD14 proteins were purified sequentially, using nickel-conjugated affinity chromatography followed by polymoxin-B-sulfate affinity chromatography. Because LPS causes changes in cytokine profiles in animals infected with Gram-negative bacteria, competitor DNA molecules for IL-8, TNF-α , IL-1β, IL-6 and IL-10 were used to study mRNA transcriptional changes by competitive RT-PCR, and better elucidate the roles of rbsCD14 and the rbsCD14:LPS complex in attenuating the effects of LPS on infected animals.

139.3

IL-4 Regulates Platelet-activating factor (PAF) Synthesis in Human Endothelial Cells

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PAF is a potent phospholipid autocoid that participates in leukocyte recruitment during inflammation. Human umbilical vein endothelial cells (HUVEC) synthesize and express PAF in response to several agonists including histamine and thrombin. In this study we examined the role of cytokines in regulating PAF synthesis. HUVEC were treated for 24 hr with IL-4 then stimulated for 5 min with histamine or thrombin. Total PAF synthesis was determined by measuring [^{3}H]-acetate incorporation into newly synthesized PAF. IL-4 stimulation primed HUVEC for increased PAF production in response to either histamine or thrombin. Other cytokines including TNFα, IL-1β and oncostatin M had no priming effects. IL-4-mediated priming was not mediated by changes in PLA_2 protein as measured by western blotting or activity as measured by prostaglandin synthesis. IL-4 also increased the sensitivity of HUVEC for histamine. Histamine induced changes in intracellular calcium and PAF synthesis occurred at 10-100 fold lower concentrations in IL-4-stimulated cells as compared to control cells. These results suggested that IL-4 may be increasing the number of histamine receptors on HUVEC. Since histamine-induced PAF synthesis occurs via H1 receptors on HUVEC, we examined the IC^{50} for the H1 antagonist pyrilamine. IL-4 increased the IC^{50} for pyrilamine suggesting that more histamine receptors are present on IL-4-stimulated cells. Studies are underway to directly measure histamine receptor expression on these cells. These data show that IL-4 primes HUVEC for total PAF synthesis in response to both histamine and thrombin. Priming for histamine may, in part, reflect an increase in histamine receptors.

139.4

NF-kB1 (P50) GENE KNOCKOUT ATTENUATES IONIZING RADIATION-INDUCED IN VIVO NF-kB ACTIVATION AND IL-1α, IL-1b AND IL-6 mRNA EXPRESSION.

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We tested the hypothesis that ionizing radiation (IR) induced in vivo inflammatory cytokine production is mediated through NF-kB activation. Using the gel shift and RNase protection assays, it was found that exposure of mice to total body IR activated NF-kB and upregulated TNFα, IL-1α, IL-1b and IL-6 mRNA expression in a tissue-specific manner, e.g. in the spleen, mesenteric lymph nodes and bone marrow. The activation of NF-kB preceded the increased expression of TNFα, IL-1α, IL-1b and IL-6 mRNA. The existence of such a temporal relationship suggests that IR-induced NF-kB activation may play a pivotal role in the induction of these cytokines. This was supported by the findings that p50 knockout mice (p50-/-) showed significant reduction in IR-induced activation of NF-kB and increases in IL-1α, IL-1b and IL-6 mRNA expression, as compared with that of the wild type (p50+/+) mice. However, p50-/- mice exhibited no significant changes in TNFα mRNA expression after IR. Thus, this indicates that the NF-kB containing p50 subunit plays an important role in mediating IR-induced IL-1α, IL-1b and IL-6 production, while it has limited influence on the expression of TNFα mRNA. This study provides new insight into the molecular mechanisms whereby IR induces the production of these inflammatory cytokines. This may lead to the development of new strategies to inhibit the production of these cytokines following IR treatment for tumor therapy or bone marrow transplant pre-conditioning (Supported by grants from NIH and VA Medical Center).

139.5

Pro-inflammatory Cytokine Expression in Surgical Wounds

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Surgical wounds provoke an inflammatory cascade which includes the temporally ordered production of cytokines and chemokines. This process of secretory events orchestrates the sequential infiltration of inflammatory leukocytes and regulates their function. The objective of this study is to define the cause and effect relationships between cytokine expression patterns, leukocyte infiltration, and normal and pathogenic wound healing outcomes. The expression of cytokines and chemokines and the pattern of leukocyte infiltration were monitored in the skin and peritoneal wall over time in a 20 mm full thickness abdominal incision in anesthetized C57BL6 mice. Cytokine/chemokine mRNA expression was detected in 3 temporal waves. Within 2 hours IL-1α, IL-1β , MIP-1α , MIP-1β , MIP-2, KC, Eotaxin and RANTES were detected; while some mRNAs were sustained (IL-1β , MIP-1α , MIP-2) others were transient (KC, Eotaxin, RANTES). No TNFα expression was detected. An intermediate wave of chemokine expression including MCP-1 followed the early wave by approximately 2-4 hr. A third wave of expression represented by IL-6 and TGFβ was observed 3-7 days following injury and may represent a transition from inflammation to healing. In general, cytokine/chemokine protein levels detected in tissue reflected levels of the corresponding mRNA. Neutrophil infiltration was detected within 3-4 hr while monocytes appeared between 8-16 hr. Neutralization of IL-1α and β markedly reduced the subsequent chemokine expression and leukocyte infiltration. Further studies will determine the impact of regulating early inflammatory events on the transition from an inflammatory to a wound healing phenotype.

139.6

The study of syndecan-1 molecule expression on a human endothelial cell line: Effects between the pro-inflammatory cytokine and the virus

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Dengue virus belongs to Flaviviridae, has four serotypes and could cause dengue fever. This disease could be catalogued into dengue fever, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) depending on the progression of the disease severity. The manifestations of dengue fever are pain of the extremities and of the post eyeball, lymphoadenopathy and rashes. Clinically, DHF/DSS also has the same initial manifestations as dengue fever. Endothelial cells have functions for maintaining the balance of body fluids which may be related to the development of DHF/DSS. It is known that glycosaminoglycans on the cell surface could play an important role of the cellular integrity and syndecans may have a role in this process. Therefore, we explored the possible relationships between the expression of syndecan-1 molecules on the surface of a human endothelial cell line, ECV 304, infected by a type 2 local strain dengue virus, PL046, and treated with TNFαnalpha, respectively. Using a FACSCalibur flow cytometer and anti-syndecan-1 monoclonal antibody, we found that the expression of syndecan-1 molecules on the ECV 304 cells was decreased in both treatments. TNF alpha showed a more profound reduction on the expression of syndecan-1 molecule than the virus alone did. These results suggested that ECV 304 endothelial cells could be infected by a type 2 dengue virus, PL046, and the pro-inflammatory cytokine, TNFαnalpha, might have a more positive contribution in the pathogenesis of DHF/DSS in terms of the regulation of syndecan-1 molecule expression on the surface of ECV304 cells.