41

**Interleukin-6 stimulates 92 kDa matrix metalloproteinase (MMP-9) activity in mice lymphocytes in vitro** A. E. Haratym-Maj, S. Tos-Luty, J.J. Tomaszewski, J.G. Maj. Department of Pathology, Institute of Agricultural Medicine, Lublin, Poland, Department of Clinical Biochemistry & Environmental Toxicology, School of Medicine, Lublin, Poland

Interleukin-6 (IL-6) is produced by T and B cells, fibroblasts, endothelial cells, monocytes and macrophages. This cytokine is one of the strongest stimulators of acute phase proteins. The aim of this work was to study the effect of IL-6 on MMP-9 activity in mice lymphocytes culture. Mice lymphocytes were isolated from peripheral blood. Lymphocytes were cultured in RPMI 1640 medium with different concentrations of IL-6. The activity of MMP-9 was estimated in a cell culture medium using zymography technique. We showed, that IL-6 stimulates MMP-9 activity in culture of mice lymphocytes. The results suggest that MMP-9 may be involved in inflammatory processes an may help lymphocytes to migrate into inflammatory sites.

42

**IN VIVO EFFECT OF SOMATOSTATIN ANALOGUE (OCTREOTIDE) ON PERITONEAL AND HEPATIC MACROPHAGES**

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Our previous studies have shown that somatostatin and its synthetic analogue octreotide modulate *in vitro* the function of peritoneal macrophages and hepatic macrophages (Kupffer cells). In the present study, male Sprague-Dawley male rats were injected subcutaneously with 10 μg/kg of octreotide twice day for one day. Peritoneal macrophages and Kupffer cells were isolated and then treated with 0.1 μg/ml of LPS. After 18 hours treatment, the amount of nitric oxide released by peritoneal macrophages and Kupffer cells obtained from octreotide-treated rats increased 18.9% and 39.9% as compared to the control group (injected with normal saline). TNF-α mRNA and TGF-β1 mRNA expresion after treatment with LPS for 3 hours were detected by RT-PCR. TGF-β1 mRNA expression in both peritoneal macrophages and Kupffer cells in octreotide-treated group was not different from that of control group. TNF-α mRNA expression in Kupffer cells was not changed by octreotide treatment. However, TNF-α mRNA expression in peritoneal macrophages of octreotide-treated rats increased as compared to controls. These findings demonstrate that octreotide differentially regulates the function of peritoneal macrophages and Kupffer cells.

43

**EFFECTS OF MACROMOLECULAR ACTIVATORS OF PHAGOCYTOSIS FROM PLATELETS (MAPP) ON FCγ RECEPTORS OF NEUTROPHILS.**

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MAPP (l-MAPP, 300 kDa; s-MAPP, 150 kDa) which are glycoproteins released from stimulated platelets enhance human neutrophilic phagocytosis through Fcγ receptors (FcγR). This function of MAPP was obscured with the increase in phagocytosis of both stimulated and control neutrophils when those adhered on surface were centrifuged with IgG-sensitized sheep red blood cells (EA) before phagocytosis reaction. Binding ability of neutrophils with EA examined by spontaneous rosette formation in suspension was increased by pretreatment of neutrophils with MAPP (12.3 % rosette positive cells with more than 8 EA in control neutrophils; 21.0 % in l-MAPP and 30.7 % in s-MAPP stimulated neutrophils), although expressions of CD16 and CD32 were not affected by MAPP. Pretreatment of neutrophils with anti CD32 monoclonal antibodies depressed phagocytosis of both MAPP stimulated (83 % depression in l-MAPP and 81 % in s-MAPP stimulated neutrophils) and control neutrophils (48 % depression) markedly.

These observations suggest that FcγR-mediated neutrophil phagocytosis is augmented by MAPP through enhancement of binding ability of FcγR-II and not by an increase in expressions of FcγR.

44

**ARSENIC ENHANCEMENT OF SKIN NEOPLASIA BY CHRONIC STIMULATION OF GROWTH-PROMOTING CYTOKINES**

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Although arsenic is a human carcinogen, there is currently no known mechanism for its action or an established animal model for its study. Previous studies in our laboratory demonstrated increased mRNA transcripts and secretion of keratinocyte-derived growth factors, including TGFα and GM-CSF and increased cell proliferation in human keratinocytes cultured in the presence of arsenite. As recent reports demonstrate that overexpression of TGFα serves to enhance skin tumors, we hypothesized that similar events are responsible for those associated with arsenic exposure. The influence of arsenic on mouse skin tumor development was studied in transgenic TG.AC mice which carry the v-Ha-*ras* oncogene (a genetically initiated model for skin carcinogenesis). Following low-dose application of TPA, a marked increase in the number of skin papillomas occurred in transgenic mice receiving arsenic compared to control drinking water, while no papillomas developed in arsenic-treated transgenic mice that had not received TPA or arsenic-treated wild-type FVB/N mice. Consistent with earlier observations using primary human keratinocyte cultures, increases in GM-CSF and TGFα mRNA transcripts were found throughout the epidermis of arsenic-treated mice. Immunohistochemical staining localized the increases in GM-CSF and TGFα to the hair follicles. Analysis of cytokine gene expression in samples of lesioned skin obtained from humans chronically exposed to arsenic via their drinking water also showed alterations in cytokine gene expression consistent with those seen *in vitro* with primary human keratinocyte cultures and the transgenic mouse model. These results suggest that arsenic enhances development of skin neoplasias via the chronic stimulation of keratinocyte-derived growth factors and may be a unique example of a chemical carcinogen which acts in this manner.

45

**MOLECULAR REGULATION OF IL-6 BY ASBESTOS.**

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Pro-inflammatory cytokines and growth factors are involved in the pathophysiological responses of asbestos-related lung diseases. Although interleukin-6 (IL-6) has been characterized as a pleiotropic cytokine with multiple biological activities, its induction and role in asbestos diseases have not been studied. Asbestos fibers were found to stimulate IL-6 secretion and mRNA transcripts in pulmonary type II-like epithelial A549 cells. IL-6 induction was dependent on the intracellular redox-oxidative state since asbestos, as well as $H_2O_2$-induced IL-6 secretion was abrogated by intracellular-acting hydroxyl scavengers and N-acetylcysteine. IL-6 induction paralleled increased DNA binding activity to the NF-kB and NF-IL-6 recognized sites in the IL-6 promoter. The NF-kB and NF-IL-6 DNA binding proteins were immunochemically characterized as a heterodimer p65/p50 and a homodimer C/EBP β, respectively. Asbestos and $H_2O_2$-induced DNA binding activity to the NF-kBand NF-IL-6 binding sites of the IL-6 promoter were inhibited by antioxidants. The role of local IL-6 production in the pathophysiological processes of fiber-induced lung disorders was examined. Although less active than fibroblast growth factor, IL-6 stimulated in vitro lung fibroblasts growth. Furthermore, elevated IL-6 levels were found in bronchoalveolar lavage fluids from patients diagnosed with lung fibrosis and work-related histories of long-term asbestos exposure. Taken together, the results suggest that asbestos-induced oxidative stress is involved in the activation of NF-kB and NF-IL-6 transcription factors recognizing IL-6 promoter. The resulting increase in IL-6 expression may be involved in both inflammatory and fibrotic processes in the lung.

46

**EXPRESSION OF CYTOKINE RECEPTORS ON NORMAL AND TRANSFORMED HEMATOPOIETIC CELLS**

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We have tested the reactivity of monoclonal antibodies (MoABs) against cytokine receptors CD25 (IL-2Rα, 55kD), CD115 (*M*-CSF-1R, 150kD), CD116 (hGM-CSRF, 145kD), CD117 (CSFR, 145kD), CDw119 (IFN-R, 90kD), CD120a (TNFR, 55kD), CD120b (TNFR, 75kD), CD121a (IL-1R, typeI, 80kD), CDw121b (Il-1R, typeII, 68KD), CD122 (IL-2Rβ, 75kD), CD123 (IL-3R), CD124 (IL-4R, 140kD), CD126 (IL-6R, 80kD), CDw127 (IL-7R, 75kD), CDW128 (IL-8R), CD130 (gp130) and CD134 with normal and transformed pathological cells from 42 patients with leukemias/lymphomas. Staining for cell surface cytokine receptor complexis was done by imunocytochemical and immunofluorescence methods evaluated by light microscopy and flow cytometry. The results indicate that some cytokine receptors are present on the pathological cells in individual cases, but with very variable expression. The enhanced receptor expression on leukemia/lymphoma cells can form a target for the diagnosis of the disease process.
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