1538 TRANSFORMING GROWTH FACTOR β (TGF-β)-INDUCED INHIBITION OF BONE MARROW (BM) STROMAL CELL PRODUCTION OF INTERLEUKIN-7 (IL-7). L W Updyke, K S Cocke, and D Wierda. Toxicology Research Laboratories, Lilly Research Laboratories, A Division of Eli Lilly and Company, Greenfield, IN.

IL-7 is produced and released by BM stromal cells and is required for pre-B cell proliferation. The current studies demonstrated that TGF-β_1 (0.1-10.0 ng/ml) caused significant toxicity, as measured by decreased cell number, viability, and [^{3}H]-thymidine incorporation, to freshly isolated Ly5/B220$^+$ murine pre-B cells and to an IL-7-dependent murine pre-B cell line SCID/FC-7 (SCID). Comparable toxicity was noted in both the presence and absence of IL-7 indicating no direct interaction of TGF-β_1 with exogenously added IL-7. Moreover, TGF-β_1 pretreatment of adherent long term BM cultures and a BM stromal cell line (SCL 160), each of which produce IL-7, caused a concentration-dependent decrease in support of proliferation of co-cultured SCID cells. This decrease was correlated with a 17% and 80% reduction, compared with control, in expression of mRNA for IL-7 by adherent SCL 160 cells exposed to 1 and 10 ng/ml TGF-β_1, respectively. TGF-β-mediated decreases in BM stromal cell production of IL-7 may represent an important pathway for feedback inhibition of pre-B cell development.

1539 CHARACTERIZATION OF IN VITRO CANINE BONE MARROW STEM CELL ASSAYS USING HUMAN DERIVED GROWTH FACTORS. E I Fischer and J R Hincks. Molecular Toxicology Lab, Toxicology Dept., Sterling Research Group, Rensselaer NY.

In vitro bone marrow (BM) stem cell assays have been well characterized in mice and humans, and to a lesser extent in dogs. However, the canine colony forming units-granulocyte, macrophage (CFU-GM) assay has been hindered by the difficulties in obtaining canine growth factors. In this study, we investigated the cross reactivity of human derived growth factors to stimulate the growth of canine CFU-GM. Canine BM samples were separated by density-gradient centrifugation and plated in IMDM medium containing 1% methylcellulose, 20% fetal bovine serum, and up to 20% human MO-T cell (T cell leukemia) or 20% human 5637 cell (bladder carcinoma) conditioned medium as a source of colony-stimulating factor. After 14 days of culturing, conditioned medium from 5637 cells stimulated better growth than MO-T cells, producing approx. 150 colonies per 10^5 cells plated. Colonies were identified as granulocyte (G), macrophage (M), or mixed (GM) by morphology and esterase staining techniques. Busulfan, a myelosuppressive agent, produced a dose-dependent inhibition of GM colony formation after incubating BM cells in vitro prior to plating. This improved canine CFU-GM assay, coupled with the CFU-E (erythroid) assay, represents an in vitro technique to assess myelosuppression in the dog.

1540 **CHANGES IN FREE INTRACELLULAR CALCIUM AND PRODUCTION OF REACTIVE OXYGEN METABOLITES BY PARTICULATE AND SOLUBLE STIMULI.** **M Tuomala, M-R Hirvonen, K Kurvinen and K Savolainen. Natl Publ Hlth Inst, Dept Env Hyg Toxicol, Kuopio, FINLAND.**

Quartz, chrysotile, phorbol myristate acetate (PMA) and chemotactic peptide (fMLP) activate phagocytic cells with a consequent production of reactive oxygen metabolites (ROM). All of these stimuli excluding PMA, which stimulates protein kinase C (PKC), also release calcium from intracellular stores. Activation mechanisms of human polymorphonuclear leucocytes (PMNL) were studied in vitro after exposure of cells (5 x 10^5 cells/ml) to graded doses of soluble and particulate stimuli (fMLP, PMA, quartz, chrysotile). Free intracellular calcium ($[Ca^{2+}]_i$) was measured with a fluorometer applying fura-2-AM and chemiluminescence, caused by ROM, was measured with a luminometer. All stimuli, except PMA, dose-dependently increased $[Ca^{2+}]_i$ whereas all of the stimuli dose-dependently increased the production of ROM. These findings are in agreement with the assumption that quartz, chrysotile and fMLP elevate $[Ca^{2+}]_i$ and induce ROM production via inositol lipid signalling pathway. PMA-induced ROM production is likely due to PKC activation, also a result of activation of inositol lipid signalling in phagosytic cells. Supported by the Finnish Work Environment Fund.

1541 CYTOKINE AND PHARMACOLOGICAL REGULATION OF LUNG FIBROBLAST PROLIFERATION. RH Reist, J Blackford, K Vrana, V Castranova, and R Dey. Depts. of Physiol., Anatomy, & Biochem., West Virg. Univ., & Div. Resp. Dis. Studies, NIOSH, Morgantown, WV.

Our objective was to study the role of macrophage-derived cytokines in fibroblast proliferation. [^{3}H] thymdine incorporation was shown to correlate well with fibroblast proliferation. In vitro treatment of alveolar macrophages with silica (150μg/ml) resulted in the production of an agent(s) which inhibits fibroblast proliferation. Indomethacin, a prostaglandin synthetase inhibitor, reversed this inhibition and unmasked a previously unseen competence factor(s). Further, the proliferative action of exogenous PDGF was reduced when fibroblasts were treated with supernate from silica-exposed macrophages, presumably due to the inhibitory action of prostaglandins. Silica-induced pulmonary fibrosis may result from decreased release of prostaglandins and/or increased release of proliferative factors from alveolar macrophages. Indeed, macrophages harvested 40 days after intratracheal instillation of silica (42mg/rat) produced an agent(s) which significantly stimulated [^{3}H] thymidine incorporation by pulmonary fibroblasts. The antifibrotic drug, tetrandrine, inhibited [^{3}H] thymidine incorporation and fibroblast proliferation in response to either serum or PDGF plus plasma. Thus, tetrandrine may be a useful probe to study fibrogenesis. (BOM-5431)

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