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1.037

INTRACELLULAR CALCIUM CONCENTRATION IN HUMAN GRANULOCYTES IS INFLUENCED BY PRIMING OF THE CELLS WITH CYTOKINES.

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Interferon-γ (IFN-γ) and granulocyte macrophage colony-stimulating factor (GM-CSF) are known to pre-activate human polymorphonuclear neutrophilic granulocytes (PMN) to increased physiological responses, e.g. oxygen metabolism, exocytosis and phagocytosis, when subsequently stimulated by a secondary agent. The mechanism of this priming effect is still unknown.

We report here effects of IFN-γ (100 U/ml) and GM-CSF (625 U/ml) on PMN calcium homeostasis, which may be instrumental to the priming process. Measured by fluorescence spectroscopy of indo-1 loaded cells ($10^7$ cells/ml), a possible but not significant difference could be observed between pre-treated cells and control cells in basal intracellular calcium ion concentration ($[Ca^{2+}]_i$) (n=11). No effect could be seen on maximum $[Ca^{2+}]_i$ upon stimulation with the bacterial peptide analogue formyl-methionyl-leucyl-phenylalanine (fMLP).

To evaluate possible differences at the single cell level, cells loaded with the fluorescent $Ca^{2+}$-indicator fura-2 were examined in a digital imaging system. Three separate experiments (174 cells examined) indicated a significant difference between IFN-γ pre-treated cells and control cells. Intracellular calcium was elevated in two of the experiments, 16 % and 37 %, (p=0.064 and p=0.0039, Student t-test) and significantly lowered in the third, -18 %, (p=0.027). No differences were observed concerning the $[Ca^{2+}]_i$ increase upon stimulation with fMLP or latency time before this response.

Basal $[Ca^{2+}]_i$ was consequently examined in a greater number of cells with flow cytometry and fluo-3 as a calcium indicator. Occasionally, for unknown reasons, a lowered basal level of calcium in pretreated cells could also be observed with flow cytometry, but in all experiments taken together cells pretreated with IFN-γ had 17% higher basal $[Ca^{2+}]_i$ than control cells (p=0.001, Wilcoxon paired-comparison test), (n=7), and GM-CSF treated cells 22% higher concentration than control cells (p=0.015) (n=6).

In conclusion, the basal $[Ca^{2+}]_i$ in human PMN is significantly increased following priming of the cells with IFN-γ and GM-CSF. The difference was not detectable, however, unless single cells were examined.

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## *IN VIVO* INTERNALIZATION OF RAT MAST CELL GRANULES BY INTRAPERITONEAL TRANSPLANTED YOSHIDA TUMOR CELLS AND RESIDENT MACROPHAGES

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Mast cells are easily identified in rat peritoneal fluid smears by May Grunwald - Giemsa staining. These cells contribute to host defence against cancer being source of TNF-α (1). Under light microscope examination, mast cell granules can be detected within the cytoplasm of Yoshida ascitic tumor cells and resident macrophages in tumor bearing rats during the first 4-5 days after tumor transplantation. Adherence of the granules to the membrane of tumor cells and host macrophages is also detectable as well as the engulfment of the granules by cytoplasmic projections. In some cells, granules appear to undergo degradation. The granules are metachromatic as determined by toluidine blue staining. Previously (2), the ability to phagocytose and degrade mast cell granules has been demonstrated in rat and human cultured fibroblasts and bovine endothelial cells.

1) Gordon, J.R. and Galli, S.J. Nature, 346, 274-276, 1990
2) Atkins, F.M., Friedman, M.M., and Metcalfe, D.D. Laboratory Invest., 52, 278-286, 1985

1.039

THE GENERATION OF MITOCHONDRIAL OXYGEN RADICALS AS A CRUCIAL STEP IN THE CYTOTOXIC ACTION OF TNF

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The macrophage-derived cytokine Tumor Necrosis Factor exerts a cytotoxic action on a variety of tumor cells by largely unknown mechanisms. We have analyzed the pathway of TNF cytotoxicity by cotreatment of L929 fibrosarcoma cells with various inhibitors of mitochondrial electron transport. Inhibition of the electron transport before the ubiquinone entry site rendered the cells markedly TNF-resistent. Inhibition, however, after the ubiquinone pool with antimycin A, resulted in a strong potentiation of TNF-cytotoxicity. Furthermore, measurement of oxygen consumption in TNF-treated cells revealed that TNF induced an early inhibition of the electron transport, most probably at complex III of the mitochondrial chain. The TNF-induced damage of the electron transport became detectable after 60 min, and thus preceded the onset of cell death for several hours.

The different effects of the mitochondrial chain inhibitors on TNF cytotoxicty and the direct impairment of mitochondrial electron transport by TNF provide suggestive evidence that the production of oxygen radicals, which are mainly derived from the ubisemiquinone site inside the mitochondria, is a causal mechanism of TNF cytotoxicity. Indeed, the direct analysis of reactive oxygen species confirmed that the generation of oxygen radicals in isolated submitochondrial particles was increased severalfold in TNF-treated cells.

1.040

PGE$_2$ AND IL-2 MODULATE THE CYTOTOXICITY OF TUMOR BEARING MICE MACROPHAGES

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MØ cytotoxic activity is modulated "in vitro" by numerous cytokines.We demonstrated that supernatants obtained from cultures of splenocytes of tumor bearing mice,Lewis Lung carcinoma,can induce a cytotoxic activity on peritoneal MØ althoug the presence of high amounts of PGE$_2$. On the other hand, we detected in the same supernatants, the presence of IFNγ and IL-2, both playing an important role in modulating the PGE$_2$ release;in fact IL-2 increases the PGE$_2$ release by MØ whereas IFNγ display an inhibitor effect. It is interesting to note that both IL-2 and IFNγ activate MØ cytotoxicity cutting the PGE$_2$ suppressor effect off.The data presented show that PGE$_2$ synthesis is important for modulating, in a negative direction, the MØ cytotoxic activity,but the augment or the suppression of MØ cytotoxicity is determined by the functional balance of cell products; such as PGE$_2$ and the lymphokines(IL-2 and IFNγ). Grants M.P.I.40%,1989

1.041

## Immunomagnetic cell isolation.

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The immunomagnetic cell separation technique described, is based upon the magnetic particles developed by Ugelstad and coworkers at SINTEF. These particles are monosized, and they have a coating which permits binding of antibodies. In addition this coating will prevent non-specific binding. By coupling antibodies to the particles, it is possible to isolate a cell type from a mixed population of cells. For instance, this technique is used for removal of tumor cells from bone marrow in autologous transplantation, and for isolation of T and B cells for different qualitative and quantitative analyses. This method has been adapted for separation of human natural killer (NK) and lymphokine activated killer (LAK) cells by using antibodies against CD56. The CD56+ cells remained unactivated after separation and preserved their funtional characteristics. Incubating the CD56+ cells with IL-2 resulted in high LAK activity.

1.042

CONTRADICTION BETWEEN NK AND AUTO-TUMOR EFFECTOR CELLS ACTIVITY LOCALLY AND ON PERIPHERY IN THE LUNG CANCER PATIENTS.

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It was found that tumor infiltrating lymphocytes /TIL/ as well as from lymph nodes draining site of lung tumors /LNG/ exhibit marked depression of NK cells activity when compared to PBL population in the same patients. On the contrary TIL and LNG lymphocytes expressed cytotoxicity against autologous tumor cells while PBL population exhibit weaker activity against same targets. It was also shown that NK and LAK activity is decreased in PBL of cancer as well as in patients with chronic non tumor lung diseases in comparison with PBL population derived from healthy donors. These dates may suggest that NK cells are not true effectors in the immunity against cancer.