854.7

USING MOLECULAR BEACONS™ FOR QUANTITATING GENE EXPRESSION DURING MACROPHAGE ACTIVATION. Danny Hoang, Brenda Rogers, and Douglas McKenzie. Stratagene Inc., La Jolla, CA. 92037

Activated macrophages are thought to exert pathological effects in rheumatoid arthritis and atherosclerosis. Recent studies suggest that agonists of the peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear receptor superfamily, inhibit the production of pro-inflammatory lymphokines by activated macrophages. In adipose tissue, pro-inflammatory lymphokines such as TNFα counteract PPARγ agonist-induced adipogenesis and lipolysis. These data suggest the existence of counter-regulatory interactions between pro-inflammatory lymphokines and endogenous PPARγ ligands such as 15d-PDJ$_2$ in the control of gene expression. In this study, macrophages derived from the human monocytic leukemia cell line THP-1 were exposed to pro-inflammatory stimuli or PPARγ agonists and quantitative assessment of gene expression was performed using Molecular Beacons™. The Molecular Beacon™ format allowed us to conduct real-time analysis of the RT-PCR. Through the use of unique gene-specific positive controls, we were able to assign copy number to the PCR input templates. We evaluated the expression of a battery of target genes, and were able to identify distinct gene expression patterns induced by each of these agents.

854.8

Alpha 1-acid Glycoprotein-induced Tumor Necrosis Factor Secretion of Human Monocytes is Enhanced by Serum Binding Proteins and Depends on Protein Tyrosine Kinase Activation. Trai-Ming Yeh and Shu-Jem Su. Department of Microbiology and Immunology and Department of Medical Technology, College of Medicine, National Cheng Kung University, Tainan, Taiwan 70101, R.O.C.

The acute phase protein, alpha 1 acid glycoprotein (AGP), stimulated human mononuclear cells as well as monocytes to secrete tumor necrosis factor-alpha (TNF) which was demonstrated by ELISA, RT-PCR and functional assays. AGP-induced TNF secretion of monocytes was enhanced in the presence of human plasma and inhibited by protein kinase inhibitors, indicating it is serum and tyrosine kinase dependent. The activation of tyrosine kinase in AGP-stimulated monocytes was further confirmed by immunoblotting of tyrosine phosphorylated proteins of monocytes at different time after AGP stimulation. Furthermore, several serum proteins such as C3, sCD14 and IgG were able to bind to AGP and enhanced TNF secretion of human monocytes induced by AGP. Taken together, these results suggest serum protein binding to AGP enhance its ability to stimulate human monocytes to secrete pro-inflammatory cytokines through a tyrosine kinase dependent pathway.

854.9

EXPRESSION OF COSTIMULATORY MOLECULES BY CANINE ANTIGEN PRESENTING CELLS.

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The CD28/B7 costimulatory system has been identified as critical in the development of an optimal T lymphocyte response in both humans and rodents. Costimulation of T lymphocytes by antigen presenting cells is expected to be essential in the immune responses of dogs. A panel of commercially available monoclonal antibodies (Mabs) directed against human and murine CD80 (B7-1) and CD86 (B7-2) molecules was employed in an indirect immunofluorescent flow cytometric assay to investigate the surface expression of these molecules. Freshly isolated or recombinant canine interferon (IFN)-gamma-stimulated canine pulmonary alveolar macrophages and unstimulated and IFN-gamma-stimulated cells of the canine monocyte/histiocyte cell line, DH82, were evaluated. Pulmonary alveolar macrophages activated by IFN-gamma bound Mabs 16-10A1 (anti-CD80) and IT2.2 (anti-CD86), demonstrating increases of 21% and 39% over constitutive levels, respectively. Interferon-gamma treatment of DH82 cells induced binding by Mabs 16-10A1, BB-1 (anti-CD80), and IT2.2 with resultant increases over constitutive expression of 18%, 22%, and 39%, respectively. These antibodies will be useful in further investigations of the canine T lymphocyte response to infectious agents and neoplastic cells.

854.10

SURVANTA DOES NOT INHIBIT NITRIC OXIDE (NO) PRODUCTION INDUCED BY LIPOPOLYSACCHARIDE (LPS) AND INTERFERON-γ IN TWO MACROPHAGE CELL LINES. K.M.K. Rao, L. Bowman and T. Meighan. PPRB/HELD/NIOSH, Morgantown, WV 26505.

We have previously shown that lung surfactant and Survanta (a surfactant substitute) inhibit NO production induced by LPS in rat alveolar macrophages (Miles et al., Am.J.Physiol., in press). We studied the effect of Survanta (200 μg phospholipid/ml) on NO production in two monocytic cell lines, J-774A.1 and Raw-264.7, and found that it does not inhibit NO production induced by LPS (1 μg/ml) plus interferon-γ (25 U/ml). The effect on NO was characterized by measuring NO production by the Griess reaction, NO message by a ribonuclease protection assay and NO protein synthesis by Western blotting. Survanta had no effect on any of the parameters. In contrast, interleukin-13 (IL-13), a cytokine known to inhibit NO production by decreasing NO message and protein synthesis, had the expected effect in these two cell lines. These observations indicate that the effect of lung surfactant on NO production is cell-specific. In alveloar macrophages, lung surfactant decreases NO production by decreasing NO protein levels without decreasing NO message levels, indicating that the regulation may be occurring at the translational level. The differential effects of Survanta on rat alveolar macrophages and these monocytic cell lines may offer a useful model for delineating the molecular mechanisms regulating NO production in inflammatory cells.

854.11

T CELL-MEDIATED INDUCTION OF MACROPHAGE FUNCTION IN AGED MICE.

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Reduction in T and B cell functions are known to contribute to immune senescence. However, it is not clear if age-related changes in macrophage functions occur. Contact-dependent signaling during T cell:macrophage adhesion is the triggering event in activation of macrophage cytokine production and effector function. CD40 ligation is known to be a critical signal in T cell-mediated macrophage function. Flow cytometric analysis of CD40L expression reveals no significant difference on CD4+ T cells from aged (22-25 mos.) versus young (5-10 wks.) mice. Aged CD4+ T cells activated for 6, 24, and 48 hours on immobilized anti-CD3 prior to paraformaldehyde fixation were capable of inducing nitric oxide production by IFN-γ primed peritoneal macrophages, suggesting no deficiency in CD40-dependent (6 hr) or CD40-independent (48 hr) contact signaling by aged CD4+ T cells. The ability of macrophages from aged mice to respond to a variety of stimuli was also assessed. Peritoneal macrophages from aged mice responded as well as macrophages from young mice to combinations of either TNF-α+IFN-γ or LPS+IFN-γ. The results indicate that no functional deficiency in the ability of T cells to induce macrophage nitric oxide responses exists in resident peritoneal macrophages from healthy aged mice. This research was supported by R03-AG16120 from the NIA.

854.12

Analysis of MAP kinase activation during SIV infection

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Attempts to interpret and integrate the results of cumulative studies relating MAP kinase (MAPK) activation to HIV infection have been confounding. For example, HIV binding to CD4/CXCR4 has been shown to induce MAPK activation and activated MAPK have been suggested to phosphorylate the HIV Vif protein thereby regulating Vif function. Further, MAPK has been detected in HIV virions and activation of MAPK in producer cells has been shown to enhance the infectivity of progeny virus. In apparent contrast, other studies have demonstrated that HIV Nef inhibits MAPK activity. As Nef is also a virion-incorporated protein required for optimal infectivity, it is presently unclear how to integrate the activition/inhibition of MAPK into a single coherent model of HIV regulation. Because HIV and SIV Nef proteins are functionally interchangeable, we examined MAPK activation during SIV infection. Our results indicate that SIV infection of rhesus macaque macrophages neither activates erk-1/2, nor precludes the activation of erk-1/2 by the addition of exogenous LPS. Interestingly, SIV infection induced the phosphorylation of p54 MAPK, which was rapidly dephosphorylated in response to LPS. The relationship of p54 MAPK activation to virion infectivity will be discussed.

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Experimental Biology 99®
Washington, D. C.
April 17-21, 1999

ABSTRACTS
PART II

Abstracts 487.1-857.4
Tutorials T1-T15

Official Publication of the Federation of American Societies for Experimental Biology
Volume 13, Number 5, March 15, 1999