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The Clinical Immunology Society

5084

PRODUCTION OF INFLAMMATORY PROTEINS BY MURINE MACROPHAGE TUMOR P388D1. C. Martin and M. E. Dorf. Dept. of Pathology, Harvard Medical School, Boston, MA 02115.

The Macrophage tumor P388D1 was stimulated with lipopolysaccharide (LPS), gamma interferon (IFN$_\gamma$), phorbol myristic acetate (PMA), interleukin-1 (IL-1) or combinations of these agents to induce the production of IL-1, interleukin-6 (IL-6), and tumor necrosis factor$_\alpha$(TNF). IL-1 did not induce IL-6 as has been reported for human fibroblasts. In dose response studies IFN$_\gamma$ and PMA synergized with LPS to increase IL-1, IL-6 and TNF production as detected by bioassays using the IL-1 dependent D10.G4.1 clone, the IL-6 dependent T1165 plasmacytoma cell line and TNF sensitive L929 cells respectively. Northern blot analysis showed that TNF mRNA accumulation peaked 1-2 hours after stimulation with LPS alone, while IL-1 and IL-6 mRNA accumulation was maximal at 4-8 hours. Kinetic studies on the secretion of inflammatory proteins by activated P388D1 revealed that TNF secretion peaked within the first 8 hours of culture while IL-6 secretion was maximal in the first 8-12 hours and IL-1 secretion peaked only after 12 hours of culture. The induction of IL-1 and IL-6 mRNA were found to be sensitive to the effects of dexamethasone (Dex) but not to cyclosporine A (CSA). In contrast, IL-6 mRNA expression in T cells was sensitive to both Dex and CSA. The data imply that although IL-1, IL-6 and TNF could be similarly induced by LPS or by LPS in combination with IFN$_\gamma$ or PMA, they are differentially regulated.

5085

DYNAMICS OF INTERLEUKIN 1 α, β AND TUMOR NECROSIS FACTOR PRODUCTION DURING THE CHRONIC PERITONEAL EXUDATIVE RESPONSE. S.W. Chensue, I.G. Otterness and S.L. Kunkel. VAMC and University of Michigan Medical School, Ann Arbor, MI; Pfizer Central Research, Groton, CT.

This study examined the changes in IL-1 and TNF production by murine peritoneal macrophages (MΦ) during an exudative response to Freund's complete adjuvant (FCA). MΦ were obtained by peritoneal lavage of CBA mice and adherence at designated intervals over a 32 day study period following i.p. injection of FCA. The MΦ were then tested for their capacity to produce IL-1 and TNF in response to 1 μg/ml LPS using the thymocyte coproliferation and LM cell lysis assays, respectively. On day 1, MΦ produced no detectable TNF and minimal levels of IL-1. By days 4-8, maximal levels of IL-1 were produced and then declined thereafter. TNF was first detected on day 4 but maximum production occurred from days 16-20. Detailed kinetic analysis at selected points confirmed these findings. Using immunoaffintiy columns with specificity for IL-1 α,β and TNFα, we showed no interference of TNF with IL-1 bioactivity. Also both forms of IL-1 were detected in supernates, though IL-1β predominated α in about a 2:1 ratio. Maximal IL-1 production corresponded to the time of maximum inflammatory cell accumulation in the peritoneal cavitiy. This preceeded the time of maximal TNF production which occurred during the plateau and early resolution phase of the response. Thus, IL-1 may be involved in early recruitment and cellular activation whereas TNF may be involved in effector and/or regulatory activities.(Supported by the Veterans Administration)

5086

CELLS INFECTED WITH ADENOVIRUSES LACKING THE 14.7KD GENE ARE SUSCEPTIBLE TO LYSIS BY ACTIVATED MACROPHAGES. N. A. Zachariades, *W. S. M. Wold and L. R. Gooding. Emory University School of Medicine, Atlanta, GA 30322 and *Institute for Molecular Virology, St. Louis, MO 63110.

We are investigating the cytolysis of adenovirus infected cells by activated macrophages (AM). Our results show that infection with adenovirus mutants lacking the 14.7Kd gene in the E3 region results in a susceptible target while infection with wild type virus does not. Previously, we have shown that infection with 14.7Kd deletion mutants renders a cell susceptible to lysis by TNF. Furthermore, we have shown that lysis of susceptible targets by TNF results only in ^{51}Cr release but not ^{3}H-TdR release implying cytoplasmic destruction without nuclear disintegration. However, in this report we show that lysis by AM is accompanied by both ^{51}Cr and ^{3}H-TdR release. This is important because adenovirus DNA is infectious and thus only AM, but not soluble TNF have the potential for eliminating the virus. Although cytolysis by AM and soluble TNF show different characteristics, we conclude that cytolysis by AM may still involve TNF since it is inhibited by the 14.7Kd gene product. Therefore, AM may be using membrane bound TNF to kill these targets, implying that either membrane TNF delivers a different cytolytic signal than does soluble TNF or the macrophage contributes additional mechanisms to the lytic process.

This work is supported by grants CA40266, AI26035 and CA48219 from the National Institutes of Health.

5087

CHANGES IN cAMP-DEPENDENT PROTEIN KINASES DURING LPS-INDUCED TUMORICIDAL ACTIVATION OF MACROPHAGES. T. Suzuki, and H. Yamamoto. Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

Changes in cAMP-dependent protein kinases during LPS-induced activation for tumoricidal activity were investigated with two different murine macrophage cell lines, P388D$_1$ and J774. The basal enzymatic activity present in the cytosol of J774 cells was almost 10-fold over that found in P388D$_1$ cells. Photoaffinity labeling of RI and RII showed that the ratio of RI to RII in P388D$_1$ cells was about 1.0, whereas that in J774 cells was about 4.0. The 24 hr incubation of J774 cells with LPS ($\leq$ 10 μg/ml) activated them for killing of P815 or EL4 cells, whereas P388D$_1$ cells did not develop tumoricidal activity with the similar LPS treatment. cAMP-dependent protein kinase ratio (-cAMP/+cAMP) in the cytosol of LPS-treated J774 changed from 0.69 to 0.77 (at 10 ng/ml LPS) to 0.95 (at 10 μg/ml LPS), suggesting the occurrence of the activation of this enzyme in response to LPS. The activity ratio in the cytosol of LPS-treated P388D$_1$ remained, on the other hand, at about 0.5. In addition, the total cAMP-dependent protein kinase activity in the cytosol of LPS-treated J774 decreased to about 1/3 of the control, probably due to translocation of the enzymes from cytosol to other part of the cells. These results thus suggest that cAMP-dependent protein kinases may play a role in the development of LPS-induced tumoricidal activity of murine macrophages. (Supported by NIH grants. CA35977-05 and AI 20742-04)

5088

CHARACTERIZATION OF MLC-GENERATED SUPPRESSOR MACROPHAGES. S.R.S. Gottesman, J.M. Edington, and G.J. Thorbecke. New York University Medical Center, New York, NY 10016.

During an allogeneic mixed lymphocyte culture (MLC), nonspecific suppressor macrophages are generated from the responder C57Bl/6 splenocytes. They inhibit proliferative responses and generation of cytotoxicity to irrelevant alloantigens. The activity appears rapidly, is present at maximal levels after a 2 day generation period and is maintained through 4 days. It can also be generated from peritoneal exudate cells. The cells are Thy-1$^-$, L3T4$^-$, Ly2$^-$ and surface Ig$^-$, before and after generation. Following generation, the activity is enriched in the low density cells from percoll gradients and in plastic adherent cells. FACS analyses shows this adherent population to be enriched for MAC-1$^+$, Asialo-GM-1$^+$ large cells. These cells do not absorb out IL-2 but appear to act through prostaglandin production as indomethacin overcomes suppressor activity in fresh assay cultures. Addition of interferon-γ (IFN-γ) to the generation cultures decreases suppressor activity, however IFN-γ in the assay cultures is without effect. The ability to generate these suppressor macrophages from spleen cells is defective in aged mice. This defect is not overcome by addition of exogenous IL-2 or IFN-γ to the generation. These results suggest that at least certain aspects of macrophage function are defective in aged mice. (Supported by AG-04860).

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MACROPHAGE RESPONSES TO THE INGESTION OF AGED NEUTROPHILS L. Meagher, J. Savill, A. Baker, R. Fuller and C. Haslett (Spon. P.M. Henson) Dept. Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, UK.

We have previously reported that monocyte-derived macrophages (Mø) ingest intact aged neutrophils (PMN) which are undergoing programmed cell death: a process which may limit tissue injury and favour resolution of inflammation (Haslett et al Faseb J., 1988, 2: A1448). Here we show that Mø ingestion of aged PMN does not induce release of thromboxane B_2 (TxB_2) by contrast with the effect of IgG-opsonized ox erythrocytes (OE) or serum-opsonized zymosan (OZ) uptake. The release of TxB_2 was assessed by specific RIA and release of the lysosomal enzyme N-acetyl-β-D-glucosaminidase (NAG) by colorimetry of supernatants from Mø which had been exposed to PMN, OE or OZ for 1 hour. Phagocytosis was confirmed by microscopy and appropriate staining. Dose-dependent increases in TxB_2 release were seen in response to stimulation with OZ and OE, but not to PMN. Mø, in 24-well plates, exposed to OZ (1 mg/well) released 364.7 $\pm$ 67.4 ng/well of TxB_2, and 211.7 $\pm$ 40.4 on exposure to OE (20×10^6/well) whereas exposure to PMN (20×10^6/well) failed to elevate TxB_2 levels above those seen with unstimulated Mø (39.6 $\pm$ 8.9 ng/well, n=4 $\pm$ SD). OE and OZ caused a dose-related release of NAG from Mø whereas phagocytosis of PMN caused little release above background levels. These observations lend further support to the concept of Mø phagocytosis of aged PMN as an injury-limiting neutrophil disposal mechanism.