Expression of genes for interleukin-1α and tumour necrosis factor-α in newborn mice

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SUMMARY

Spleen cells from newborn mice are immunologically non-reactive and do not respond by proliferation to T- or B-cell mitogens. However, they synthesize significant levels of mRNA for interleukin-1α (IL-1α) and tumour necrosis factor-α (TNF-α) after mitogen stimulation. Since these two cytokines mediate many protective activities in the body, they may be important in ensuring the survival of immunologically immature newborns.

Murine fetuses and newborns are immunologically poorly reactive, and many aspects of the immune response are not manifested by their immune system. Immaturity of both T and B cells, defects in the antigen-presenting function of macrophages and/or high activity of natural suppressor cells have all been considered as reasons for their non-reactivity (Chiscon & Golub, 1972; Sidman & Unanue, 1975; Skowron-Cendrzak & Ptak, 1976; Lu, Calamai & Unanue, 1979).

In spite of the immunological non-reactivity, newborns survive under conventional conditions. There must, therefore, be a mechanism providing for their survival. The expression of mRNA for the cytokines interleukin-1α (IL-1α), tumour necrosis factor (TNF)-α and TNF-β, which mediate several events of acute and chronic inflammation and are involved in defence mechanisms, was tested. It is shown below that bacterial lipopolysaccharide (LPS)-stimulated spleen cells from immunologically non-reactive newborn mice synthesize significant levels of mRNA for IL-1α and TNF-α, and it is proposed that these cytokines may be involved in their survival.

Spleens were aseptically removed from newborn (age less than 20 hr) or adult (7–8 week old) B10.A mice (from the breeding colony of our Institute) and single-cell suspensions were prepared in Eagle's minimal essential medium supplemented with antibiotics, glutamine, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES buffer and 10% fetal calf serum. Cells adjusted to a concentration of 3×10^6/ml were stimulated, or not, with 20 μg/ml of LPS (Difco Lab., Detroit, MI) for 8 hr or with 5 μg/ml of concanavalin A (Con A; Sigma Chemical Co., St Louis, MO) for 24 hr. Total RNA was isolated from 2×10^7 cells using the quanidinium isothiocyanate method (Chirgwin *et al.*, 1979), subjected (30 μg per lane) to electrophoresis in 1% agarose and blotted to Hybond N nitrocellulose membrane (Amersham International, Amersham, Bucks, U.K.) (Maniatis *et al.*, 1982). The RNA isolated from cells stimulated with LPS was hybridized with a ^{32}P-labelled IL-1α probe (Lomedico *et al.*, 1984) or TNF-α probe (Fransen *et al.*, 1985); RNA isolated from Con A-stimulated cells was hybridized with a TNF-β probe (Li *et al.*, 1987). The conditions of hybridization were as described elsewhere (Lipoldová *et al.*, 1989). The membranes were exposed at −70° on Kodak XAR-5 film. The blots were then stripped and rehybridized with an actin probe.

Figure 1a shows that no detectable mRNA for TNF-α was produced by unstimulated spleen cells from either newborn or adult mice. After 8-hr stimulation with LPS, cells from both adult and newborn mice synthesized significant amounts of TNF-α mRNA, as is evident from hybridization bands slightly above 18 S. Similarly, LPS-stimulated spleen cells from adult and newborn mice synthesized significant levels of mRNA for IL-1α (Fig. 1b), even if the level of mRNA for IL-1α was lower in cells from newborn than from adult animals. To demonstrate comparable quantities of RNA in individual lanes, the same gel that was used to determine IL-1α and TNF-α mRNA, was, after stripping, hybridized to an actin probe (Fig. 1c).

In contrast to cells stimulated with LPS, Con A-stimulated spleen cells from newborn mice did not produce any detectable amount of mRNA for TNF-β, while cells from adult mice did (Fig. 2a). It has been demonstrated that TNF-β is produced by T cells (Svedersky *et al.*, 1985), while TNF-α is a product mainly of macrophages (Carswell *et al.*, 1975). The findings presented here thus show the ability of macrophages, but not of T cells, from newborn mice to produce TNF.

The results thus demonstrate that spleen cells from newborn mice that did not respond by proliferative response to LPS or Con A stimulation (data not shown) synthesized significant levels of mRNA for IL-1α and TNF-α after the stimulation. The synthesis of these two cytokines may have importance in securing the survival of newborns. IL-1 and TNF have been shown to play a central role in host defences by virtue of their

Abbreviations: IL-1, interleukin-1; LPS, bacterial lipopolysaccharide; TNF, tumour necrosis factor.

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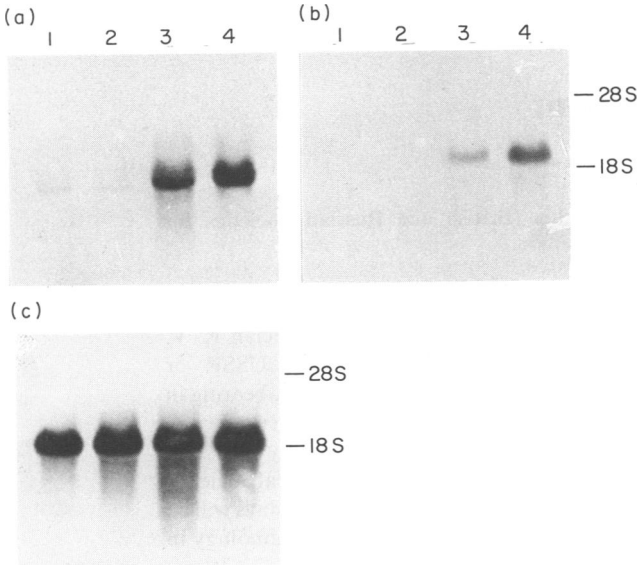

Figure 1. Synthesis of mRNA for TNF-α and IL-1α by spleen cells from newborn and adult B10.A mice. Cells were stimulated or not with LPS for 8 hr and then the RNA was isolated and hybridized with an TNF-α (a), IL-1α (b) or actin (c) probe. Unstimulated (lanes 1 and 2) and LPS-stimulated (lanes 3 and 4) cells were from newborn (lanes 1 and 3) or adult (lanes 2 and 4) mice.

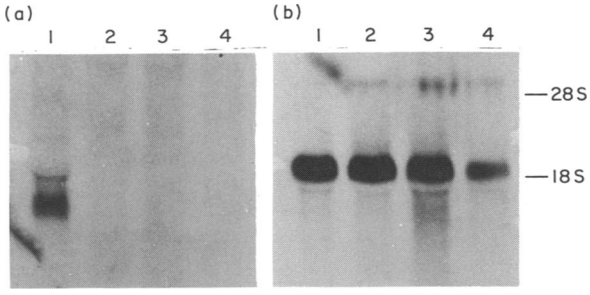

Figure 2. Synthesis of mRNA for TNF-β by spleen cells from newborn and adult mice. RNA was isolated from cells that were stimulated with Con A (lanes 1 and 2) or were not stimulated (lanes 3 and 4), and was hybridized with a TNF-β (a) or an actin (b) probe. Cells were from adult (lanes 1 and 3) or newborn (lanes 2 and 4) B10.A mice.

ability to augment the replication of activated T and B lymphocytes and to mediate the events of inflammation. Direct involvement of these factors in haemopoiesis (Mochizuki *et al.*, 1987), synthesis of acute-phase proteins (Ramadori *et al.*, 1985), release of degradative enzymes from connective-tissue cells (Durum, Schmidt & Oppenheim 1985), and in defence against bacterial infections (Czuprynski & Brown, 1987) or against tumour cell growth (Lachman *et al.*, 1986; Haranaka, Satomi & Sakurai, 1984) has been documented. Recently it was shown that TNF is also involved as a regulatory cytokine in embryogenesis (Mizuno & Soma, 1988). Altogether this emphasizes the biological role of IL-1 and TNF, and it is therefore important that newborns do have the capability to synthesize these agents, even if they are not yet able to mount specific immune responses.

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REFERENCES

Carswell E.A., Old L.J., Kassel R.L., Green S., Fiore N. & Williamson B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. natl. Acad. Sci. U.S.A.* **72,** 3666.

Chirgwin J.M., Przybyla A.E., Macdonald R.J. & Rutter W.J. (1979) Isolation of biologically active nucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18,** 5294.

Chiscon M.O. & Golub E.S. (1972) Functional development of the interacting cells in the immune response. I. Development of T cell and B cell function. *J. Immunol.* **108,** 1379.

Czuprynski C.J. & Brown J.F. (1987) Recombinant murine interleukin-1 enhancement of nonspecific antibacterial resistance. *Infect. Immun.* **55,** 2061.

Durum S.K., Schmidt J.A. & Oppenheim J.J. (1985) Interleukin 1: an immunological perspective. *Ann. Rev. Immunol.* **3,** 263.

Fransen L., Müller R., Marmenout A., Tavernier J., Van Der Heyden J., Kawashima E. *et al.* (1985) Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. *Nucl. Acids Res.* **13,** 4417.

Haranaka K., Satomi N. & Sakurai A. (1984) Antitumour activity of murine tumor necrosis factor (TNF) against transplanted murine tumours and heterotransplanted human tumours in nude mice. *Int. J. Cancer*, **34,** 263.

Lachman L.B., Dinarello C.A., Llansa N.D. & Fidler I.J. (1986) Natural and recombinant human interleukin 1β is cytotoxic for human melanoma cells. *J. Immunol.* **16,** 3098.

Li C.-B., Gray P.W., Lin P.-F., McGrath K.M., Ruddle F.H. & Ruddle N.H. (1987) Cloning and expression of murine lymphotoxin cDNA. *J. Immunol.* **138,** 4496.

Lipoldová M., Londei M., Grubeck-Loebenstein B., Feldmann M. & Owen M.J. (1989) Analysis of T-cell receptor usage in activated T-cell clones from Hashimoto's thyroiditis and Grave's disease. *J. Autoimmun.* **2,** 1.

Lomedico P.T., Gubler U., Hellman C.P., Dukovich M., Giri J.G., Pan Y.C.E., Collier K., Semionow R., Chua A.O. & Mizel S.B. (1984) Cloning and expression of murine interleukin-1 cDNA in E. coli. *Nature (Lond.)*, **312,** 458.

Lu C.Y., Calamai E.G. & Unanue E.R. (1979) A defect in the antigen-presenting function of macrophages from neonatal mice. *Nature (Lond.)*, **282,** 327.

Maniatis T., Fritsch E.F. & Sambrook J. (1982) *Molecular Cloning: A Laboratory Manual*, p. 202. Cold Spring Harbor University, New York.

Mizuno S. & Soma G.-I. (1988) Endogenous and exogenous TNF therapy (EET therapy): conceptual and experimental grounds. *Ann. Inst. Pasteur/Immunol.* **139,** 282.

Mochizuki D.Y., Eisenman J.R., Conlon P.J., Larsen A.D. & Tushinski R.J. (1987) Interleukin-1 regulated hematopoietic activity, a role previously ascribed to hemopoietin 1. *Proc. natl. Acad. Sci. U.S.A.* **84,** 5267.

Ramadori G., Sipe J.D., Dinarello C.A., Mizel S.B. & Colten H.R. (1985) Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL 1) and purified human IL 1. *J. exp. Med.* **162,** 930.

Sidman C.L. & Unanue E.R. (1975) Receptor mediated inactivation of early B lymphocytes. *Nature (Lond.)*, **257,** 149.

Skowron-Cendrzak A. & Ptak W. (1976) Suppression of local graft-versus-host reactions by mouse foetal and newborn cells. *Eur. J. Immunol.* **6,** 451.

Svedersky L.P., Nedwin G.E., Goeddel D.V. & Palladino M.A. (1985) Interferon-γ enhances induction of lymphotoxin in recombinant interleukin 2-stimulated peripheral blood mononuclear cells. *J. Immunol.* **134,** 1604.