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Inability of mitogen-stimulated spleen cells from newborn mice to synthesize interleukin-2 receptors

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SUMMARY

Spleen cells from newborn mice do not respond by proliferation to concanavalin A (Con A) or bacterial lipopolysaccharide (LPS) stimulation. This non-reactivity cannot be reversed to a positive response by exogenous interleukin-2 (IL-2). The stimulation with Con A of spleen cells from newborn mice, in contrast to cells from adult animals, does not result in synthesis of mRNA for inducible 55,000 molecular weight (MW) IL-2 receptors (IL-2R). The failure of neonatal spleen cells to synthesize IL-2R mRNA is an intrinsic property of the cells themselves, and it is not due to activity of natural suppressor cells present in newborn animals. Since the expression of functional IL-2R represents one of the early and pivotal events in immune cell activation, we propose that the inability to synthesize IL-2R may be one of the primary reasons for the immunological immaturity of newborns.

INTRODUCTION

Newborn animals are non-reactive or profoundly hyporeactive in all immunological tests *in vivo* and *in vitro* (Argyris, 1968; Olding & Oldstone, 1974; Mosier & Johnson, 1975; Sidman & Unanue, 1975; Hardy & Mozes, 1978; Rodriguez *et al.*, 1979). Defects in the antigen-presenting function of macrophages (Argyris, 1968; Lu, Calamai & Unanue, 1979) or immaturity of both T and B cells (Chiscon & Golub, 1972; Sidman & Unanue 1975) have been considered as possible causes of this deficiency. On the other hand, active inhibitory mechanisms, represented by neonatal suppressor cells and their factors (Olding & Oldstone, 1975; Skowron-Cendrzak & Ptak, 1976; Murgita *et al.*, 1981; Mazur-Kolecka *et al.*, 1988), have also been described in newborn animals. All these passive and active mechanisms might thus contribute to the immunological non-reactivity of newborns.

Development of a normal immune response after immunization depends on the expression of functional interleukin-2 receptors (IL-2R) (Waldmann, 1986; Diamantstein & Osawa, 1986). Their expression represents one of the early and pivotal events in lymphoid cell activation. We tested the expression of IL-2R in mitogen-stimulated immunologically non-reactive cells from newborn mice. We found that these cells, in contrast to cells from adult animals, were not able to synthesize mRNA for the inducible 55,000 MW IL-2R. We therefore propose that the inability to synthesize IL-2R may be one of the primary factors responsible for the immunological immaturity of newborns, and we show that this failure is an intrinsic property of their cells.

Abbreviations: Con A, concanavalin A; IL-1, interleukin-1; IL-2, interleukin-2; IL-2R, interleukin-2 receptor(s); LPS, bacterial lipopolysaccharide; SF, suppressor factor(s).

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MATERIALS AND METHODS

Animals

Mice of inbred strains B10.A and AKR/J, 2–3 months of age, and their offspring were from the breeding colony of the Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague.

Interleukins

Recombinant human interleukin-1α (rIL-1α, specific activity 1×10^8 U/mg) was obtained from Dr P. T. Lomedico, Hoffmann-La Roche Inc., Nutley, NJ. The activity of IL-1 was tested in the classical thymocyte co-stimulatory assay (Mizel, Oppenheim & Rosensteich, 1978).

Recombinant human interleukin-2 (rIL-2, specificity activity 2×10^6 U/mg) was obtained from Dr E. Gren, Institute of Organic Synthesis, Riga, Latvia. The activity of IL-2 was assessed by its ability to support the growth of IL-2-dependent CTL (Gillis & Smith, 1977).

Proliferative assay

Spleen cells (2×10^5/well) from newborn (less than 24 hr old) or adult B10.A mice were stimulated with concanavalin A (Con A; 5 μg/ml; Sigma Chemicals Co., St Louis, MO) or bacterial

lipopolysaccharide (LPS; 40 μg/ml; Difco Laboratories, Detroit, MI) in RPMI-1640 medium supplemented with antibiotics, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES buffer and 10% foetal calf serum, as described elsewhere (Holáň *et al.* 1985). Recombinant IL-1 (100 U/ml) or rIL-2 (100 U/ml) was added to some cultures. The plates were incubated at 37° in a humidified atmosphere of 5% CO_2 in air, and cell proliferation was determined by incorporation of [^{3}H]thymidine (TdR) added for the last 6 hr of a 72-hr incubation period (Holáň *et al.*, 1985).

Northern blot analysis

Total RNA was isolated from 2×10^7 cells, which were stimulated or not with Con A (5 μg/ml) for 16 hr. The RNA was extracted using the guanidinium-isothiocyanate method (Chirgwin *et al.*, 1979) and subjected (30 μg per lane) to electrophoresis in 1% agarose, blotted to Hybond-N nitrocellulose membranes (Amersham, Amersham, Bucks, U.K.) (Maniatis, Fritsch & Sambrook, 1982), and hybridized with a ^{32}P-labelled IL-2R probe (Shimizu *et al.*, 1985) for 16 hr at 42°. The conditions of hybridization have been described elsewhere (Lipoldová *et al.*, 1989). Membranes were exposed at −70° to Kodak XAR-5 film. The blots were then stripped and rehybridized with a cDNA probe for actin to demonstrate comparable quantities of RNAs in individual lanes.

Cell separation

Spleen cells from adult (3 months old) AKR/J mice (Thy-1.1$^+$) were mixed with spleen cells from newborn B10.A mice (Thy-1.2$^+$) at a ratio of 2:1 and were cultured unstimulated or stimulated with Con A (5 μg/ml). After 16 hr of culture, the T cells of AKR/J and B10.A origin were separated by the panning technique (Wysocki & Sato, 1978) using plastic Petri dishes coated with purified anti-Thy-1.1 and anti-Thy-1.2 monoclonal antibodies (Lake *et al.*, 1979).

Demonstration of receptors for Con A

The presence of receptors for Con A on spleen cells from newborn and adult mice was determined by the ability of cells to adhere to glass or plastic surfaces coated with Con A (1 mg/ml), as described by Mattes *et al.* (1983). After a 30-min incubation of cell suspensions on Con A-coated surfaces, the non-adherent cells were removed and the proportion of adherent cells was determined.

RESULTS

Non-reactivity of spleen cells from newborn mice

Spleen cells from newborn mice (age less than 24 hr) did not respond by proliferation to stimulation with mitogens Con A or LPS (Fig. 1). This non-reactivity was not reversed to a positive response even if the cells from neonatal mice were stimulated in the presence of exogenous rIL-1 or rIL-2 (Fig. 1) and was not dependent on a concentration of mitogens (data not shown).

Neonatal spleen cells do not synthesize IL-2R mRNA after mitogen stimulation

Since spleen cells from newborn mice did not respond by proliferation to mitogen stimulation, we next studied activation of these cells at the level of expression of the gene for IL-2R. We

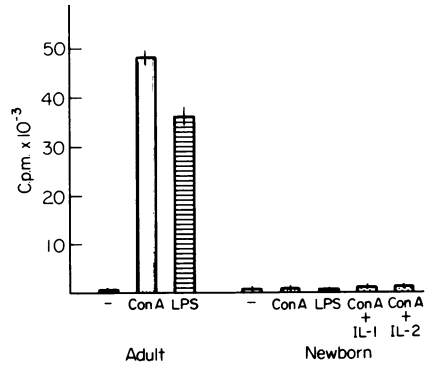

Figure 1. Absence of proliferation in cultures of mitogen-stimulated spleen cells from newborn mice and inability of exogenous IL-1 or IL-2 to induce responsiveness.

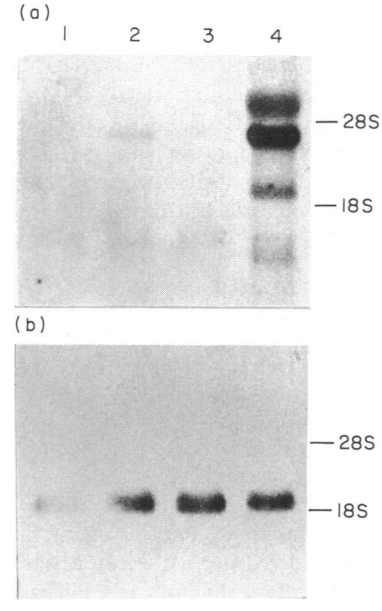

Figure 2. Expression of mRNA for the 55,000 MW IL-2R in spleen cells from newborn (lanes 1 and 3) and adult (lanes 2 and 4) B10.A mice. Cells were not stimulated (lanes 1 and 2) or stimulated with Con A (lanes 3 and 4) for 16 hr. Total RNA was isolated, divided by electrophoresis, blotted and hybridized with an IL-2R probe (a) and an actin probe (b). The positions of molecular weight markers are indicated.

tested the synthesis of IL-2R mRNA in spleen cells for newborn and adult (8–9-week-old) B10.A mice. After stimulation of spleen cells from adult donors we found five bands of mRNA hybridizing with a cDNA probe for IL-2R (Fig. 2, lane 4) that were typical of the different types of mRNA coding for IL-2R (Miller *et al.*, 1985). However, none of these bands was found after hybridization of RNA isolated from similarly stimulated spleen cells from newborn mice (Fig. 2, lane 3). No detectable IL-2R mRNA was found either when the neonatal spleen cells were stimulated with phorbol myristate acetate (PMA), Con A plus PMA or phytohaemagglutinin (data not shown). In addition, even if two or three times more RNA from stimulated newborn cells was used in a Northern analysis, no evidence of

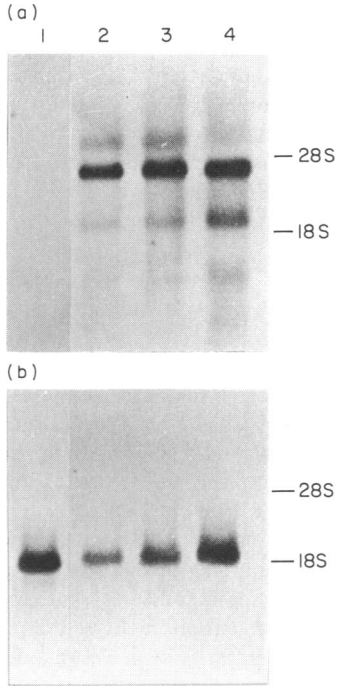

Figure 3. Effect of neonatal spleen cells and their factor(s) on the expression of 55,000 MW IL-2R mRNA by spleen cells from syngeneic adult mice. Spleen cells from adult B10.A mice were cultured unstimulated (lane 1) or stimulated with Con A (lanes 2–4) in the presence of spleen cells from newborn mice (lane 3) or in the presence of SF (lane 4) SF was prepared by 24-hr culture of spleen cells from newborn mice as described by Mazur-Kolecka *et al.* (1988). RNA was hybridized with an IL-2R probe (a) and an actin probe (b).

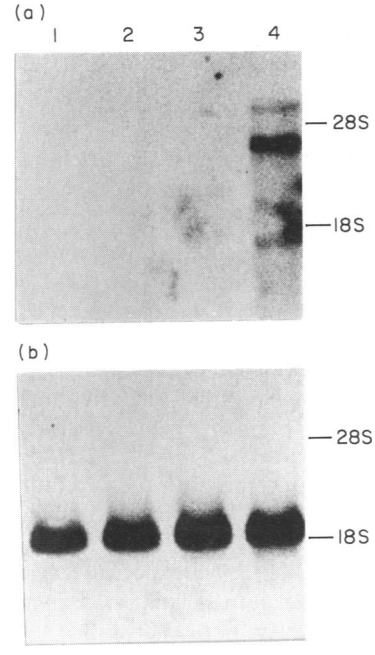

Figure 4. Synthesis of IL-2R mRNA in a mixture of spleen cells from newborn and adult mice. Spleen cells from adult AKR/J mice (Thy-1.1$^+$) were mixed with cells from newborn B10.A mice (Thy-1.2$^+$) at a ratio of 2:1 and were cultured for 16 hr unstimulated or stimulated with Con A. After the culture, T cells of AKR/J and B10.A origin were separated. Total RNA was isolated from the cell populations obtained and was hybridized with an IL-2R probe (a) and an actin probe (b). RNA was from unstimulated (lanes 1 and 2) or Con A-stimulated (lanes 3 and 4) cells derived from adult AKR mice (lanes 2 and 4) or from newborn B10.A mice (lanes 1 and 3).

mitogen-induced expression of IL-2R mRNA was obtained. All these observations support the suggestion that mitogen-activated newborn spleen cells do not have the ability to synthesize mRNA for IL-2R.

Failure to synthesize IL-2R mRNA after Con A stimulation is an intrinsic property of newborn cells

We next tested whether the inability of spleen cells from newborn mice to synthesize IL-2R mRNA is an intrinsic property of these cells or whether it is due to an activity of natural suppressor cells present in the neonatal spleen cell population. Spleen cells from adult mice were stimulated with Con A alone, or in the presence of spleen cells from syngeneic newborn donors at a ratio of (adult:newborn) 2:1, which was found to be effective in suppression of proliferative responses, or in the presence of suppressor factors (SF) produced by spleen cells from newborn mice. Even if the neonatal suppressor cells or suppressor factors produced by them strongly inhibited mitogen-induced proliferation of spleen cells from adult donors (Mazur-Kolecka *et al.*, 1988), there was no inhibition of synthesis of mRNA for IL-2R in adult cells stimulated in the presence of spleen cells from newborn donors (Fig. 3).

To test the possibility that the absence of IL-2R expression in cells from newborn animals could be due to a defect in the activity of antigen-presenting cells, we co-cultured spleen cells from adult and newborn mice and then separated and tested both populations for the presence of mRNA for IL-2R. Spleen cells from adult AKR/J mice (expressing Thy-1.1 antigen) were co-cultured for 16 hr with spleen cells from newborn B10.A mice (Thy-1.2$^+$), not stimulated or stimulated with Con A, and then the Thy-1.1$^+$ and Thy-1.2$^+$ cells separated. A significant level of mRNA for IL-2R was found only in stimulated Thy-1.1$^+$ cells, while no detectable mRNA for the IL-2R was present in Thy-1.2$^+$ cells from newborn mice (Fig. 4).

Spleen cells from newborn mice express Con A receptors

To exclude the possibility that the non-reactivity of the cells from newborn mice is due to their inability to bind Con A, we next tested the expression of Con A receptors. Spleen cells from newborn or adult mice were cultured on glass or plastic surfaces coated with Con A. After washing of the non-adherent cells, the number of the remaining cells was determined. The proportion of adherent cells with respect to the number of cells before culture was similar in samples of cells from adult and newborn donors and was more than 60% (data not shown).

DISCUSSION

Immunological hyporeactivity or even non-reactivity of newborns has been well documented (Argyris, 1968; Olding & Oldstone, 1974; Mosier & Johnson, 1975; Sidman & Unanue, 1975; Hardy & Mozes, 1978; Rodriguez *et al.*, 1979). Numerous attempts have been made to explain this immunological immaturity. Defects in the functions of macrophages, immaturity of T

and B lymphocytes and/or high activity of neonatal suppressor cells have all been considered as possible causes of this deficiency (Chiscon & Golub, 1972; Skowron-Cendrzak & Ptak, 1976; Lu *et al.*, 1979; Murgita *et al.*, 1981; Mazur-Kolecka *et al.*, 1988). However, detailed studies of the non-reactivity of newborns at the level of expression of genes for individual immunoregulatory molecules have not yet been described.

Production of IL-1 and synthesis of IL-2R represent two of the earliest events in lymphoid cell activation. We have recently shown that mitogen-stimulated spleen cells from newborn mice do synthesize mRNA for IL-1 (Lipoldová & Holáň, 1990). Here we demonstrate that synthesis of mRNA for the inducible 55,000 MW IL-2R does not occur in mitogen-stimulated spleen cells from newborn mice, while similarly stimulated spleen cells from adult animals synthesize high levels of IL-2R mRNA. The inducible IL-2R under study binds the IL-2 molecule (Waldmann, 1986) and, together with the constitutively synthesized 75,000 MW IL-2R chain, forms a high-affinity IL-2R (Nishi, Ishida & Honjo, 1988). Functional IL-2R, which are inducibly expressed on T and B lymphocytes, are essential for the development of the normal immune response. We therefore propose that the failure of newborns to synthesize mRNA for the 55,000 MW IL-2R could be the primary cause of their immunological immaturity.

Theoretically, the inability of mitogen-stimulated spleen cells from newborn animals to synthesize IL-2R mRNA could represent an intrinsic property of the cells, or it might be due to the high activity of natural suppressor cells present in the lymphoid organs of newborns (Skowron-Cendrzak & Ptak, 1976; Murgita *et al.*, 1981; Mazur-Kolecka *et al.*, 1988). Indeed it was shown in another model, i.e. in an *in vitro* system of alloantigen-activated non-specific suppressor cells, that suppressor cells diminished the occurrence of IL-2R in alloantigen-stimulated immunocompetent cells from adult donors (Loertscher & Strom, 1989). The natural suppressor cells present in newborn mice, which have been shown to inhibit mitogen-induced proliferation of lymphoid cells from adult donors (Olding & Oldstone, 1974; Mazur-Kolecka *et al.*, 1988), did not suppress the very beginning of cell activation represented by the synthesis of mRNA for IL-2R. There must therefore be another step of cell activation, beyond the expression of the gene for IL-2R, that is inhibited by neonatal suppressor cells and their factors.

It has been shown that thymus cells from mouse foetuses transiently express IL-2R (Ceredig *et al.*, 1975; Raulet, 1985; Boyer, Diamond & Rothenberg, 1989). We observed that thymus cells from newborn mice synthesized low, but detectable, levels of mRNA for IL-2R after stimulation with IL-2, but not after Con A stimulation. We also found low synthesis of IL-2R mRNA in the IL-2-stimulated spleen cells from newborn mice (data not shown). This was in contrast to stimulation of the same cells with Con A, despite the fact that both neonatal spleen and thymus cells bind Con A. There must be different pathways of transduction of the activation signals leading to the IL-2R gene expression after stimulation of cells with mitogens or IL-2. We have obtained evidence that suppressor cells present in newborn mice selectively inhibit mitogen- or antigen-induced cell proliferation, but not cell activation induced by IL-2 (V. Holáň, M. Lipoldová and A. Zajícová, manuscript submitted for publication). The observations thus show distinct regulatory mechanisms of the immune responses to exogenous stimuli (mitogens, antigens) and to endogenous ones represented by growth factors (interleukins). Furthermore, newborn mice have impaired production of IL-2 (Ishizaka & Stuttman, 1983; Aygyris, De Stefano & Zamkoff, 1985). These defects in IL-2 and IL-2R synthesis may be important in the establishment of self-tolerance. This is supported by observations that administration of exogenous IL-2 into newborn mice counteracts allotransplantation tolerance induction (Malkovský *et al.*, 1984; Holáň, 1988).

The securement of immunological non-reactivity for foetuses and newborns is of great biological importance. First, absence of an immune response is important at this stage because the animal is still developing its receptor repertoire. Second, the immunological immaturity enables the establishment of self-tolerance, as has been demonstrated by the ready susceptibility of foetuses and newborns to induction of immunological tolerance to foreign tissues (Billingham, Brent & Medawar, 1953; Hašek, 1953).

In conclusion, we suppose that more mechanisms could contribute to the immunological non-reactivity of newborns. But we propose that the inability to synthesize IL-2R after mitogen or antigen stimulation might be the primary cause of the immunological immaturity of newborn animals.

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REFERENCES

ARGYRIS B.F. (1968) Role of macrophages in immunological maturation. *J. exp. Med.* **128,** 459.

ARGYRIS B.F., DE STEFANO M. & ZAMKOFF K.W. (1985) Interleukin-2 production in neonatal mouse. *Transplantation*, **40,** 284.

BILLINGHAM, R.E., BRENT L. & MEDAWAR P.B. (1953) Actively acquired tolerance of foreign cells. *Nature (Lond,)*, **172,** 603.

BOYER P.D., DIAMOND R.A. & ROTHENBERG, E.V. (1989) Changes in inducibility of IL-2 receptor α-chain and T cell receptor expression during thymocyte differentiation in the mouse. *J. Immunol.* **142,** 4121.

CEREDIG R., LOWENTHAL J.W., NABHOLZ M. & MACDONALD H.R. (1985) Expression of interleukin-2 receptor as a differentiation marker on intrathymic stem cells. *Nature (Lond.)*, **314,** 98.

CHIRGWIN J., PRZYBYLA A., MACDONALD R. & RUTTER W. (1979) Isolation of biologically active ribonucleic 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.

DIAMANTSTEIN T. & OSAWA H. (1986) Interleukin-2 receptor, its physiology and a new approach to a selective immunosuppressive therapy by anti-interleukin-2 receptor monoclonal antibody. *Immunol. Rev.* **92,** 5.

GILLIS S. & SMITH K.A. (1977) Long-term culture of tumour-specific cytotoxic T cells. *Nature (Lond.)*, **268,** 154.

HARDY B. & MOZES E. (1978) Expression of T cell suppressor activity in the immune response of newborn mice to a T-independent synthetic polypeptide. *Immunology*, **35,** 757.

HAŠEK M. (1953) Vegetative hybridization of animals by joining their blood circulations during embryonic life (in Czech). *Čs. Biol.* **2,** 265.

HOLÁŇ V. (1988) Modulation of allotransplantation tolerance induction by interleukin-1 and interleukin-2. *J. Immunogenet.* **15,** 331.

HOLÁŇ V., LIPOLDOVÁ M., TAKÁČ M., ČERNÁ J., VANČATOVÁ A., ČECHOVÁ D., VESELSKÝ L. & HAŠEK M. (1985) Establishment and characterization of a permanent T-cell line producing an antigen non-specific suppressor factor. *Immunology*, **56,** 275.

ISHIZAKA S.T. & STUTTMAN O. (1983) Analysis by limiting dilution of interleukin 2-producing T cells in murine ontogeny. *Eur. J. Immunol.* **13,** 936.

LAKE P., CLARK E.A., KHORSHIDI M. & SUNSHINE G.H. (1979) Production and characterization of cytotoxic Thy-1 antibody secreting hybrid cell lines. Detection of T cell subsets. *Eur. J. Immunol.* **9,** 875.

LIPOLDOVÁ M. & HOLÁŇ V. (1990) Expression of genes for interleukin-1α and tumour necrosis factor-α in newborn mice. *Immunology*, **70,** 136.

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 Graves' Disease. *J. Autoimmun.* **2,** 1.

LOERTSCHER R. & STROM T.B. (1989) Differential regulation of activation-associated receptor expression on CD4 and CD8 positive T lymphocytes by allosensitized suppressor T cells. *Transplantation*, **48,** 473.

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.

MALKOVSKÝ M., MEDAWAR P.B., HUNT R, PALMER L. & DORE C. (1984) A diet enriched in vitamin A acetate or *in vivo* administration of interleukin-2 can counteract a tolerogenic stimulus. *Proc. Roy. Soc. (Lond.)*, **B 220,** 439.

MANIATIS T., FRITSCH E.E. & SAMBROOK J. (1982) *Molecular Cloning: a Laboratory Manual*, p. 202. Cold Spring Harbor University, New York.

MATTES M.J., TANIMOTO M., POLLACK S.M. & MAURER D.H. (1983) Preparing monolayers of non-adherent mammalian cells. *J. immunol. Meth.* **61,** 145.

MAZUR-KOLECKA B., KUBERA M., ŠTĚDRA J., HOLÁŇ V., SKOWRON-CENDRZAK A. & BUBAK-SATORA M. (1988) Characteristics of the neonatal spleen cell subpopulation in mice which suppresses lymphocyte proliferative response to mitogen stimulation. *Folia Biol. (Krakow)*, **36,** 127.

MILLER J., MALEK T.R., LEONARD W.J., GREENE W.C., SHEVACH E.M. & GERMAIN R.N. (1985) Nucleotide sequence and expression of a mouse interleukin 2 receptor cDNA. *J. Immunol.* **134,** 4212.

MIZEL S.B., OPPENHEIM J.J. & ROSENSTEICH D.L. (1978) Characterization of lymphocyte-activating factor (LAF) produced by macrophage line, $P388D_1$. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120,** 1497.

MOSIER D.E. & JOHNSON B.M. (1975) Ontogeny of mouse lymphocyte function. II. Development of the ability to produce antibody is modulated by T lymphocytes. *J. exp. Med.* **141,** 216.

MURGITA R.A., HOOPER D.C., STEGAGNO M., DELOVITCH T.L. & WIGZELL H. (1981) Characterization of murine newborn inhibitory T lymphocytes: functional and phenotypic comparison with an adult T cell subset activated *in vitro* by alpha-feto-protein. *Eur. J. Immunol.* **11,** 957.

NISHI M., ISHIDA Y. & HONJO T. (1988) Expression of functional interleukin-2 in human light chain/Tac transgenic mice. *Nature (Lond.)*, **331,** 267.

OLDING L.B. & OLDSTONE M.B.A. (1974) Lymphocytes from human newborns abrogate mitosis of their mother's lymphocytes. *Nature (Lond.)*, **249,** 161.

RAULET D.H. (1985) Expression and function of interleukin-2 receptors on immature thymocytes. *Nature (Lond.)*, **314,** 101.

RODRIGUEZ G., ANDERSON G., WIGZELL H. & PECK A.B. (1979) Non-T cell nature of the naturally occurring spleen associated suppressor cells present in the newborn mouse. *Eur. J. Immunol.* **9,** 737.

SHIMIZU A., KONDO S., TAKEDA S., YODOI J., ISHIDA N., SABE N., OSAWA H., DIAMANTSTEIN T., NIKAIDO T. & HONJO T. (1985) Nucleotide sequence of mouse IL-2 receptor cDNA and its comparison with the human IL-2 receptor sequence. *Nucl. Acids Res.* **13,** 1505.

SIDMAN C.L. & UNANUE E.R. (1975) Receptor mediated inactivation of early B lymphocytes. *Nature (Lond.)*, **257,** 147.

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

WALDMANN T.A. (1986) The structure, function, and expression of interleukin-2 receptors on normal and malignant lymphocytes. *Science*, **232,** 727.

WYSOCKI L.J. & SATO V.L. (1978) 'Panning' for lymphocytes: a method for cell selection. *Proc. natl. Acad. Sci. U.S.A.* **75,** 2844.