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Reduced expression of the interleukin-2-receptor γ chain on cord blood lymphocytes: relationship to functional immaturity of the neonatal immune response

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SUMMARY

Mutation of the interleukin-2 (IL-2) receptor γ chain, which also serves as a component of the receptor complexes for IL-4, 7, 9 and 15, results in severe immune deficiency. We hypothesized that the immunological immaturity of healthy neonates might be associated with low levels of expression of this receptor molecule. Using monoclonal antibody and a highly sensitive immunofluorescence method, we showed that IL-2 receptor γ chain is expressed at significantly lower levels on cord blood cells compared with adult cells. IL-2-dependent T-cell activation *in vitro* was reduced in cord blood cells compared with adult cells, but B-cell responses to IL-4 were not obviously impaired. The lower level of expression of the γ chain and some other cytokine receptor chains may contribute to the immunological immaturity of the newborn, by selectively depressing particular immunological mechanisms.

INTRODUCTION

The cytokine interleukin 2 (IL-2) acts on cells through a complex receptor consisting of three independent proteins.[1] One of these, the γ chain, also forms part of the cellular receptor complexes for IL-4,[2,3] IL-7,[4] IL-9[5] and IL-15.[6] While the immune system appears to be able to compensate for loss of a number of cytokine functions,[7] mutation of the γ-chain gene is associated with X-linked severe combined immune deficiency, probably reflecting its involvement in the receptor complexes for several cytokines.[8]

The ability of cells to respond to IL-2 appears to be regulated largely by control of expression of the receptor α chain (also known as CD25 or p55). CD25 is absent or present at very low levels in cells that have not recently been activated, but is rapidly up-regulated by a variety of stimuli.[9] The IL-2 receptor β chain is constitutively expressed[10] and relatively refractory to regulation,[11] although ligation of co-stimulatory signalling molecules such as CD28 do increase β-chain expression.[12] The γ chain is constitutively expressed by monocytes and lymphocytes,[13] and is relatively resistant to regulation.[11]

Immune responses in the healthy neonate are restricted in a number of ways.[14] Neonates make effective responses to a limited variety of antigens, failing in particular to mount an antibody response to encapsulated bacteria.[15] Even for protein antigens, which do elicit an antibody response, neonates show a restricted use of the immunoglobulin V, D and J gene repertoire,[16] which limits the diversity of the response, and a limited ability to switch from IgM to IgG or IgA responses,[17] which restricts the range of antibody-mediated functions available to the neonate. Neonatal antibody responses show little evidence of somatic mutation of immunoglobulin genes[18] and a poor maturation of antibody affinity,[19] factors that imply a limited ability to develop immunological memory. This is reflected in the use of periodic repeated injections in routine immunization of infants.

The immaturity of the neonatal immune system reflects in part a lack of immune stimulation *in utero*, but appears to be in part developmental,[14] and involves a number of immunological mechanisms. The cellular composition of neonatal blood differs in a number of respects from the mature state. The majority of B cells belong to the CD5-positive subset,[20] associated with low affinity, cross-reactive antibody responses; T cells show a number of phenotypic differences from mature cells, including a low level of expression of the CD45R0 isoform,[21] which is associated with prior activation. CD40 ligand, which is involved in T-cell/B-cell interactions, is not induced upon activation of neonatal T cells, under conditions that induce its expression in adult T cells.[22,23] The expression of a number of cytokine receptors, including the α and β chains of the IL-2 receptor, is reduced, compared with adult cells.[24]

Since the IL-2 receptor γ chain is central to immune functions mediated through a number of cytokines, and

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mutation of the gene for the receptor is associated with severe immune deficiency, it is possible that the immunological immaturity of the nonate is associated with low levels of the receptor. To test this hypothesis, we used a monoclonal antibody against IL-2-receptor γ chain, combined with an immunofluorescence procedure capable of detecting receptors at levels around 100 molecules/cell, to examine IL-2-receptor γ chain expression on cord and adult blood cells. This was followed by *in vitro* functional assays to test the ability of neonatal cells to respond to recombinant IL-2 and IL-4.

MATERIALS AND METHODS

Samples

Cord blood was obtained from healthy full-term babies, and adult blood from healthy laboratory staff. The mononuclear cell fraction was isolated by centrifugation on Ficoll hypaque. Cord cells obtained in this way were significantly contaminated with red cell precursors; these were removed where necessary by lysis with isotonic ammonium chloride for 5 min at room temperature. In the majority of phenotypic studies this step was not necessary because two-colour analysis was performed, to determine IL-2-receptor γ-chain expression specifically on CD4-positive, CD8-positive or CD19-positive cells, thus excluding the red cell precursors from analysis.

Antibodies

The monoclonal antibody TUGh4[13] directed against the γ chain of the IL-2 receptor was kindly provided by Dr K. Sugamura (Department of Microbiology, Tohoku University School of Medicine, Sendai, Japan). Other monoclonal antibodies used were Mik-b1 against the IL-2 receptor β chain,[25] 7G7B6 against the α chain,[26] fluorescein-conjugated CD3, CD4, CD8 (Becton Dickinson, San Jose, CA), CD19 (Silenus Laboratories, Melbourne, Australia) and CD23 (Coulter, Hialeah, FL), CyChrome-CD19 (PharMingen, San Diego, CA) and a number of other antibodies available in our laboratories.

Immunofluorescence and flow cytometry

Cytokine receptors were detected by a high-sensitivity procedure that requires the use of selected batches of reagents and optimization of staining and analysis conditions, as described previously.[27,28] Two-colour analysis was performed as described,[27,28] except that analysis of B cells after culture (Table 1) used CD19-CyChrome to identify the B cells.

In vitro *functional assays*

Mononuclear cells were isolated on Ficoll hypaque and monocytes were depleted by adherence on plastic petri-dishes. Cells (10^6/ml) were cultured in flat-bottomed microtitre plates with preservative-free CD3 antibody (OKT3) with or without added IL-2 (Roche, Nutley, NJ) as indicated in Fig. 1. After 3 days, cultures were pulsed with tritiated thymidine and harvested 16 hr later. For IL-4 responses, cells (0·5 M/ml, 3 ml per tube) were cultured in 10 ml round-bottomed culture tubes for 24 or 48 hr, in the presence or absence of IL-4 (Genzyme, Boston, MA). Cells were harvested and analysed by immunofluorescence as described above.

Table 1. Modulation of B-cell surface molecules by IL-4. Mean fluorescence intensities are shown for B-cell expression of CD23, MHC class II and surface IgM after 48 hr in culture with IL-4 or control medium. Data are from one experiment representative of nine, carried out on different cord and adult blood samples.

	Adult		Cord	
Marker	Control	IL-4	Control	IL-4
CD23	13	47	49	367
MHC class II	521	856	783	835
Surface IgM	101	206	149	401
Surface IgD	52	73	61	81
CD98	59	66	129	124
CD40	208	226	195	219
X63	10	8	10	18

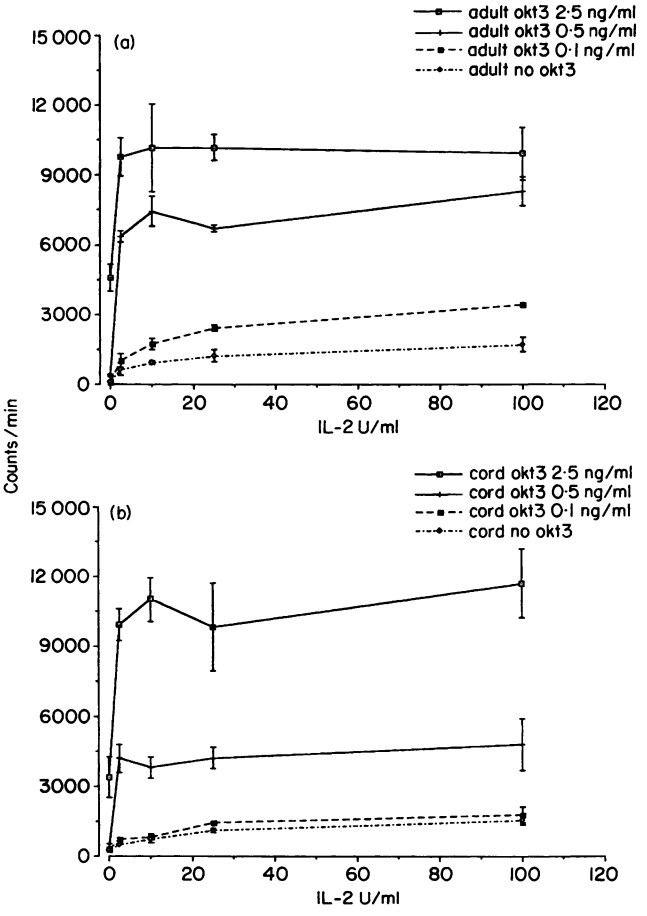

Figure 1. Lymphocyte proliferation in response to CD3 antibody with and without IL-2. (a) shows the response of adult cells whilst (b) shows cord cells. The data are representative of two experiments using different blood samples. The differences between the adult and cord responses were statistically significant ($P < 0·01$) for the two lower concentrations of CD3 antibody, where the response is IL-2 dependent. At the highest concentration of CD3 and in the absence of CD3, the responses of cord and adult cells were not statistically significantly different.

RESULTS

IL-2-receptor γ chain was detected on lymphocytes and monocytes, in both cord and adult blood (Figs 2(a) and (b)). The staining intensity of monocytes showed considerable overlap with the negative control antibody, indicating a low level of expression, and there was no significant difference between cord and adult monocytes in reactivity with the antibody. Adult lymphocytes showed clear expression on the majority of cells, while cord lymphocytes showed significantly lower expression (Figs 2(a) and (b)). Two-colour analysis showed IL-2-receptor-γ-chain expression in B lymphocytes and T lymphocytes, including CD4 and CD8 subsets, in both adult and cord blood (Figs 2(a) and (b)). However, as can be seen in Figs 2(a) and (b) and Table 2, levels were significantly lower in cord blood. Figure 3 shows that the other two chains of the IL-2 receptor were also reduced in cord compared with adult cells, with the exception of a population of cells expressing the β chain, which was increased in cord cells. The cells showing high levels of β chain have been identified as large granular

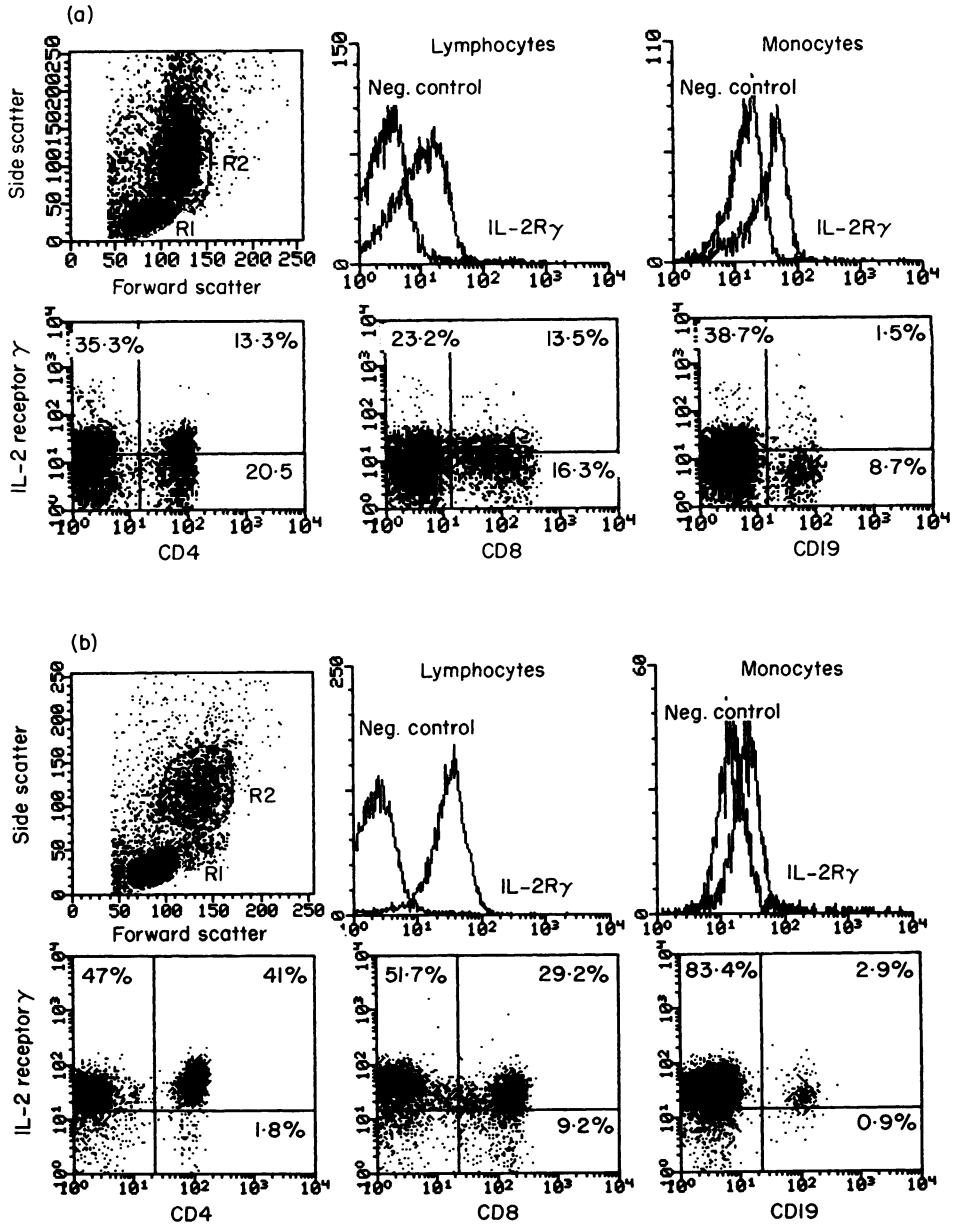

Figure 2. Flow cytometric analysis of IL-2 receptor (IL-2R) γ chain on cord (a) and adult (b) blood cells. The top left panel shows dual scatter parameters, allowing identification of lymphocytes (R1) and monocytes (R2). The next two panels show fluorescence histograms (x-axis is fluorescence intensity and y-axis is relative cell frequency) for IL-2R γ chain on lymphocytes and on monocytes, in each case superimposed on the negative control antibody trace. The lower traces show two-colour fluorescence analysis, for the lymphocyte population, of IL-2R γ-chain expression (y-axis) against markers (x-axis) for the major T-cell subsets (CD4 and CD8) and B cells (CD19). Numbers represent the percentage of total lymphocytes in each quadrant. Comparison of Figs 2(a) and 2(b) shows that staining intensities on lymphocytes and proportions of lymphocytes staining are higher on adult than on cord cells. The data in these figures are representative of at least 10 data sets, which are analysed statistically in Table 2.

Table 2. Expression of IL-2-receptor γ on cord and adult lymphocytes and subsets. Values represent percentage positive ± SD, with the number of samples analysed given in brackets.

Population analysed	Cord blood	Adult blood	*P* value
CD4	47 ± 15 (12)	85 ± 11 (12)	0·0001
CD8	58 ± 18 (12)	82 ± 8 (10)	0·0008
CD19	42 ± 16 (12)	66 ± 17 (11)	0·002

leucocytes in adults,[29] although it is not clear whether the cells showing strong IL-2-receptor β-chain staining in cord are also large granular leucocytes.

Staining of tonsil sections with antibody against the IL-2-receptor γ chain showed uniformly distributed weak staining, with somewhat higher staining intensities in the T-cell-rich interfollicular zones, compared to the B-cell-rich follicles. Tissue from an 8-year-old tonsillectomy and autopsy tissue from a 6-week-old accident victim showed qualitatively similar staining, but tissue staining was too weak to allow any reliable conclusion on differences in staining intensity between the tissues.

Adult peripheral blood and cord blood lymphocytes were depleted of monocytes and cultured with CD3 antibody, with and without IL-2. As can be seen from Fig. 1, the proliferative response, particularly at lower CD3 concentrations, was IL-2 dependent. Cord cells were responsive to IL-2, but responses were significantly lower than adult cell responses. We examined the ability of CD3 antibody, and IL-2, to regulate the expression of IL-2-receptor component chains. CD3 antibody and IL-2, together or separately, modulated expression of the α, β and γ chains of the IL-2 receptor in adult cells. In cord cells, the effects were minimal (Table 3).

Adult and cord blood lymphocytes were cultured in the presence of IL-4. After 48 hr in culture, there was an increase in expression of CD23, major histocompatibility complex (MHC) class II and surface IgM by B cells in both adult and cord cells (Table 1). Changes for other markers (IgD, CD98 and CD40) were much smaller. A 24-hr culture period showed similar, but smaller, changes. While the increase in MHC class II was greater in the adult cells, increases in CD23 and IgM were marked in both adult and cord cells, and it is difficult to conclude whether cord cells were any less responsive, especially since starting levels were higher in cord cells. Since these results indicate that the IL-4 receptor is functional on cord blood cells, we determined the effect of culture in IL-4 on IL-2-receptor γ-chain expression. IL-4 did not increase IL-2-receptor γ expression (not shown).

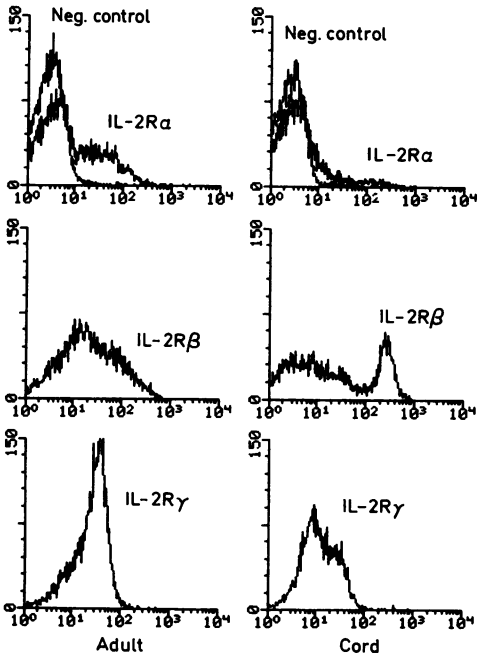

Figure 3. Direct comparison of cord and adult lymphocyte staining for the three chains of the IL-2 receptor. The top traces include a negative control antibody, superimposed over the α-chain antibody histogram. The x-axis represents fluorescence intensity, the y-axis relative cell frequency. The data are representative of more than 10 data sets, and the data for the IL-2-receptor (IL-2R) γ chain are analysed statistically in Table 2 whilst statistical data for α and β chains have been reported elsewhere.[24]

DISCUSSION

The data presented in Figs 2(a) and (b), and Table 2 show that IL-2-receptor γ chain is expressed at significantly lower levels on cord as compared with adult cells. Figure 3 shows that the IL-2-receptor α and β chains are also expressed at lower levels on cord than on adult cells, and we have shown elsewhere that cord cells express lower levels of several other cytokine receptors, including IL-4, IL-6 and tumour necrosis factor.[24] The functional consequences of the deficit in IL-2 receptor were explored by activating T cells in a system where proliferation is IL-2 dependent. As can be seen in Fig. 1 at the lower concentrations of CD3 antibody, where proliferation is more dependent on IL-2, proliferative responses were lower in cord than in adult cells. The intrinsic proliferative ability of T cells was unimpaired, as can be seen from the highest concentration of CD3 antibody. Thus cord cells show an impaired responsiveness to IL-2. Interestingly, the small but significant proliferative response of cord cells to low concentrations of IL-2 (25 U/ml) and CD3 antibody (0·5 ng/ml) (Fig. 1) occurs despite any detectable effect on IL-2-receptor concentrations. The receptor concentrations are mean values for the population; it is possible that a small subpopulation of cells does respond to IL-2 and accounts for the proliferation seen, without significantly affecting the mean fluorescence intensities.

Cord blood B cells were able to respond to IL-4 with an increase in expression of CD23, IgM and a smaller increase in MHC class II. These markers characteristically increase in response to IL-4,[30–32] and the effect is selective, since other markers (CD98, surface IgD and CD40 were used in this study) do not change markedly in response to IL-4. In the case of IL-4, it is difficult to compare the responsiveness of cord and adult cells quantitatively, because starting levels of CD23, IgM and MHC class II differ between cord and adult B cells, but there is no obvious and consistent impairment of IL-4-responsiveness in cord cells. We have previously shown that the IL-4-specific chain of the receptor (CDw124) is also expressed at lower levels on cord compared with adult B cells.[24] The situation for IL-4 is complicated by the possibility that the α chain may transduce

Table 3. Modulation of IL-2 receptor (IL-2R) chains by CD3 antibody (0·5 ng/ml), IL-2 (25 U/ml) and CD3 + IL-2. Mean fluorescence intensities are tabulated for reactivity with antibodies against the three chains of the IL-2 receptor, after culturing cells in the absence (control) or presence of IL-2, CD3 antibody and both together. Data are from one experiment representative of two, carried out on different cord and adult blood samples.

	Adult				Cord			
Receptor	Control	CD3	IL-2	CD3 + IL-2	Control	CD3	IL-2	CD3 + IL-2
IL-2Rα	8·2	11·9	13·1	13·7	3·5	3·5	3·3	3·7
IL-2Rβ	22·4	17·4	11·0	11·1	4·2	4·5	3·9	4·1
IL-2Rγ	14·7	16·7	17·0	20·1	4·1	4·1	4·5	4·7

signals in the absence of the γ chain, acting either alone or in conjunction with an unknown second chain.[33] It is interesting to note that the existence of alternative IL-4 receptors has been postulated on the basis of the much greater sensitivity of adult cells to IL-4 if detected by the increased expression of surface IgM, as compared with the increased expression of CD23.[31] The results in Table 1 suggest that in cord cells CD23 is more sensitive than IgM to IL-4-mediated control, while the opposite is true for adult cells. A minimal interpretation of our results is that cord blood B cells respond to IL-4, in spite of both the α and the γ (common) chain being expressed at low levels.

The low level of IL-2 receptor on cord cells was not significantly increased by culture in the presence of IL-2 (with or without CD3 antibody) or IL-4, whilst adult cells did show some responsiveness to IL-2 (Table 3) and IL-4.[27] IL-2 down regulated expression of the β chain of the receptor. We did not examine the effect of reduced common-chain expression on responses to IL-7, 9 and 15. IL-7-receptor chain is present on cord T cells at levels close to those on adult cells,[24] and IL-7 is functional on cord T cells, regulating the level of expression of the co-stimulator molecule B7.[34]

The reduction in response to IL-2 may contribute to the functional immaturity of the healthy neonate. The more obvious impairment of IL-2 responses as compared with IL-4 (this study) or IL-7[34] suggests either that the response to IL-2 is limited by the α or β chain, rather than the γ chain, or that these cytokines have alternative signalling mechanisms.

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