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Role of interleukin-2 and interferon-γ in inducing production of IgG subclasses in lymphocytes of human newborns

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SUMMARY

Unlike lymphocytes from adults, lymphocytes from cord blood of neonates cannot synthesize immunoglobulin G (IgG) in response to pokeweed mitogen (PWM). By using this mitogen in concert with interferon-γ (IFN-γ), interleukin-2 (IL-2), or interleukin-6 (IL-6), we studied the induction of IgG subclass molecules in lymphocytes of human neonates. IFN-γ induced a limited, but substantial, enhancement of IgG2 production by neonatal lymphocytes. IL-2 dose dependently increased the production of each neonatal IgG subclass, whereas IL-6 did not. However, in adult lymphocytes, and under specific conditions, IL-6 or IL-2 each increased the production of all four IgG subclasses. Early in the culture IFN-γ synergized with IL-2 during the latter or whole culture period to enhance cord blood IgG2 levels. This finding contrasted with the adult IgG2 synthesis synergistically up-regulated by IFN-γ and IL-6. IL-2 caused a graded increase in immunoglobulin production in neonatal lymphocytes with IgG3 being the highest and IgG2 the lowest, thus corresponding to the differential increase of serum levels of IgG3/IgG1 and IgG4/IgG2 early in childhood. Results suggest that IL-2, but not IL-6, is critical to the development of human IgG subclass production.

INTRODUCTION

Neonatal lymphocytes are defective in their ability to produce immunoglobulins,[1] which may contribute to the newborn's increased susceptibility to severe infection.[2] Lymphocytes from cord blood show a lower immunoglobulin production than that of adult lymphocytes when stimulated with pokeweed mitogen (PWM), Epstein–Barr virus (EBV), lipopolysaccharide (LPS) or *Staphylococcus aureus* Cowan I (SAC).[3,4] The poor ability of neonates to produce immunoglobulin may be due to functionally immature B cells,[5,6] few helper T cells,[6–9] and/or increased suppressor T cell activity.[5,6,9–11] Despite the immaturity of neonatal lymphocytes, cytokines can ameliorate their diminished function.[12–15]

Human IgG is divided into four subclasses based on the antigenicity of the heavy chains,[16,17] each expressing different functions, including antimicrobial activity.[18–21] However, it is still unclear how newborn infants develop the pathways necessary to produce the requisite IgG subclasses. The staggered production of different subclasses merely demonstrates the complexity of neonatal development; IgG3 and IgG1 appear before IgG4 and IgG2.[22,23] These results are supported by *in vitro* studies that investigated IgG subclass production in normal children of various ages.[3]

Previous studies clearly indicated that the *in vitro* induction of IgG subclass is markedly affected by the specific stimulus used, including PWM,[24–26] SAC,[25,27] LPS,[25,28] EBV,[25] CD3 antibody,[15] phorbol myristate acetate (PMA) with mouse thymoma cell lines (EL4),[29] and CD40 antibody.[30] These reports indicated that the *in vitro* induced subclass distribution resembled that found in normal serum (IgG 1 > 2 > 3 > 4) when the cultures were stimulated with PWM,[25,26] but not with the others.

We recently described the regulation of IgG subclass induction from adult lymphocytes by interferon-γ (IFN-γ) and interleukin-6 (IL-6) by using PWM as the cell-culture stimulus.[31,32] IFN-γ significantly suppressed the production of IgG1, whereas IgG2 secretion was spontaneously enhanced, thus demonstrating the specificity of IFN-γ for IgG2.[31] IL-6, however, acted as a differentiation factor on both T and B cells to enhance IgG production with differential expression of the IgG subclass molecules.[32] In the experiments evaluating the combined effect of these two cytokines, IFN-γ was shown to antagonize the enhanced production of IgG1 by IL-6 and to act synergistically with IL-6 to produce IgG2.[31]

Despite the functional immaturity of the neonatal B lymphocytes, neonatal T lymphocytes readily produce IL-2. Other lymphokines are not produced unless the cells are re-stimulated *in vitro*.[33–35] These results are reminiscent of the cytokine profile of naive T cells in mice.[36]

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Abbreviations: IFN-γ, interferon-γ; IgG, immunoglobulin G; IL-2, interleukin-2; IL-6, interleukin-6; PWM, pokeweed mitogen; SAC, *Staphylococcus aureus* Cowan I.

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We evaluated the effect of IL-2, IFN-γ and IL-6 on the differentiation of lymphocytes from cord blood of human neonates to clarify the development of the cellular mechanisms resulting in IgG production.

MATERIALS AND METHODS

Reagents

Recombinant IL-6 was the kind gift of Dr H. Karasuyama of Tokyo University (Tokyo, Japan). Recombinant IFN-γ was purchased from Shionogi Co. (Osaka, Japan) and IL-2 (TGP-3) was purchased from Takeda Co. (Osaka, Japan).

Separation of peripheral blood mononuclear cells

Mononuclear cells were isolated from heparinized whole blood by Ficoll–Conray density gradient centrifugation.[37] Whole blood consisted either of cord blood that was collected immediately after an uncomplicated birth, or of venous blood obtained from healthy adult volunteers.

Induction of immunoglobulin synthesis

Mononuclear cells (1×10^6) were suspended in 1 ml of RPMI-1640 medium supplemented with 20 mM L-glutamine (Sigma Chemical Co, St. Louis, MO), 5×10^{-5} M 2-mercaptoethanol (Sigma Chemical Co), 50 μg/ml gentamycin (Shionogi) and 10% fetal calf serum (FCS; Gibco, Grand Island, NY) in the presence or absence of 10 μl/ml PWM (Gibco). The cells were cultured for 4 days, after which they were washed three times, plated on fresh medium and cultured for another 5 days. Recombinant IFN-γ, IL-2 or IL-6 was added on the days indicated and its effect on IgG subclass production was evaluated. The culture supernatant was collected and the content of IgG or the IgG subclass molecules was quantified.

Measurement of IgG subclasses

IgG subclasses were quantitated as described previously.[31,32] Briefly, flat-bottomed 96-well microtitre plates (Limbro, McLean, VA) were coated with a 50-μg/ml combination of goat anti-human κ and λ antibodies (Cappel, Malvern, PA) in 60 μl of carbonate buffer (pH 9·6). To minimize non-specific binding, the anti-human κ and λ antibodies were absorbed with normal mouse immunoglobulin and normal goat immunoglobulin prior to use. Following an overnight coating with antibody, at 4° in a humidified atmosphere, the plates were washed with phosphate-buffered saline (PBS), and each well was saturated with 300 μl of 0·25% gelatin (Wako, Osaka, Japan) for 3 hr at 37°. After another wash, 60 μl of each sample was placed in individual wells, after which the plates were incubated for 2 hr at room temperature. Following the incubation, the plates were washed thoroughly and allowed to react sequentially with the following reagents: 60 μl of murine anti-human IgG subclass monoclonal antibody, 60 μl of biotinylated goat anti-mouse IgG antibody (Tago, Burlingame, CA), and 50 μl of a 1000-fold dilution of a streptavidin–horseradish peroxidase conjugate (BRL, Gaithersberg, MD). *o*-Phenylenediamine (OPD, BRL) was then added and the optical density (OD) at 490 nm was read with an enzyme-linked immunosorbent assay (ELISA) reader (APR-A4; Tosoh, Tokyo, Japan). Data were quantitated by comparison to the absorbance of standard purified IgG samples (Protogen, Laufelfingen, Switzerland). We used the following five murine monoclonal antibodies against human immunoglobulin: anti-IgG (8a4), anti-IgG1 (NL16), anti-IgG2 (HP6014), anti-IgG3 (HP6050), and anti-IgG4 (RJ4), all obtained from Oxoid (Basingstoke, Hants, UK). Specificity of each antibody was confirmed previously.[31,32] Goat anti-mouse IgG antibody used as the secondary antibody was passed through a goat immunoglobulin- and human immunoglobulin-coupled Sepharose 4B column to avoid non-specific binding, then diluted 200-fold for use. The sensitivity limits of the assays for IgG1, IgG2, IgG3, and IgG4 were 170, 540, 270, and 600 pg/ml, respectively.

Calculation of % increase of production of neonatal IgG subclass

The differential amplifications of each subclass by IL-2 were calculated according to the following formula:

$$\%\ \text{increase} = \frac{\text{increase of neonatal IgG subclass by 1U/ml of IL-2}}{\text{increase of adult IgG subclass by 1U/ml of IL-2}} \times 100.$$

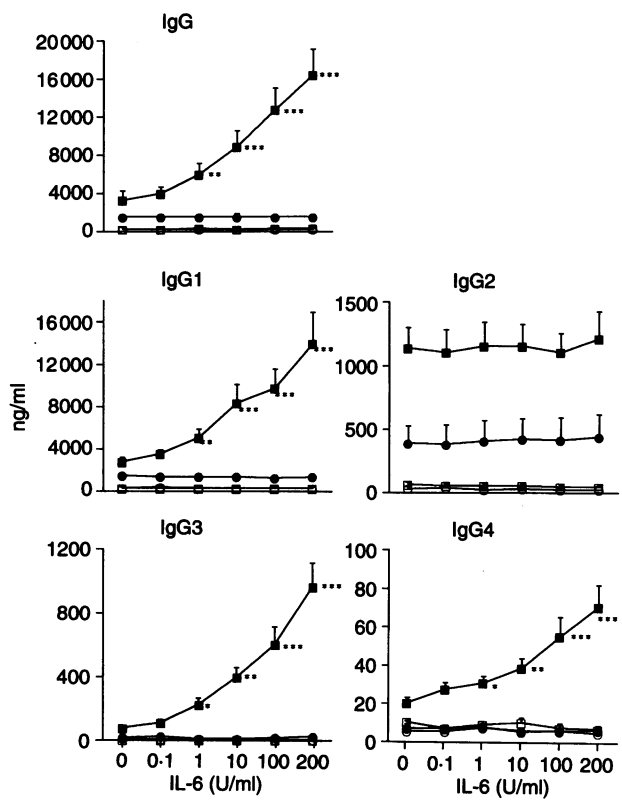

Figure 1. Dependency of IgG subclass production on IL-6. Mononuclear cells from adult or cord blood were cultured with the indicated doses of IL-6 for 9 days in the presence or absence of PWM. IgG subclass proteins were then measured by ELISA. Values are mean ± SD, where $n = 4$. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$ show the statistically significant difference between corresponding cultures with and without IL-6. (■) adult lymphocytes in the presence of PWM; (□) cord blood lymphocytes in the presence of PWM; (●) adult lymphocytes in the absence of PWM; (○) cord blood lymphocytes in the absence of PWM.

Data analysis

Data are expressed as mean ± SD. A two-tailed Student's *t*-test was used in analysis. The significant difference was set at $P < 0.05$.

RESULTS

IL-6-induced production of IgG subclass by cord blood lymphocytes

Cord blood lymphocytes produced significantly smaller amounts of total IgG than adult lymphocytes in the presence of PWM (644 ± 58·5 versus 6480 ± 518 ng/ml) as well as in its absence (345 ± 94·7 versus 1870 ± 173 ng/ml). Similar findings were obtained by analyzing each subclass. Unstimulated cord blood cells elicited a reduced production of IgG1 (214 ± 68·7 versus 1850 ± 144 ng/ml), IgG2 (30·0 ± 14·5 versus 361 ± 62·9 ng/ml), IgG3 (2·13 ± 1·01 versus 26·5 ± 3·40 ng/ml), and IgG4 (3·01 ± 2·00 versus 6·41 ± 2·34 ng/ml) compared with quiescent adult cells. In PWM-driven cultures, both neonatal and adult lymphocytes showed measurable increases in IgG subclasses; however, adult cell production was significantly elevated in all cases. IgG1 (244 ± 66·2 versus 4125 ± 477 ng/ml), IgG2 (35·3 ± 8·54 versus 1120 ± 151 ng/ml), IgG3 (4·23 ± 1·01 versus 36·8 ± 3·44 ng/ml), and IgG4 (9·60 ± 4·60 versus 21·4 ± 7·33 ng/ml).

The effect of IL-6 on IgG subclass production by adult lymphocytes varies depending upon when it is added to the culture. During 9 days in culture, IL-6 did not induce the production of any immunoglobulins in neonatal lymphocytes. Conversely, adult lymphocytes demonstrated increased IgG1, IgG3, and IgG4 production (Fig. 1). When IL-6 was administered during the second half of the culture period, only adult lymphocytes were induced to show higher IgG1, IgG2, and IgG4 levels (Fig. 2).

IFN-γ-induced production of IgG2 subclass by cord blood lymphocytes

As shown in Fig. 3, IgG production by adult lymphocytes was profoundly suppressed by IFN-γ (500 U/ml), whereas that of cord blood lymphocytes was enhanced. Analysis of subclasses

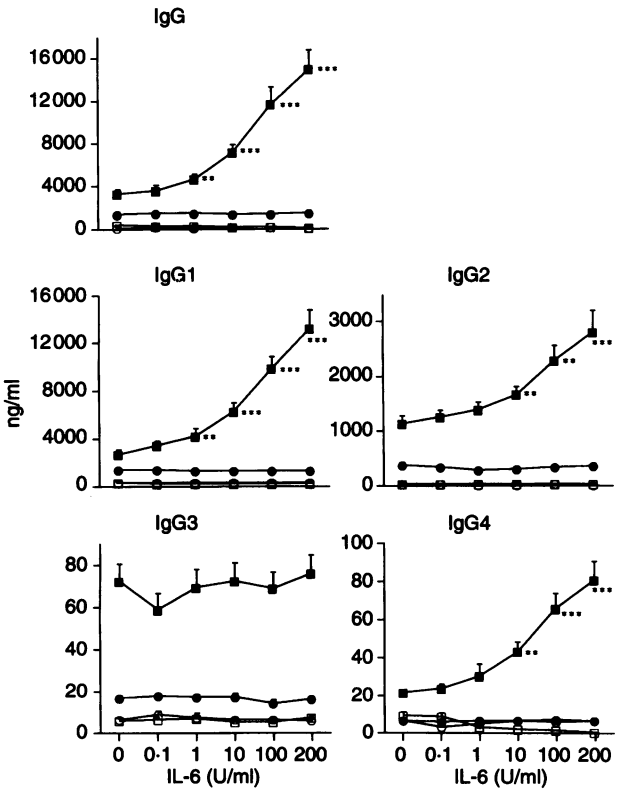

Figure 2. IL-6-enhancement of IgG subclass production in the late phase. Mononuclear cells from adult or cord blood, stimulated with or without PWM, were cultured for 4 days without IL-6. All cells were then co-cultured with the indicated doses of IL-6 for another 5 days. Values are expressed as mean ± SD, where $n = 4$. $^{**}P < 0.01$, and $^{***}P < 0.001$ show the statistically significant difference between corresponding cultures with and without IL-6. (■) adult lymphocytes in the presence of PWM; (□) cord blood lymphocytes in the presence of PWM; (●) adult lymphocytes in the absence of PWM; (○) cord blood lymphocytes in the absence of PWM.

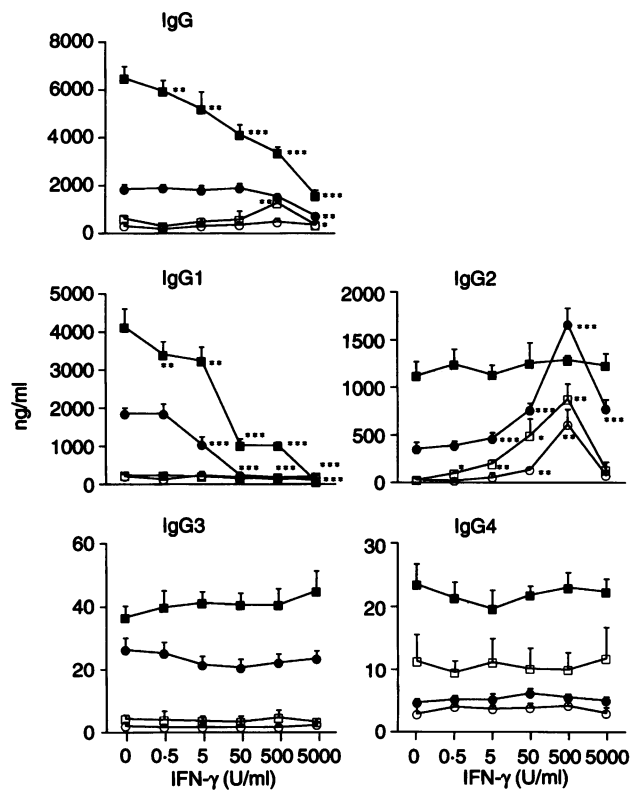

Figure 3. Selective stimulation of IgG2 synthesis by IFN-γ. Mononuclear cells from adult or cord blood were cultured for 9 days with the indicated doses of IFN-γ. Values are mean ± SD, where $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ show the statistically significant difference between corresponding cultures in the presence and absence of IFN-γ. (■) adult lymphocytes in the presence of PWM; (□) cord blood lymphocytes in the presence of PWM; (●) adult lymphocytes in the absence of PWM; (○) cord blood lymphocytes in the absence of PWM.

indicated that, in contrast to adult lymphocytes, whose production of IgG1 was dose-dependently suppressed by IFN-γ, the release of IgG1 by cord blood lymphocytes was unaffected by IFN-γ. This was most likely because of the small amount of IgG1 produced by those lymphocytes. In contrast to the enhancement of IgG2 production from adult lymphocytes without PWM, IgG2 synthesis by cord blood lymphocytes was up-regulated by IFN-γ, both with or without PWM. However, the enhanced IgG2 level in cord blood lymphocytes did not approach those of the adult. IFN-γ was ineffective in the induction of the subclasses IgG3 and IgG4. These data suggest that in neonatal lymphocytes, the enhancement of IgG2 production by IFN-γ specifically effects total IgG. It is possible that the excess of IgG2 compared with the low amount of IgG1 masked any detectable induction of IgG1 in the cord blood lymphocytes.

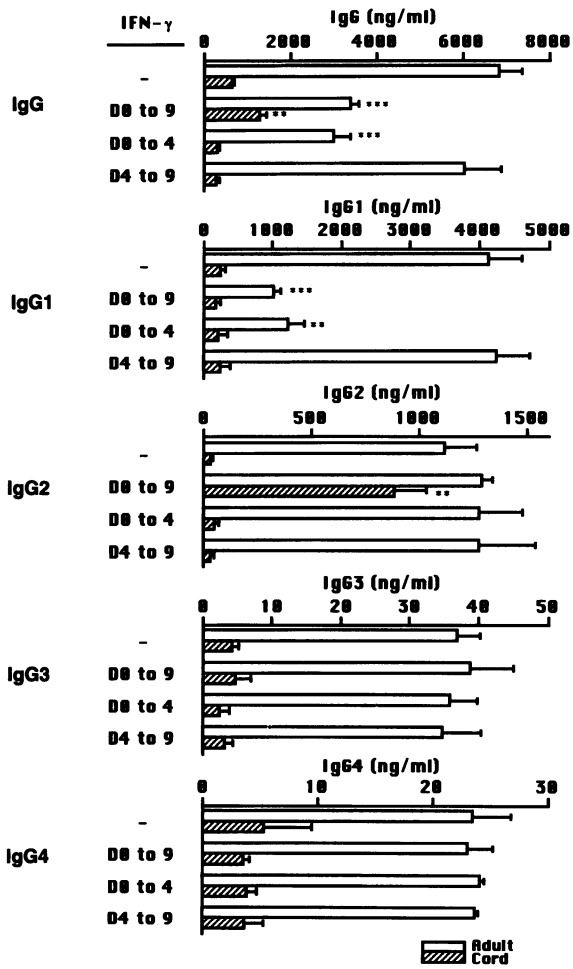

Figure 4. Kinetic studies of IgG2 induction by IFN-γ. PWM-stimulated mononuclear cells from adult or cord blood were cultured in the presence of 500 U/ml of IFN-γ. IFN-γ was added for the first half (day 0 to day 4), last half (day 4 to day 9), or all (day 0 to day 9) of the culture period. Values are expressed as mean ± SD, where $n = 4$. $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$ show statistically significant difference between corresponding cultures with and without IFN-γ.

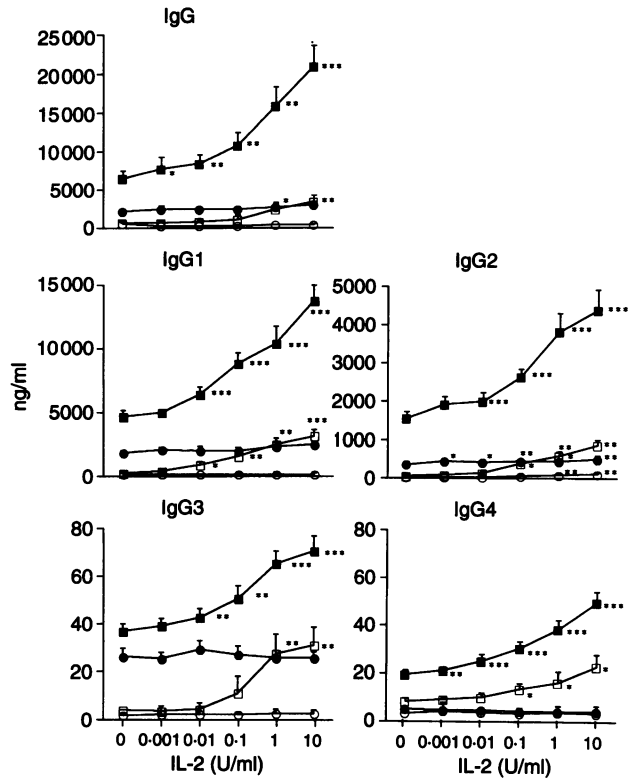

Figure 5. IL-2-induced up-regulation of IgG subclass secretion. Mononuclear cells from adult or cord blood were cultured with the indicated doses of IL-2 for 9 days in the presence or absence of PWM. Values are expressed as mean ± SD, where $n = 4$. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$ demonstrate the statistically significant difference between corresponding cultures with and without IL-2. (■) adult lymphocytes in the presence of PWM; (□) cord blood lymphocytes in the presence of PWM; (●) adult lymphocytes in the absence of PWM; (○) cord blood lymphocytes in the absence of PWM.

Kinetic studies of IgG2 induction by IFN-γ

As shown in Fig. 4, the up-regulation of IgG2 in cord blood lymphocytes required IFN-γ treatment throughout the culture period. This induced level of IgG2 did not yet approach that seen in unstimulated adult lymphocytes. The total IgG response in cord blood paralleled that of IgG2; this agrees with our above-mentioned results.

Neonatal IgG subclass production enhanced by IL-2

IL-2 enhanced the production of all four IgG subclasses by adult PWM-stimulated lymphocytes to levels approaching those seen with IL-6 (Figs 1 and 2). However IL-2, not IL-6, significantly increased the production of all IgG subclasses by cord blood lymphocytes dose dependently (Fig. 5). While both lymphocyte populations responded to IL-2 treatment, the effect was less pronounced in the cord blood cells. None-the-less, these results were statistically significant. Preliminary experiments show that 0·1 to 1 U/ml of IL-2 sufficiently enhances the proliferation of lymphocytes initiated by concanavalin A.

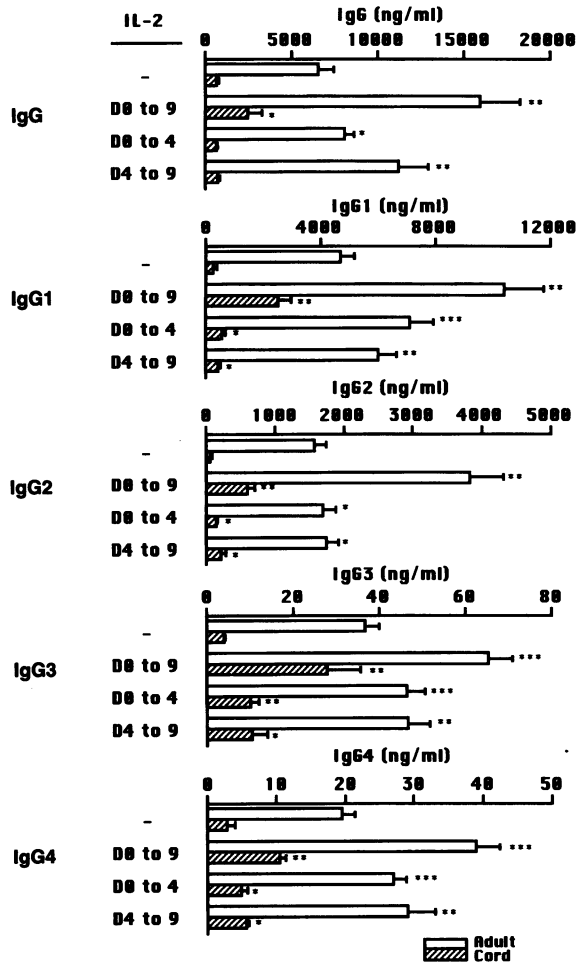

Figure 6. Kinetics of IL-2-induced subclass production. PWM-stimulated mononuclear cells from adult or cord blood were cultured in the presence of 1 U/ml of IL-2 during the first half (day 0 to day 4), last half (day 4 to day 9), or entire (day 0 to day 9) culture period. Values are expressed as mean ± SD, where $n = 4$. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, and $***P < 0{\cdot}001$ illustrate the statistically significant difference between corresponding cultures in the presence or absence of IL-2.

Although the up-regulation of neonatal IgG subclass production by IL-2 was not subclass-specific, the percentage increase by IL-2 (1 U/ml) of each subclass, calculated according to the formula in the Materials and Methods, was maximal with IgG3 (83·2%), followed by IgG1 (39·1%), IgG4 (39·0%) and IgG2 (23·5%).

Time-dependent enhancement of IgG subclass production by IL-2

The up-regulation of IL-2-related IgG subclass production was highest when this cytokine was added for the entire culture period (day 0 to day 9). While shorter incubation times (0–4 days or 4–9 days) produced less IgG induction, these smaller increases were still statistically significant relative to untreated cultures. The enhancing effect of IL-2 was observed in both adult and cord blood lymphocytes, although the adult lymphocytes showed a more pronounced response (Fig. 6).

Combined effect of IFN-γ and IL-6 on IgG subclass induction in cord blood lymphocytes

IFN-γ in the first half and IL-6 in the last half exerted antagonistic effects on IgG1 production and co-operative action on IgG2 synthesis in adult lymphocytes stimulated with PWM. However, in cord blood lymphocytes, no such effects were observed (Fig. 7).

IFN-γ modulates the IL-2-induced up-regulation of IgG subclass production in cord blood lymphocytes

Combined effects of IFN-γ and IL-2 on the generation of each subclass induced by PWM were then examined. In the production of IgG1 by adult lymphocytes, IFN-γ counteracted IL-2. The inhibitory actions of IFN-γ were observed even when it was present at least during the first half of the culture period, although they were more prominent when IFN-γ was present during the whole cultures. The inhibition by IFN-γ of adult IgG1 production was less marked in the presence of IL-2 during the entire culture period than in its presence only in the first half or in the last half alone. Similar combined effects of IFN-γ and IL-2 on IgG1 synthesis were seen in cord blood lymphocytes; notably IFN-γ acted synergistically with IL-2 in causing IgG2 production (Fig. 8). To elicit this synergy, IFN-γ was only required early in the culture period, whereas IL-2 was required at least during the latter half. IFN-γ in the first half, IL-2 in the last half, and both generated 50·5 ± 21·2, 213·5 ± 73·3, and 794·3 ± 72·8 ng/ml of neonatal IgG2 production, respectively. Moreover, IFN-γ early in the culture synergized with IL-2 during the whole culture to enhance cord blood IgG2 levels; both cytokines induced 934 ± 73·84 ng/ml of IgG2, compared with only IFN-γ (50·5 ± 21·2) and IL-2 alone (592 ± 111).

DISCUSSION

Consistent with previous findings,[3] the present study clearly demonstrates that lymphocytes from cord blood produce smaller amounts of all IgG subclasses than do lymphocytes from the venous blood of adults. The production of each IgG subclass in neonatal lymphocytes was unaffected by IL-6, in contrast to the differential up-regulation in adult cells.[32] Three hypotheses should be considered to explain this issue. First, the inability of IL-6 to promote immunoglobulin by the neonatal mononuclear cells may have been due to poor activation of B cells by the PWM. However, this contention is unlikely, because neonatal IL-2 responsiveness was up-regulated by the stimulation with PWM as shown in Fig. 5. Second, it is possible that IL-6 fails to augment cord blood IgG secretion because IL-2 is limiting, based on the effects of IL-6 which is dependent on IL-2.[38] This possibility is contradictory to the efficient production of IL-2 by cord blood lymphocytes (data not shown). Third, this lack of responsiveness could be attributed to a reduced expression of IL-6 receptor complexes or to diminished signal-transduction pathways,[39] although further studies are required to elucidate the mechanism.

We have shown here, as well as in an earlier report, that exogenous IFN-γ enhances the production of IgG2 by cord blood lymphocytes. Our data suggest that, regardless of PWM

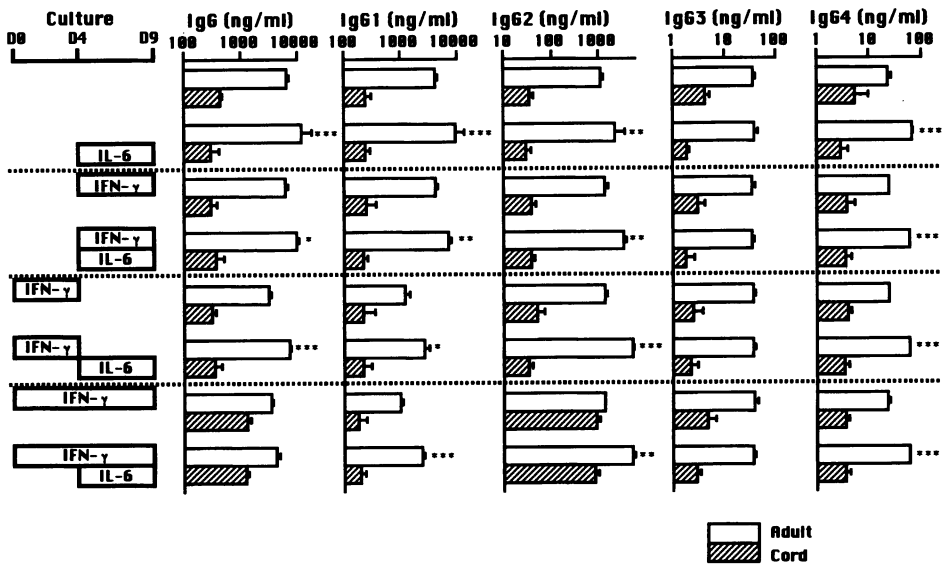

Figure 7. Combined effects of IFN-γ and IL-6 on immunoglobulin production. PWM-stimulated mononuclear cells from adult or cord blood were cultured in the presence of IFN-γ (500 U/ml) and IL-6 (100 U/ml) as indicated. Values are expressed as mean ± SD, where $n = 4$. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$ show the statistically significant difference between corresponding cultures with and without IL-6.

stimulation, IFN-γ enhances the production of IgG2 in lymphocytes from cord blood of newborn infants. This may not be due to the failure of PWM to activate cord blood lymphocytes *in vitro* because PWM induced significantly enhanced responsiveness to IL-2 as compared to that of unstimulated cells (Fig. 5). Apparently similar responsiveness to IFN-γ in PWM and non-PWM-stimulated cord blood cells may be caused by the decreased production of IFN-γ in lymphocytes from cord blood versus those from adult venous blood (data not shown), consistent with previous reports.[33–35]

Similar studies of the kinetics and of the dose-response using adult and neonatal cells suggest that IFN-γ may also

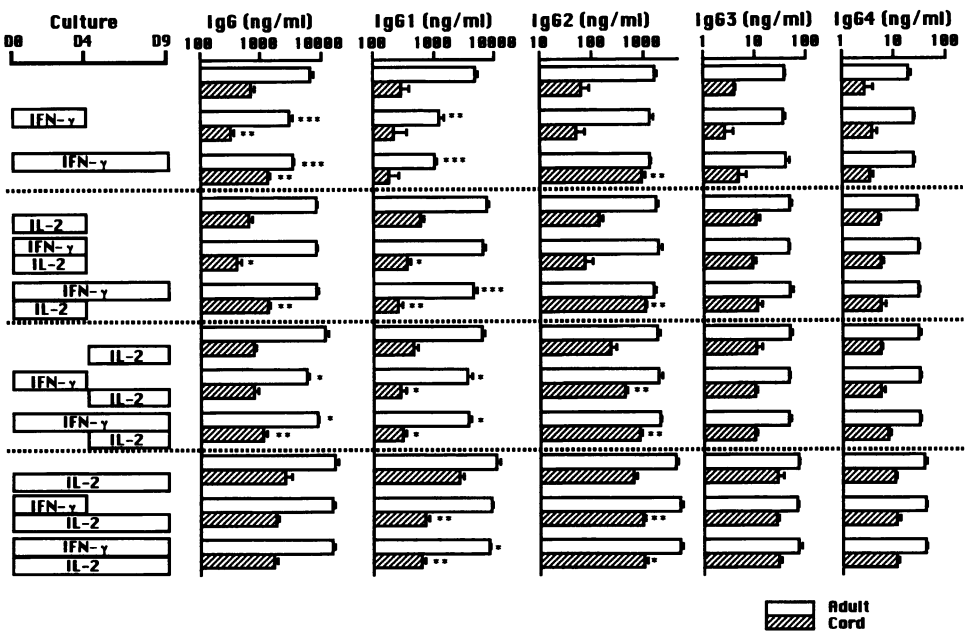

Figure 8. Modulation of IgG subclass induction by IFN-γ and IL-2. PWM-stimulated mononuclear cells from adult or cord blood were cultured in the presence of IFN-γ (500 U/ml) and IL-2 (1 U/ml) as indicated. Values are expressed as mean ± SD, where $n = 4$. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, and $^{***}P < 0{\cdot}001$ demonstrate statistically significant difference between corresponding cultures with and without IFN-γ.

serve as a non-switching factor specific for IgG2.[31] Physiologically, the enhancement of IgG2 by IFN-γ in neonatal lymphocytes becomes more important in light of the fact that neonatal T cells produce IFN-γ when restimulated by CD3 antibody.[34] These data underscore the potential importance of IFN-γ in the development of IgG2 during infancy.

IL-2 is thought to be a factor in the differentiation of B cells.[38,40–47] The importance of IL-2 is further emphasized by the previous work using CD3-activated lymphocytes by Splawski and co-workers,[15] in that the addition of IL-2 to neonatal lymphocytes overcame the inability to produce immunoglobulin in stimulation with CD3 antibodies.

Current literature supports a possible link between IL-2 and IL-6 in the development of the lymphocyte.[38,45] One report suggests that IL-6 can mediate the IL-2-induced B-cell differentiation[45] while a second paper reports that B-cell differentiation induced by IL-6 actually depends on IL-2.[38] Our data reconcile this contradiction by demonstrating that neonatal lymphocytes are unresponsive to wide range (0·1 to 200 U/ml) of IL-6, thus removing any prerequisites for IL-2/IL-6 interdependence.

CD3-stimulated cultures respond to IL-2 by up-regulating their IgG levels associated with the expression of IL-2 receptors (CD25) on neonatal T and B cells.[15,34] These findings raise the possibility that IL-2 may also participate in the conversion of naive into memory T cells thereby expressing helper cell functions,[48] while it stimulates B-cell differentiation.

The combined actions of IFN-γ and IL-6, which elicited additive effects for the induction of adult IgG2 production, were negligible relative to neonatal IgG2 induction (Fig. 7). These data further strengthen the relevance of the unresponsiveness of cord blood lymphocytes to IL-6, even when coupled to the co-stimulatory agent, IFN-γ.

In contrast, IFN-γ and IL-2 showed a synergistic enhancement of IgG2 production in cord blood lymphocytes, but not in adult lymphocytes. Synergy was maximal when IFN-γ was added only during the first half of the culture period and IL-2 was present, at least, in the latter half of the culture period (Fig. 8). As previously described, IFN-γ alone enhanced IgG2 to almost maximal levels when present throughout the culture period. Such conditions may be unsuitable for visualizing the overriding actions of IL-2. These findings were consistent with previous data reporting that adult B cells, initially activated by SAC plus IL-2 or IFN-γ, subsequently secreted maximal levels of immunoglobulins in response to IL-2.[43] This study substantiates our data (Fig. 6) wherein IL-2, either in the initial culture, or in the subsequent culture, generated a weaker enhancement of IgG subclasses than IL-2 present during the entire culture period. Thus, IL-2 may be able to stimulate both B-cell proliferation and B-cell differentiation.

Although the ability of IL-2 to enhance neonatal IgG subclass production was not subclass specific, there is a sizeable distinction between the extent of the IL-2-induced enhancement of the subclasses IgG3 > 1 > 4 > 2. This differential up-regulation of neonatal IgG subclass production was consistent with the fact that ontogenically serum levels of IgG3 and IgG1 increase faster than those of IgG4 and IgG2.[22,23] Therefore, our results may strongly suggest that IL-2 is a major potentiator of the development of the IgG subclasses and is the driving force that increases the age-related clonal diversity in IgG antibodies to *Streptococcus pyogenes* group A carbohydrate (A-CHO) antibodies.[49] This suggestion is further supported by the observation that neonatal T cells preferentially produce IL-2, not other cytokines, upon primary stimulation with CD3 antibodies.[33–35] Adult T lymphocytes can generate cytokines in abundance, including IL-6, IFN-γ and IL-2. This could effectively amplify the generation of the IgG subclasses. In contrast, the sole dependence of neonatal subclass induction on IL-2 may not favour their defence mechanisms. Neonatal lymphocytes could easily lose the ability to synthesize IgG subclass molecules when the IL-2/IL-2R system is inhibited by an immunosuppressant such as cyclosporine A, whereas adult lymphocytes may overcome such immunosuppressive effects by using the IL-6/IL-6R system as an alternative pathway.

Our study clearly illustrates the central role of IL-2 in the induction of the IgG subclass in neonatal lymphocytes. The involvement of other cytokines, including the ones secondarily induced by IL-2, in such IgG induction has yet to be revealed. It seems likely that the delay in the maturation of the IgG subclass-producing ability in childhood can be explained by the lower levels of these cytokines induced in lymphocytes during infancy.

It is important to note the functional identity of the individual subclasses in adult and cord blood lymphocytes. This should be considered in that anti-*Streptococcus pyogenes* group A carbohydrate (A-CHO)[49] and anti-pneumococcal antibodies[19] of adults are strikingly restricted to the IgG2 subclass, whereas those of children are found in both the IgG1 and IgG2 subclasses. This maturational isotypic shift from IgG1 to IgG2 during infancy may be caused in part by the delayed appearance of IFN-γ as compared with the easily inducible IL-2.[33–35] Our data, showing the combined effects of IFN-γ and IL-2 (Fig. 8), support the idea that IgG1, which is driven by IL-2 even in the early neonatal period, is suppressed, and that IgG2, which is less effectively induced by IL-2, is synergistically up-regulated when IFN-γbecomes available at a specific time during development. IFN-γ may therefore modulate the induction of the IgG subclass by IL-2 early in life. Finally, we emphasize that IL-2 may work in newborn lymphocytes to stimulate these cells to synthesize IgG subclass since it is the most readily inducible cytokine in neonatal T cells.

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