Delineation of the Functional Capacity of Human Neonatal Lymphocytes

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Abstract

Neonatal T cell–B cell collaboration was investigated utilizing a system of T cell–dependent polyclonal B cell activation and Ig secretion. In this system, T cells activated by immobilized anti-CD3 provide a potent stimulus for Ig production by adult lymphocytes. By contrast, anti-CD3 stimulation of cord blood lymphocytes generated minimal numbers of Ig-secreting cells. Ig production by neonatal lymphocytes was enhanced by the addition of *Staphylococcus aureus* or secreted factors from mitogen-stimulated adult T cells. Supplementation with IL-2 resulted in the production of large amounts of IgM and small amounts of IgG and IgA, with less Ig produced than by comparable cultures of adult lymphocytes. Neonatal T cells proliferated and produced IL-2 in response to immobilized anti-CD3, and supported B cell proliferation and Ig secretion by adult B cells, although not as effectively as adult T cells. Supernatants from activated neonatal T cells were markedly limited in their capacity to support Ig production by adult B cells. Neonatal B cells could be induced to differentiate in response to anti-CD3-stimulated adult T cells. However, the amounts of IgG and IgA secreted were small compared to adult levels. These studies indicate a relative, but not absolute, functional deficiency of both neonatal B and T cells. (*J. Clin. Invest.* 1991. 87:545–553.)
Key words: CD3 • immunoglobulin • interleukin 2 • neonatal B cells • T cells

Introduction

Neonates are deficient in the ability to generate humoral immune responses (1). Part of this deficiency may relate to lack of previous exposure to exogenous antigen. However, the inability to respond to antigens, such as bacterial capsular polysaccharides, even after repeated exposure suggests that the deficient immune responses of neonates may be the result of immaturity of some elements of the immune system at birth (1–3). Delineation of the responsiveness of neonatal cord blood lymphocytes to polyclonal mitogens in vitro has supported the concept that they are functionally deficient compared to adult lymphocytes. For example, pokeweed mitogen (PWM) stimulates the generation of IgM-, IgG-, and IgA-producing cells by adult lymphocytes in vitro, but generates minimal Ig-producing cells (ISC)[1] from neonatal lymphocytes (4–8). However, recent studies have demonstrated that the B cells induced to secrete Ig in response to PWM are largely surface IgD–negative, postswitch memory B cells (9–11). Therefore, the absence of Ig secretion by the neonatal B cells in response to PWM is consistent with the lack of memory B cells in cord blood, but does not provide information about the functional capabilities of naive neonatal B cells.

Stimulation of cord blood B cells with *Staphylococcus aureus* (SA), a polyclonal activator that induces Ig production by both memory and naive B cells (11, 12), and factors secreted by activated adult T cells (TF) induces the secretion of small amounts of IgM (13). Similarly, Epstein-Barr virus (EBV) transformation also induces neonatal B cells to secrete Ig in vitro, but IgM is the only Ig class produced. These results support the conclusion that some neonatal B cells are functionally competent, but limited to IgM secretion (7, 8, 14).

In vitro analysis of the functional capabilities of neonatal T cells has also suggested that they are functionally immature. Early studies utilizing PWM as the polyclonal stimulator demonstrated suppression of adult Ig secretion by the neonatal T cells (5, 8, 15, 16). This suppression was mediated by $CD4^+$ T cells rather than $CD8^+$ suppressor T cells (17–19). A subpopulation of naive $CD4^+$ T cells has been identified in adult peripheral blood by its expression of a number of different proteins, including CD45RA, the high molecular weight isoform of CD45 (20). As with neonatal T cells, stimulation of the $CD45RA^+$ population from adult peripheral blood with PWM fails to induce help for B cells but rather elicits suppression (21). The majority of cord blood T cells express CD45RA, suggesting that these neonatal T cells may be comparable to the $CD4^+$, $CD45RA^+$ suppressor/suppressor-inducer T cells in adult peripheral blood (19, 22–24). Irradiation of the neonatal T cells has been shown to abolish suppressive activity, but they still remain deficient in the capacity to provide help (8, 24, 25).

Recently a new method of generating antibody responses in vitro has been described which is unique in that the majority of adult peripheral blood B cells can be induced to secrete Ig, indicating that naive as well as memory B cell populations are induced to differentiate (26). In this system, T cells activated by immobilized monoclonal antibody (MAb) to the CD3 molecular complex support the activation and differentiation of B cells to ISC in the absence of other polyclonal B cell activators or cell types (27). Helper activity can be elicited from both $CD45RA^+$ (naive) and $CD45RO^+$ (memory) T cell subsets after stimulation with anti-CD3 (28). The magnitude of adult B cell responses induced by the anti-CD3-stimulated T cells is far greater than that resulting from activation with SA and TF or

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1. *Abbreviations used in this paper:* ISC, immunoglobulin-secreting cells; SA, *Staphylococcus aureus,* Cowan I strain; TF, supernatants of mitogen-activated T cells.

PWM. T cell contact, but not HLA identity is required for these responses (27). This model system, therefore, appeared to be an appropriate one to analyze the capacity of neonatal lymphocytes to participate in T cell–B cell collaboration leading to the secretion of antibody. Utilizing this system, the functional capabilities of neonatal T and B cells were delineated.

Methods

Cell preparation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood of healthy adult volunteers by centrifugation over sodium diatrizoate/ficoll gradients (Pharmacia, Inc., Piscataway, NJ) as described (29). Neonatal lymphocytes were collected from the cord blood of healthy newborns at the time of delivery and mononuclear cells were obtained as above.

Culture medium. All cultures were carried out in medium RPMI 1640 Gibco Laboratories, Grand Island, NY), supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml, Sigma Chemical Co., St. Louis, MO), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (FBS) (Microbiological Associates, Walkersville, MD).

Reagents. SA (Pansorbin) was purchased from Calbiochem-Behring Corp., La Jolla, CA, and used at a final concentration of 1:60,000 (vol/vol). PWM (Gibco Laboratories) was used at a final concentration of 10 μg/ml. PHA was purchased from Wellcome Reagents, Division of Burroughs Wellcome Co., Research Triangle Park, NC. 4β-Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma Chemical Co. and was dissolved in ethanol for use in culture. Purified recombinant interleukin 2 (IL-2) was provided by the Cetus Corporation, Emeryville, CA.

MAb. The MAb, 64.1, is an IgG2a MAb directed at the CD3 molecular complex on mature T cells (30, 31). 64.1 was purified from ascites fluid by passage over a column of Sepharose 4B coupled with staphylococcal protein A. OKT8 (American Type Culture Collection [ATCC], Rockville, MD) is an IgG2a MAb directed at the CD8 molecule. L234 (ATCC) is an IgG2a MAb directed at monomorphic HLA-DR determinants. Anti-Tac, a MAb directed at the p55 component of the IL-2 receptor (CD25), was generously provided by Dr. T. Waldmann, National Cancer Institute, National Institutes of Health, Bethesda, MD (32).

Cell separation. PBMC from adult peripheral blood and cord blood were depleted of monocytes and natural killer (NK) cells by incubation with 5 mM L-leucine methyl ester (Sigma Chemical Co.) in serum-free medium (33, 34). After washing, B cells were depleted of T cells by two cycles of rosetting with neuraminidase-treated sheep red blood cells (SRBC) (35). Adult B cells prepared in this fashion are > 90% CD20 positive, as has been previously established (36). Cord blood B cells were 70–75% CD20 positive and 75–90% CD19 positive. Approximately 85–90% of both the cord and adult CD20-positive B cells stained positively for IgD. The B cell preparations contained < 1% CD3-positive cells. Sedimented rosette-forming cells from the first centrifugation were treated with isotonic NH_4Cl to lyse the SRBC and then passed over a nylon wool column. The resulting cell population contained < 1% esterase-staining cells and > 95% $CD3^+$ T cells (36). Cord blood T cell populations were also > 95% $CD3^+$ and were > 95% $CD45RA^+$. CD4 enriched populations were obtained after panning to remove $CD8^+$ and $HLA\text{-}DR^+$ cells as previously described (37). Monocyte depletion was demonstrated by the absence of proliferation in response to PHA by the cord and adult T cell populations. T cells were subjected to treatment with mitomycin C (40 μg/ml, Sigma Chemical Co.) or 2,000 rad of γ irradiation before culture.

Generation of mitogen-stimulated T cell supernatants (TF). T cells were suspended in medium with 10% FBS at a concentration of 5×10^6 cells/ml and incubated with 1 μg/ml of PHA and 1 ng/ml of PMA for 2 h at 37°C. The cells were then washed and resuspended in fresh culture medium at 5×10^6/ml, 2 ml per 17 × 100-mm round-bottom tube. After 48 h, the supernatants were harvested, filtered, and stored at −20°C until used (36). TF was added to the well so that the final concentration was 25% vol/vol. In some cases, T cell supernatants were generated by incubating T cells (5×10^6 cells per well, 1 ml per well) in 64.1-coated wells and harvesting the supernatants after 48 h.

Culture conditions. RPMI 1640 or 0.05 M Tris at pH 9.5 was used to dilute the 64.1, the concentration was adjusted to 4 μg/ml and 50 μl were placed in each well of 96-well microtiter plates (Costar Data Packaging, Cambridge, MA). After incubation at room temperature for 1–5 h, the wells were washed twice with medium to remove nonadherent MAb before the addition of cells (38). Routine cultures were carried out in triplicate in a total volume of 200 μl per well. T cells or $CD4^+$ cells were added at the density of 1.0×10^5 per well and B cells were added at 2.5×10^4 per well. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. At the end of a 5-d incubation period, the cells were harvested for determination of ISC. Supernatants for quantitation of Ig secretion were harvested at varying time points as indicated.

Proliferation assays. Culture conditions used for the assay of lymphocyte [^{3}H]thymidine incorporation were identical to those used for the generation of ISC. The cells were incubated for 3–5 d with 1 μCi of [^{3}H]thymidine (6.7 Ci/mM; New England Nuclear, Boston, MA) present for the last 18 h. The cells were harvested onto glass filter paper and [^{3}H]thymidine incorporation determined by liquid scintillation spectroscopy.

Detection of ISC. ISC were detected by a reverse hemolytic plaque assay that has been described in detail (39). For this assay, staphylococcal protein A–coupled SRBC are the indicator cells and the developing antiserum is a rabbit anti-human Ig (IgM, IgG, and IgA) (Cappel Laboratories, West Chester, PA) diluted 1:50, and the complement source is a 1:20 dilution of guinea pig serum (Pel-Freeze Biologicals, Inc., Rogers, AR) that had previously been absorbed with SRBC. All data are expressed as the mean number of ISC per 1,000 B cells initially cultured.

Measurement of Ig secretion. Ig in the culture supernatants was quantitated utilizing isotype specific ELISA assays as previously described (11, 40). The sensitivities of the specific assays are IgM 24 ng/ml, and IgG and IgA 12 ng/ml.

IL-2 assay. Supernatants to be assayed for IL-2 production were obtained after incubation of neonatal and adult T cells (1.0×10^5/well) for 72 h with immobilized anti-CD3 in the presence of 5 μg/ml anti-CD25 MAb. Supernatants were filtered and frozen before assay. IL-2 production was determined by incubation of CTLL cells (5×10^3 cells per well) suspended in 100 μl of medium containing 10% FBS with 100 μl of the culture supernatant to be assayed or various concentrations of recombinant IL-2 diluted in culture medium. [^{3}H]Thymidine incorporation was assessed after 28 h with [^{3}H]thymidine present for the last 8 h. IL-2 production was determined by comparing CTLL [^{3}H]thymidine incorporation supported by supernatants with that supported by known concentrations of recombinant IL-2 (41).

Statistical analysis. Statistical analysis of the data was carried out utilizing the paired *t* test.

Results

Anti-CD3 stimulation of neonatal lymphocytes is not sufficient to induce Ig secretion. The initial experiments examined the capacity of anti-CD3 stimulation to induce ISC generation by neonatal B and T cells. Although anti-CD3 induced the generation of a large number of ISC by adult lymphocytes, minimal numbers of ISC were induced from cord lymphocytes (Table I). The addition of SA to provide an additional activation signal for the B cells resulted in a consistent increase in the number of ISC generated by the anti-CD3-stimulated neonatal lymphocytes, whereas co-stimulation of anti-CD3-activated neonatal lymphocyte cultures with both SA and TF markedly increased the generation of ISC. In the absence of anti-CD3 stimulation, SA and TF induced only modest generation of ISC from neona-

Table I. Ig Production by Neonatal Lymphocytes Stimulated with Combinations of Polyclonal Activators

Lymphocyte source	Addition to culture	Without anti-CD3				With anti-CD3			
		ISC	IgM	IgG	IgA	ISC	IgM	IgG	IgA
				ng/ml				*ng/ml*	
Adult	Nil	0.0	ND	ND	55	720.0	3,920	2,240	1,920
	TF	134.0	1,560	380	296	708.6	4,480	2,000	1,872
	SA	8.0	37	240	156	849.8	4,160	2,480	1,952
	SA + TF	307.0	2,720	4,480	928	766.2	3,840	1,000	1,712
Cord 1	Nil	0.0	ND	ND	ND	0.0	ND	ND	ND
	TF	0.0	ND	ND	ND	0.0	100	ND	ND
	SA	0.0	ND	ND	ND	2.6	1,600	ND	ND
	SA + TF	3.2	800	ND	ND	163.0	1,480	ND	ND
Cord 2	Nil	0.0	ND	ND	ND	0.4	ND	ND	ND
	TF	0.0	ND	ND	ND	6.4	270	17	ND
	SA	0.0	ND	ND	ND	54.2	1,360	ND	ND
	SA + TF	19.2	510	ND	ND	232.0	1,520	ND	ND
Cord 3	Nil	0.2	ND	ND	ND	0.0	ND	ND	ND
	TF	0.0	ND	ND	ND	1.4	190	20	ND
	SA	0.0	ND	ND	ND	26.6	264	ND	ND
	SA + TF	0.4	400	ND	ND	113.8	1,720	ND	ND

Cord or adult B cells were incubated with mitomycin C-treated cord or adult $CD4^+$ cells. Supernatants were collected for determination of Ig content and cells were harvested for determination of ISC (per 10^3 B cells) on day 6. ND, not detectable.

tal lymphocytes. These results contrast with the responses noted in cultures of anti-CD3-stimulated adult lymphocytes, in which the addition of SA or TF alone or in combination had little effect on the generation of ISC.

Adult lymphocytes secreted IgG and IgA in addition to IgM after stimulation with TF or SA and TF and in response to stimulation with anti-CD3 (Table I). The addition of SA and TF to the anti-CD3-stimulated adult lymphocytes did not result in a dramatic increase in the secreted Ig, nor an alteration of the isotype of Ig secreted. In the absence of anti-CD3 stimulation, neonatal lymphocytes secreted Ig only after stimulation with SA and TF and the response was limited to IgM. Anti-CD3 stimulation of the neonatal B and T cells did not result in Ig secretion but IgM secretion was induced by the addition of either adult TF or SA to these cultures. The amount of IgM produced in response to SA and TF was markedly augmented by co-stimulation with anti-CD3. The responses of the neonatal lymphocytes varied in that some of the preparations required SA alone and others required SA and adult TF for the maximal response to anti-CD3 stimulation. Even with maximal stimulation, however, only IgM was secreted by day 6.

The addition of large concentrations of IL-2 (50 U/ml) could also augment IgM production by anti-CD3-activated neonatal lymphocytes and in addition, induced the secretion of a modest amount of IgG and IgA (Table II). Addition of smaller amounts of IL-2 (10 U/ml) had a much more modest effect, supporting a small amount of IgM and IgG secretion in one of five experiments. The amount of Ig secreted in cultures supported by 50 U/ml of IL-2 was significantly less than that produced by anti-CD3-stimulated adult B and T cells with or without supplemental IL-2.

Examination of the kinetics of Ig production in IL-2-supported cultures of neonatal T and B cells demonstrated ongoing production of Ig for up to 20 d (Fig. 1). Secretion of IgM could be observed as early as the 5th d of culture. Detection of IgG and IgA secretion was delayed compared to IgM. Although the amount of IgA increased throughout the culture period, it represented only a minor portion of the Ig produced in contrast to cultures of anti-CD3-stimulated adult B and T cells.

Functional capacity of neonatal T cells. The next experiments examined the functional capacity of neonatal T cells. Anti-CD3 induced DNA synthesis by neonatal T cells that was not significantly different from that exhibited by adult T cells (Fig. 2). Secretion of IL-2 by cord T cells in response to stimulation with anti-CD3 was also observed, although the amount of

Table II. Supplemental IL-2 Induces IgM, IgG, and IgA Secretion by Anti-CD3-stimulated Neonatal B and T Cells

Cells	Addition	Ig isotype secreted		
		IgM	IgG	IgA
			ng/ml	
Cord	Nil	11±11	3±3	6±6
	IL-2 (10)	83±44	14±10	6±6
	IL-2 (50)	2,075±615	395±143	53±1
Adult	Nil	10,174±1,692	2,520±583	8,083±886
	IL-2 (50)	11,572±4,424	3,404±1,109	6,383±1,811

Cord and adult B cells and irradiated T cells were incubated with immobilized anti-CD3 and with or without IL-2 (10 or 50 U/ml). Supernatants were harvested after a 10-d incubation for quantitation of the Ig secreted by ELISA. The results are the mean±SEM for five experiments.

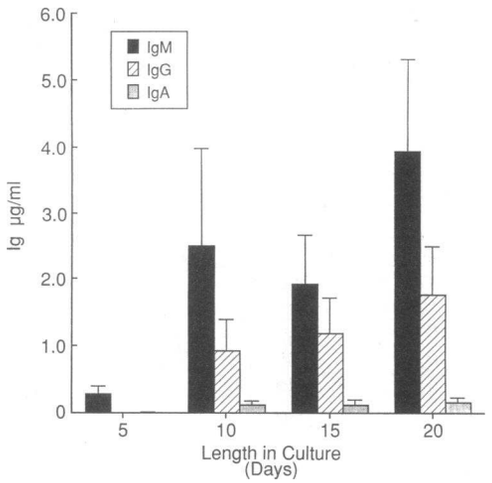

Figure 1. Kinetics of Ig production by anti-CD3-stimulated neonatal B and T cells supplemented with IL-2. Cord B cells (1.0–2.5 × 10^4 per well) were incubated with irradiated cord T cells (1.0 × 10^5 per well) in the presence of immobilized anti-CD3 and 50 U/ml of IL-2. Supernatants were harvested from triplicate wells on the indicated days for quantitation of Ig isotypes secreted by ELISA. The results are expressed as the mean±SEM for four different experiments.

IL-2 produced by the neonatal T cells was somewhat less than that produced by adult T cells (adult T cells 42 U/ml; cord T cells 27.6±3.3 U/ml) (Fig. 3).

The helper function of anti-CD3-stimulated neonatal T cells was investigated initially by examining their ability to support B cell proliferation. As can be seen in Fig. 4, anti-CD3-stimulated neonatal T cells induced proliferation of neonatal B cells, although less effectively than adult T cells. As can be seen in Table III, neonatal T cells also promoted adult B cell proliferation, but not as effectively as adult T cells. The addition of adult TF did not overcome this difference. These results indicate that anti-CD3-stimulated neonatal T cells can promote DNA synthesis by both adult and neonatal B cells, but are less effective in this regard than adult T cells even when cultures are supplemented with cytokine-rich T cell supernatants.

The capacity of neonatal T cells to promote ISC generation by adult B cells was also examined (Table IV). Anti-CD3-activated neonatal $CD4^+$ cells supported the generation of ISC by adult B cells in the absence of additional co-stimulation, although not as effectively as adult $CD4^+$ cells. The addition of

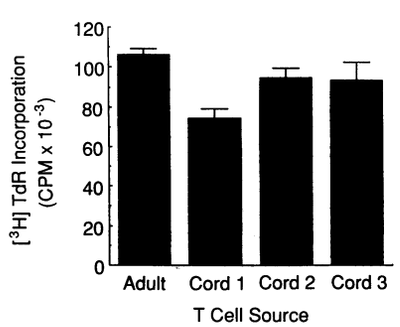

Figure 2. DNA synthetic responses of anti-CD3-stimulated adult and neonatal CD4+ T cells. T cells (1 × 10^5 per well) were incubated with immobilized anti-CD3. [^{3}H]Thymidine was added 18 h before harvest of the cells on day 5. The results are expressed as the mean±SEM of triplicate samples.

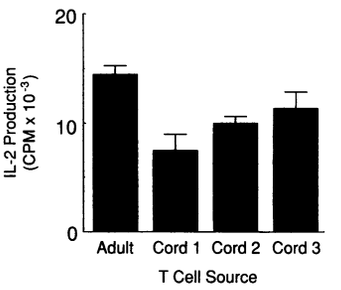

Figure 3. IL-2 production by anti-CD3-stimulated neonatal and adult T cells. Neonatal or adult T cells (1 × 10^5 per well) were incubated with immobilized anti-CD3 in the presence of 5 µg/ml of antibody to the p55 component of the IL-2 receptor. Supernatants were collected at 72 h and analyzed for the production of IL-2 by [^{3}H]thymidine incorporation of CTLL cells. The results are expressed as the mean±SEM of the triplicate samples.

adult TF augmented the response of adult B cells stimulated by anti-CD3-activated neonatal $CD4^+$ cells, although the number of ISC generated remained less than that supported by the anti-CD3-stimulated adult $CD4^+$ cells. Anti-CD3-stimulated neonatal $CD4^+$ cells promoted the secretion of IgG and IgA in addition to IgM by the adult B cells (Table V). Secretion of all Ig isotypes was augmented by the addition of adult TF. In some, but not all experiments, neonatal $CD4^+$ cells were able to support Ig secretion by adult B cells to the same extent as the adult $CD4^+$ cells when adult TF was added. These results indicate that neonatal $CD4^+$ T cells could provide help to adult B cells, but that optimal support of Ig secretion required the addition of lymphokine-rich supernatants from activated adult T cells. Thus, neonatal T cells appear to be quantitatively, but not qualitatively deficient in the capacity to provide help for B cells.

The above studies suggested that anti-CD3-stimulated neonatal T cells might be deficient in the capacity to secrete sufficient cytokines to support B cell differentiation. Therefore, the ability of supernatants generated by activated neonatal T cells to support adult B cell Ig secretion was examined (Table VI). TF from adult T cells supported the secretion of IgA, IgG, and IgM from SA-stimulated adult B cells. By contrast, TF from neonatal T cells was markedly deficient in the capacity to support the secretion of Ig by SA-stimulated adult B cells. The addition of IL-2 to the SA-stimulated B cells supported a modest amount of Ig secretion and augmented B cell differentiation supported by neonatal T cell supernatants, but not to the level

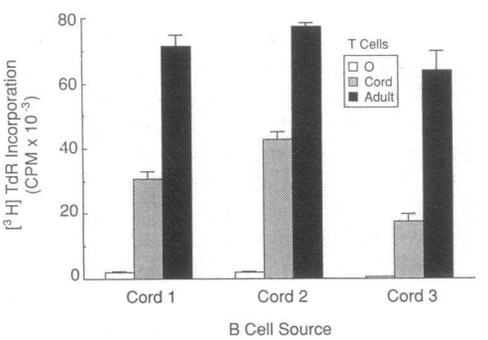

Figure 4. Neonatal B cell DNA synthesis induced by anti-CD3-stimulated neonatal T cells. Cord B cells (2.5 × 10^4 per well) were incubated with or without irradiated cord or adult T cells (1 × 10^5 per well) stimulated with immobilized anti-CD3. [^{3}H]Thymidine was present for the last 18 h of a 3-d culture. The results are expressed as the mean±SEM of the triplicate samples.

Table III. Support of Adult B Cell Proliferation by Neonatal T Cells

T cell source	Addition to culture			
	Without anti-CD3		With anti-CD3	
	−TF	+TF	−TF	+TF
	cpm × 10⁻³			
No T cells	0.1±0.0	1.5±0.2	1.0±0.1	1.7±0.1
Adult T	0.3±0.0	15.7±0.9	68.4±6.9	78.6±7.5
Adult CD4⁺	0.3±0.1	16.5±0.6	60.1±7.9	84.0±8.8
Cord 1 CD4⁺	0.5±0.1	4.6±0.5	17.9±1.3	41.3±1.7
Cord 2 T	1.8±0.3	4.5±0.7	17.7±0.2	31.3±4.0
Cord 2 CD4⁺	0.8±0.2	3.7±0.3	22.1±1.0	35.9±5.3
Cord 3 T	1.6±0.2	6.1±0.3	22.8±1.3	37.8±1.3
Cord 3 CD4⁺	0.2±0.0	4.7±0.5	23.5±2.2	54.6±6.6

Adult B cells were incubated with mitomycin C–treated adult or cord T cells or CD4⁺ cells in the presence or absence of anti–CD3 and harvested after a 5-d incubation. [³H]Thymidine incorporation by TF or anti–CD3-activated cord or adult T cells was < 1.3±0.4 × 10³ cpm.

Table IV. Neonatal CD4⁺ T Cell Support of the Generation of ISC from Adult B Cells

T cell source	Control		Anti–CD3 stimulated	
	−TF	+TF	−TF	+TF
	ISC per 10³ B cells			
Adult	0.0±0.0	84.2±24.1*	837.6±107.7	669.2±80.6
Cord	0.1±0.0	7.8±3.0‡	111.9±41.3	276.4±36.3*‡

Adult B cells were incubated with mitomycin C–treated adult or cord CD4⁺ T cells in the presence or absence of immobilized anti–CD3 or adult TF. ISC generation was determined on day 5. The results are expressed as the mean±SEM for three adult B and T cell populations and seven cord T cell populations.
* Significant enhancement of ISC generation with addition of adult TF ($P < 0.05$).
‡ Cord T cells supported significantly less ISC from adult B than adult T cells ($P < 0.05$).

supported by adult TF. By contrast, supplementation with IL-2 did not augment Ig production supported by adult TF. These results suggest that there are differences in the amounts or types of cytokines secreted by neonatal and adult T cells.

Functional capacity of neonatal B cells. The ability of neonatal B cells to respond to anti–CD3-activated adult T cells was also examined. Adult CD4⁺ cells support ISC generation by the neonatal B cells in the absence of additional co-stimulation (Fig. 5). This contrasts with the absence of ISC generation by the neonatal B cells in response to anti–CD3-stimulated neonatal CD4⁺ cells. However, the magnitude of the response was much less than that induced from adult B cells. The addition of either SA, adult TF, or SA and TF variably enhanced the response, but not to the levels observed with adult B cells. In the absence of anti–CD3 stimulation, SA and TF induced minimal numbers of ISC (< 20 ISC per 1,000 B cells).

Examination of the Ig isotypes secreted by neonatal B cells in response to the anti–CD3-stimulated adult CD4⁺ cells at day 6 demonstrated a small amount of IgG secretion in addition to IgM (Table VII). IgA secretion was observed in some experiments after a 10-d incubation (data not shown). Addition of TF or IL-2 (not shown) variably enhanced Ig secretion in the cultures supported by adult T cells in contrast to responses observed with neonatal T cells.

Discussion

The current studies were carried out to delineate the full functional potential of human neonatal B and T lymphocytes. To achieve this, a model system was employed that permits functional activation of both naive and memory T and B cells and, therefore, could be used to examine specific aspects of neonatal T and B cell collaboration (26). The results demonstrate that with optimal activation and cytokine support, neonatal B cells can be induced to differentiate and secrete all three major Ig isotypes by neonatal T cells. These findings may have clinical implications with regard to amplifying antibody responses of neonates in vivo.

Previous in vitro studies of neonatal lymphocyte responses have demonstrated deficiencies in the ability to generate antibody (1). A number of features of neonatal lymphocytes may contribute to altered responsiveness. Thus, cord blood contains predominantly naive B cells and T cells that respond poorly to

Table V. Neonatal T Cell Support of Immunoglobulin Secretion by Adult B Cells

T cell source	−TF			+TF		
	IgM	IgG	IgA	IgM	IgG	IgA
	ng/ml					
Adult	8,834±2,241	3,458±1,300	1,950±422	7,632±2,104	3,632±1,319	1,701±404
Cord	1,552±478	436±165	478±152	4,924±1,462	1,560±606	1,176±388

Adult B cells were cultured with adult or cord CD4⁺ T cells and immobilized anti–CD3 in the presence or absence of adult TF. Supernatants were harvested for the determination of Ig secretion after a 5- or 6-d incubation. The results are expressed as the mean±SEM for five experiments with adult B and T cell populations and nine cord T cell populations. Cord T supported less Ig secretion than adult T cells ($P < 0.05$). TF enhanced the Ig production supported by cord T but not adult T cells ($P < 0.05$).

Table VI. Support of Adult B Cell Ig Secretion by Cord and Adult T Cell Supernatants

Expt.	Mitogen	TF	Ig secreted by SA-stimulated adult B cells		
			IgM	IgG	IgA
			ng/ml		
1	Anti-CD3	Adult 1	1,720	>2,000	560
		Adult 2	1,400	>2,000	312
		Cord 1	500	<15	<15
		Cord 2	220	<15	<15
	PHA/PMA	Adult 1	1,520	>2,000	432
		Adult 2	1,460	640	196
		Cord 1	800	<15	<15
		Cord 2	560	<15	<15
2	PHA/PMA	IL-2 only	<24	123	39
		Adult	810	2,863	402
		Adult + IL-2	909	2,241	402
		Cord 1	96	621	150
		Cord 1 + IL-2	507	1,836	192
		Cord 2	<24	180	66
		Cord 2 + IL-2	198	1,071	150

Adult B cells were incubated with SA and TF generated from adult or cord T cells in response to immobilized anti-CD3 or PHA/PMA. Supernatants were harvested for determination of Ig secretion on day 5. IL-2 was added to the indicated wells at a concentration of 50 U/ml.

many mitogens. PWM drives primarily memory B cells and generates minimal Ig producing cells from neonatal B and T cells (4–9). EBV and the combination of SA and adult TF stimulate some Ig secretion, but the response is small and limited to IgM (7, 8, 14). These latter results have suggested that the capacity of neonatal B cells to produce Ig is deficient. Irradiated neonatal T cells can provide help for PWM-stimulated adult B cells, suggesting that neonatal T cells can serve as helper cells (8). However, they appear to be considerably less potent in this regard than adult T cells. It is difficult, however, to draw conclusions concerning the ability of neonatal T cells to function as helper T cells for neonatal B cells, inasmuch as it is clear from the current studies that neonatal B cells require a much greater array of activation signals than adult B cells. Thus, the deficient responses of the neonatal lymphocytes could represent true functional immaturity, but the degree of nonresponsiveness may not have been accurately assessed previously because of the use of stimuli that do not activate naive B cells effectively in vitro.

Cord blood B cells differ from adult B cell populations in a number of ways, including the small percentage of memory cells and the large percentages of naive and $CD5^+$ B cells (42, 43). However, in contrast to PWM, stimulation of adult lymphocytes with immobilized anti-CD3 induces Ig secretion from most adult B cells, indicating that differentiation of naive and memory B cell populations can be induced (26). Specifically, both IgD^+ naive B cells and $CD5^+$ B cells isolated from adult peripheral blood secrete Ig in response to anti-CD3 activated adult T cells (L. Vernino and P. E. Lipsky, unpublished observation). Therefore, the use of this model system was thought to be an appropriate way to evaluate the functional capabilities of neonatal B cells and their capacity to collaborate with activated T cells for the induction of antibody secretion.

Anti-CD3 stimulation of adult T cells is sufficient to induce the secretion of large amounts of Ig by adult B cells. However, anti-CD3 stimulation of neonatal B and T cells did not induce ISC generation or Ig secretion. This did not appear to relate to a diminished responsiveness of the neonatal T cells to signaling by immobilized anti-CD3 at the concentrations tested. Cord T cells express CD3 at a density that is comparable to memory T cells. Although cord T cells and naive ($CD45RA^+$) adult T cells have been reported to be deficient in their ability to be activated and to produce IL-2 in response to soluble antibody to CD3 and CD2, the results reported here indicate that neonatal T cells can be induced to proliferate and secrete IL-2 by immobilized anti-CD3 (44–47). These results are consistent with the demonstration that naive adult T cells expressing CD45RA are responsive to signaling by immobi-

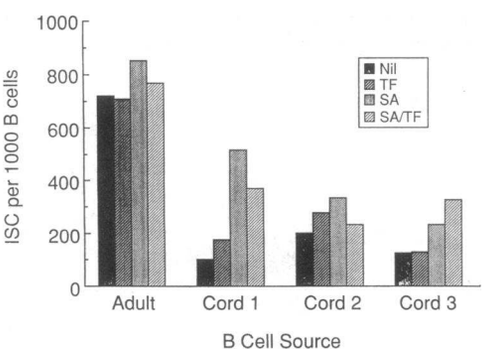

Figure 5. Anti-CD3-activated adult T cell support of neonatal ISC generation. Adult or cord B cells (2.5×10^4 per well) were cultured with mitomycin C–treated adult $CD4^+$ T cells (1.0×10^5 per well) in the presence of anti-CD3, with or without SA or mitogen-stimulated adult T cell supernatants. ISC generation was assayed on day 5.

Table VII. Adult T Cell Support of Neonatal B Cell Differentiation

B cells	TF	Immunoglubulin isotype		
		IgM	IgG	IgA
			ng/ml	
Adult	–	3,920	2,240	1,920
	+	4,480	2,000	1,872
Cord 1	–	4,800	88	<12
	+	4,320	164	<12
Cord 2	–	2,160	58	<12
	+	2,688	116	<12
Cord 3	–	3,440	118	<12
	+	4,080	182	<12

Cord or adult B cells were incubated with mitomycin C–treated adult T cells stimulated with immobilized anti-CD3 in the presence or absence of adult TF. Supernatants were collected after a 6-d incubation and analyzed for Ig content.

lized anti–CD3, and after activation are capable of producing IL-2 and supporting adult B cells proliferation and differentiation (28). Therefore, neonatal T cells can be activated through the CD3 molecular complex, although they appear to require the enhanced signaling capacity of immobilized 64.1 for the induction of proliferation and IL-2 production.

Although there was clear evidence that immobilized anti–CD3 induced neonatal T cell activation, proliferation, and IL-2 production, neonatal T cells were deficient in the capacity to function as efficient helper cells for B cell responses. Thus, anti–CD3 activated neonatal T cells induced proliferation of adult and neonatal B cells, but the responses induced by the neonatal T cells were less than those elicited by adult T cells. Both neonatal and adult $CD45RA^+$ T cells suppress adult lymphocyte responses to a variety of stimuli (6, 21, 24, 28). It seemed unlikely that suppressive influences could account for the limited capacity of neonatal T cells to provide help for B cells. In this regard, irradiation has been shown to prevent suppression of adult lymphocyte responses by PWM-stimulated neonatal T cells (8, 24). Moreover, suppression caused by anti–CD3-stimulated adult T cells is completely prevented by irradiation or mitomycin C treatment (48). In addition, the T cell populations were treated with leucine methyl ester to remove NK cells and were depleted of $CD8^+$ T cells to minimize suppression. Even after these maneuvers to limit suppression, neonatal T cells supported suboptimal B cell activation. Despite their limitations, it is clear, however, that anti–CD3-stimulated neonatal $CD4^+$ cells are capable of inducing contact-dependent B cell activation in the absence of other cell types, but do so less well than adult $CD4^+$ T cells.

The limited capacity of neonatal T cells to provide help for B cells was more apparent when production of Ig by B cells was examined and especially when the ability to provide help for neonatal B cell differentiation was assessed. This appears to reflect the increased helper cell signals required to promote Ig production by cord B cells. The diminished capacity of neonatal T cells to support Ig production by adult B cells appeared to be quantitative rather than qualitative as IgG and IgA were secreted in addition to IgM, albeit each in lesser amounts than secreted in cultures supported by adult T cells. Addition of adult TF to cultures of anti–CD3-activated cord T cells and adult B cells augmented ISC generation and the secretion of all Ig isotypes, suggesting that cytokine production by the neonatal T cells was limiting. Comparison of the ability of TF obtained from neonatal and adult T cells to support adult B cell Ig secretion confirmed the conclusion that deficient cytokine production by the neonatal T cells contributed to the submaximal support of B cell differentiation.

Previous reports indicate that $CD45RA^+$ adult T cells and cord T cells secrete IL-2 in response to a variety of stimuli (49–51). In the current studies, stimulation of neonatal T cells with immobilized anti–CD3 resulted in the production of IL-2, although the amount produced was slightly less than that produced by the adult T cells. Even though the cord T cell supernatants contained IL-2, they were markedly limited in the capacity to support Ig secretion by SA-stimulated adult B cells. Analysis of the cord T cell supernatants indicated that they were not suppressive and that the addition of IL-2 enhanced the responses of SA-stimulated adult B cells, but not to the levels noted with adult TF. These results indicate that the lesser amount of IL-2 in the neonatal T cell supernatants might contribute to their decreased capacity to support B cell responses, but could not entirely explain the deficiency. Cord blood and both adult human and murine naive T cells have been reported to be deficient in the ability to produce other cytokines, including IFN-γ, IL-3, and IL-4, that may play a role in supporting B cell responses (49, 50, 52–54). Although none of these cytokines alone promote Ig secretion by SA-stimulated B cells, each can enhance the secretion supported by IL-2 (40, 55, 56). Therefore, deficient production of one or more of these cytokines may contribute to the deficient support of B cell differentiation by the neonatal T cells.

Neonatal B cells can be induced to secrete Ig in response to SA and adult TF or EBV. However, neonatal B cell responses are deficient in both the quantity of Ig secreted and in the capacity to produce IgG and IgA. Previous results have suggested that neonatal B cells are limited in their capacity to differentiate into ISC and to switch to Ig isotypes other than IgM, although their capacity to differentiate in a system involving T cell–B cell collaboration has not been completely evaluated because PWM, the most frequently employed T cell–dependent B cell activator, is not an optimal stimulus for naive lymphocytes. Examination of the potential of neonatal B cells to collaborate with T cells indicated that they could be activated by anti–CD3-activated adult T cells. DNA synthesis of neonatal B cells induced by anti–CD3-activated adult T cells was comparable to that of adult B cells. Despite this clear-cut evidence of the capacity of neonatal B cells to receive activation signals from adult T cells, the generation of ISC and Ig secretion were much less than that observed for adult B cells. Although, preliminary analysis by limiting dilution suggests that 1 in 125 neonatal B cells can be induced to secrete immunoglobulin when supported by adult T cells in this system, this is still considerably less than the precursor frequencies obtained with adult B cells (26).

It should be noted that neonatal B cells responded more poorly to anti–CD3-activated T cells than either adult IgD^+ naive B cells or adult $CD5^+$ B cells, indicating that they exhibited diminished functional responsiveness beyond that predicted from their phenotype. Since anti–CD3-stimulated adult T cells supply all of the activation signals and cytokines necessary for the support of maximal adult B cell differentiation, addition of SA or adult TF does not usually augment the response. However, the addition of either SA or adult TF to cultures of neonatal B cells and anti–CD3-activated adult T cells enhanced the production of Ig, suggesting that differentiation of neonatal B cells requires additional signals and cytokines beyond that necessary for adult B cell differentiation. The exact nature of these signals and their role in T cell–B cell collaboration remains to be determined.

Previous results have suggested that neonatal B cells are limited in their capacity to secrete IgG and IgA. The current studies clearly indicate that neonatal B cells can be induced to produce IgG and IgA after stimulation with anti–CD3-activated adult T cells or anti–CD3-activated neonatal T cells and exogenous cytokines. The induction of IgG and IgA secretion from neonatal B cells that were uniformly IgM positive at the initiation of culture suggests that anti–CD3-stimulated T cells induced isotype switching. Induction of Ig isotype switch is also suggested by the delay in IgG and IgA production compared to IgM secretion. A significant increase in IgG and IgA, but not IgM, production between days 10 and 20 was appreciated in five experiments. Anti–CD3-activated T cells appear to be an important factor in inducing this putative isotype switch be-

cause neonatal B cells did not secrete IgG or IgA when stimulated with SA and TF in the presence of neonatal or adult T cells even when cultured for up to 20 d (data not shown). Thus, anti-CD3-activated T cells appear to provide a unique signal necessary for Ig isotype switching by a small population of neonatal B cells. The secretion of IgG and IgA was only observed when anti-CD3-activated neonatal lymphocyte cultures were supplemented with exogenous cytokines, and especially large concentrations of IL-2. Thus, one difference between adult and neonatal B cells may be the concentration of IL-2, and perhaps other cytokines, necessary to induce differentiation.

In summary, stimulation of neonatal lymphocytes with immobilized anti-CD3 has demonstrated unique functional patterns of both neonatal T cells and B cells that distinguish them from adult lymphocytes. The capacity of neonatal B cells to secrete IgM and also IgG and IgA after appropriate stimulation is greater than previously demonstrated and suggests that deficient antibody production of neonates may be overcome by provision of more optimal activation and differentiation stimuli.

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