might explain a proposed relationship between $PaCO_2$ and RLF (4).

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Response of Human Newborn Lymphocytes to Alloantigen: Lack of Evidence for Suppression Induction

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Summary

Between 1:120 and 1:180 of human newborn T cells proliferate in limiting dilution cultures with allogeneic lymphocytes or with Ia-bearing monocytic stimulator cells. The proliferating responder cells were derived from both the OKT 4^+ and OKT 8^+ subsets as determined by immunofluorescence and by thymidine uptake. Five to seven days after an exchange blood transfusion there was a slight increase in the percentage of OKT 8^+ T lymphocytes in the recipient's blood. Newborn blood also contains a population of non-T cells which proliferate in the absence of allogeneic stimulator cells. In limiting dilution cultures, the frequency of these spontaneously dividing cells was 1:3125 of mononuclear cells. Our results suggest that the newborn T lymphocyte proliferative response to alloantigen is mature by the time of birth and they provide no phenotypic explantion for the previous report of mixed lymphocyte culture-induced suppression by newborn T cells. The predominance of newborn metaphases in 2-way mixed lymphocyte cultures with adult cells (on which the previous report of suppression was based) is not seen if the non-T (stimulator) cells are irradiated. These results suggest that the data previously interpreted as evidence for suppression arose through proliferation of newborn non-T cells.

Abbreviations

AMoL, acute monocytic leukemia
HBSS, Hank's balanced salt solution

IL, interleukin
MLC, mixed lymphocyte culture
MNC, mononuclear cells
PPD, purified protein derivative

Factors that may contribute to the susceptibility of human newborns to severe Herpes virus infections include a deficiency of natural killer cells (1), a lack of maternal antibody (26), and, conceivably, the suppression of antigen specific responses by newborn T lymphocytes. Evidence for newborn T cell suppression comes principly from studies in which the mitogen response of adult lymphocytes is suppressed by addition of newborn lymphocytes (7). Examples include the predominance of newborn mitoses in mixed cultures of newborn and adult lymphocytes stimulated by phytohemagglutinin reported by Olding and Oldstone (20). Early onset in fetal life of this type of suppression and its postnatal persistence was reported recently by Unander and Olding (27). Our own (11) and Rodriguez *et al.* (22) results suggest that a subset of T lymphocytes which binds the OKT 8 monoclonal antibody is required for this suppresion. These studies did not exclude the possibility that suppression might develop primarily as a result of the mitogen activation of the newborn cells. Indeed, Tosato *et al.* (25) finding that newborn T cells did not suppress adult lymphocyte responses to Epstein-Barr virus unless a mitogen was added, and our own observation of relatively low numbers of cells with the OKT 8^+ suppressor phenotype in newborn blood (8) are consistent with the view that newborn T cells are not intrinsically suppressive, though they may become so as a result of mitogen activation. Lawler *et al.* (16)'s report of suppression of maternal T cell proliferation in 2-way MLC suggests that antigen stimulus may sometimes be sufficient to trigger suppression by newborn T cells. To explore this issue, we have re-examined the proliferative response of human newborn lymphocytes to an alloantigen stimulus using limiting dilution to determine responder cell frequencies and T-cell-subset specific antibodies to characterize the responder cell phenotype. We used responder frequency analysis because it provides a more quantitative estimate than the simple measurement of thymidine uptake in bulk cultures which, when previously applied to newborn responders, has given conflicting results (2, 3, 21). Other relevant observations include that of Asantila *et al.* (2), who showed that MLC responses by human fetal lymphocytes were immunologically specific, and Granberg *et al.* (6) who report that fewer cytotoxic T cells are generated in newborn than in adult cultures. Newborn non-T cells are known to have a high rate of spontaneous thymidine uptake (10), which could give an artifactual impression of suppression and might interfere with precursor frequency estimations by limiting dilution. To avoid confusion due to this activity we separated and irradiated the non-T cells, which were used as stimulator cells in MLCs. As an *in vivo* parallel to these studies, we investigated blood T cell subsets in newborns before and after alloantigen challenge in the form of exchange transfusion for rhesus isoimmunization.

MATERIALS AND METHODS

Blood was obtained from healthy adults or from the placentae of healthy newborns and was either defibrinated or anticoagulated with preservative-free heparin (9). For the exchange transfusion studies 5–7 ml of blood was obtained from the first syringe of blood withdrawn from the umbilical vein at the start of the transfusion and a further 2–3 ml was obtained from a peripheral vein 5–7 d later. MNC were separated on Ficoll-Hypaque gradients as before (10); the serum of healthy adults was retained for culture supplementation. Cells were routinely washed in HBSS buffered with 0.01 M HEPES and they were cultured in bicarbonate-buffered RPMI 1640 medium with 10% human serum and 10 μg/ml gentamicin (complete medium).

Stimulator cells for MLC. Mononuclear cells in HBSS were irradiated to 3000r in 2 min from a cobalt source, then washed once and resuspended at 10^6 per ml in complete medium. Pooled stimulator cells for the limiting dilution cultures were prepared by mixing mononuclear cells from four donors and depleting T cells by E-rosetting as previously described (17). These preparations contained fewer than 5% of cells which stained with OKT 3. The non-T cells were washed and resuspended at 10^8 cells in 1-ml aliquots of complete medium and were supplemented with 10% dimethyl sulfoxide before controlled freezing to −70°C. Aliquots were thawed at 37°C as required, then washed, irradiated, and resuspended in complete medium.

The AMoL line cells used as stimulators in certain experiments were originally cultured from the bone marrow of a patient with AMoL. These cells are Ia antigen positive and have the histochemical characteristics of AMoL cells: they are negative for T or B lymphocyte surface antigens.

Limiting dilution cultures for precursor cell frequency. These cultures were in U-bottom Linbro 76–013–05 plates. For MLC responser frequencies, each well contained 5×10^4 stimulator cells and the desired number of responder cells in 0.1 ml of complete medium. Wells were pulsed for 6 h on the 9th d of culture [this was the optimal duration of culture found by van Oers *et al.* (19) and in our own experience] with 0.25 μCi tritiated thymidine (TRA 61, 5 Ci/mmol, Radiochemical Centre, Amersham) and then harvested onto glass fiber discs. Background counts were determined from unstimulated wells containing responder cells only and their log mean + 3 SD calculated. Experimental wells with greater cpm than the background + 3 SD were counted as responder wells as described (13).

The frequency of newborn mononuclear cells that took up thymidine in the absence of exogenous stimulation was similarly determined by limiting dilution except that the cultures contained no stimulator cells and they were pulsed for 6 h on the 6th d of culture. The backbround controls for these cultures were E-rosette depleted newborn cells which were recombined with irradiated non-T cells.

Time course of spontaneous thymidine uptake by newborn MNC. Triplicate wells, each containing 2×10^5 newborn MNC in 0.2 ml RPMI 1640 with 1% human serum, were cultured as above and harvested each 24 h after a 4-h pulse with 1 μCi tritiated thymidine.

MLC cultures for responder cell phenotype by immunofluorescence. Single donor stimulator cells, 5×10^6, were cultured with 5×10^6 responser cells in complete medium in a Falcon 2057 tube for 6 d. The cultures were then spun down and resuspended in 60% Percoll in HBSS and overlaid with 52, 40, and 30% Percoll in HBSS. These gradients were centrifuged at 800 *g* for 10 min, then the blast-enriched fraction was recovered from the 40–52% interface. These cells were stained with monoclonal antibodies whose specificity for T cell subsets has been reviewed (15). A fluorescein-conjugated goat anti-mouse IgG (Cappel Labs) was used for a 2-stage immunofluorescence technique (8).

MLC cultures for responder cell phenotype by thymidine uptake. Stimulator and responder cells were cultured in 10 ml complete medium in Falcon 2057 tubes as above. On the 6th d of culture, 9 ml of medium was aspirated from the tube and 5 μCi of tritiated thymidine was added to the remaining 1 ml. The cells were resuspended and incubation was continued for 2 h. Two milliliters of Ficoll-Hypaque was then run under the cells and the tube was centrifuged for 10 min at 600 *g*. The cells at the interface were aspirated, washed twice, and then resuspended in 10 μL of OKT 3, 4, or 8 or a medium control. All washing and subsequent processing of the cells was in HBSS, supplemented with 0.1% sodium azide and 0.1% bovine serum albumin. After 30 min on ice the cells were washed three times and resuspended in 120 μL of HBSS. Twenty microliters of this suspension was counted on a Coulter Counter. Between 1 and 2 $\times 10^5$ cells were then added to a Falcon Microtest III (Cat # 3912) microwell pre-coated with goat anti-mouse IgG. These plates were prepared by preactivating the plastic with 0.1% glutaraldehyde, washing, and then coating with 200 μg/ml goat

anti-mouse IgG (Cappel Labs) in bicarbonate buffer, pH 9, for at least 24 h. The wells were washed extensively with HBSS immediately before adding the cells. Incubation of the cells in the wells was for 30 min at 37°, then the non-adherent cells were washed out with a continuous stream of cold HBSS. The wells were shaken dry and the adherent cells were lysed with 0.1 ml of 1% sodium dodecyl sulfate. The plates were frozen to facilitate cutting them up and transferring the wells to liquid scintillation vials. The wells and cell lysates were counted in 2.5 ml of Instagel (Packard). The results, in cpm, were divided by the number of cells $\times 10^{-5}$ added per well to give a corrected cpm figure.

Blood T cell phenotype after exchange transfusion. T cell subsets were determined by counting MNC stained with monoclonal antibodies as described previously (8). The sex of the cells was determined by quinacrine staining of metaphase spreads from 3-d phytohemagglutinin-stimulated cultures using standard methods (26). The karyotypes were examined for fluorescent Y chromosomes in metaphase spreads and for Y bodies in interphase nuclei.

Characterization of source of dividing cells in newborn-adult MLC. Newborn and adult MNC were obtained from donors of different sex and 5×10^6 cells of each were cultured in 2-way MLC. The remaining cells were separated into T- and non-T cells by E-rosetting (10). The non-T cells of each were then divided into two aliquots, one of which was irradiated to 3000r. The following combinations of cells were then cultured:

#1: 5×10^6 unseparated newborn cells + 5×10^6 unseparated adult cells

#2: 4×10^6 newborn T-cells, 4×10^6 adult T-cells; 2×10^6 newborn non-T-cells, 2×10^6 adult non-T-cells.

#3: As #2 except that the non-T-cells were irradiated to 3000r.

Colcemid was added for 90 min on the 6th d of culture and metaphase spreads were prepared as above for quinacrine staining. Only satisfactorily spread metaphases were examined and at least 26 per culture combination were scored for the presence or absence of a fluorescent Y chromosome.

RESULTS

Frequency of proliferating responder cells. Newborn and adult blood MNC were cultured in limiting dilution in 24–48 replicates with 100–800 responder cells per well. Either T-depleted lymphocytes prepared from a pool of four donors or an Ia antigen positive line of AMoL cells were irradiated and used as stimulator cells. Responder wells were defined as those in which thymidine uptake was greater than the mean + 3 SD of unstimulated control wells. The data were plotted as percentage non-responder wells (on a log axis) against the number of responder cells per well (Fig. 1) and the relationship was linear. The responder cell frequency is interpolated at the 37% non-responder level and is 1:180 for adult and newborn cells using the pooled stimulator cells and 1:120–1:150 using the AMoL stimulators.

Lack of response by newborn cells to soluble antigens was confirmed in limiting dilution using tetanous toxoid and PPD as antigens. E-rosette separated newborn T-cells were cultured at 500–50,000 cells per well with 100,000 irradiated autologous MNC as antigen presenting cells. Approximately 8% of wells had thymidine uptake above background in either the presence or the absence of antigen so no significant frequency of antigen specific precursors could be demonstrated.

Phenotype of responder cells in MLC. The phenotype of MLC responder cells was determined either by separating the blast cells from 6-d MLC on Percoll density gradients and staining them by immunofluorescence or by adhering thymidine pulsed responder cells to plastic microwells with monoclonal antibodies.

Cells with the phase contrast morphology of blasts and staining with either OKT 4 or OKT 8 were recovered from all the 1-way newborn and adult MLC (Table 1). The newborn responder populations had slightly fewer OKT 8^+ cells than the adult responders, but this difference is not significant and was no

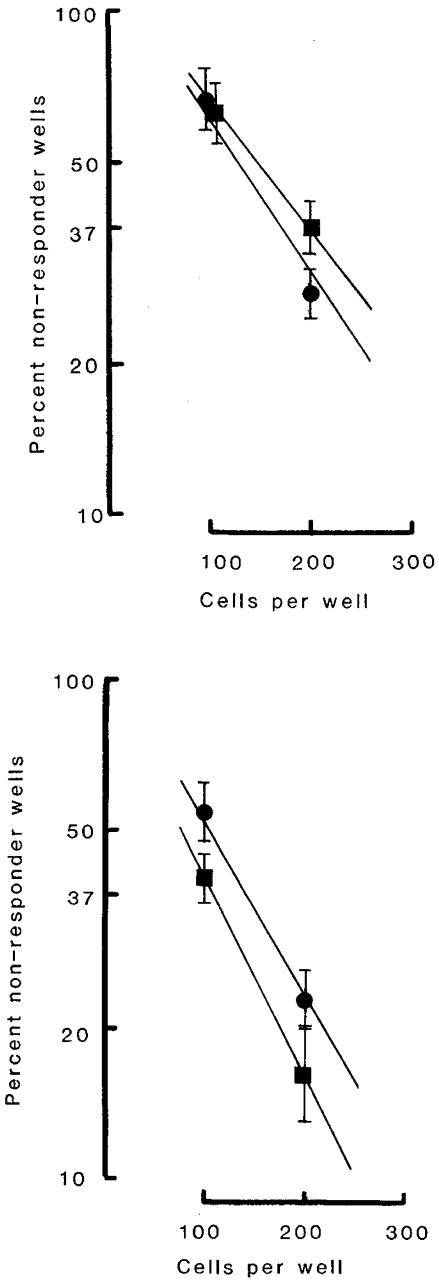

Fig. 1. Limiting dilution responder cell frequency analysis of human newborn (●) and adult (■) blood lymphocytes stimulated by irradiated T-depleted cells (*upper panel*) or AMoL cells (*lower panel*). Results are the mean ± SD for percentage non-responder wells for five samples each on newborn and adult blood.

Table 1. *T cell phenotype of newborn or adult T cell blasts recovered from mixed lymphocyte culture**

	Percentage of T cells stained with:	
Responders (n)	OKT 4	OKT 8
Newborn (9)	49 ± 3	50 ± 3
Adult (11)	46 ± 3	53 ± 4

* Newborn or adult mononuclear cells, 5×10^6, were cultured for 6 d with 5×10^6 single-donor 3000r-irradiated stimulators after which the blast cells were separated on a discontinuous Percoll gradient. The blast-enriched cells were phenotyped by immunofluorescence. The sum of percentage OKT 4^+ and OKT 8^+ cells was 92 ± 3. The proportion of each subset is expressed as a percentage of the sum of the OKT 4 + OKT 8 positive cells; n, number of responders studied.

greater than the difference in percentage OKT 8^+ cells of the newborn and adult cells originally put into culture.

To obtain more secure evidence that the cells with the buoyant density and phase contrast morphology of blasts were indeed dividing, we devised an additional measurement of responder cell phenotype. After 6 d incubation the MLC were pulsed with tritiated thymidine, then the viable cells were harvested from a Ficoll-Hypaque gradient and incubated with monoclonal antibodies. The antibody-coated cells were then recovered by their adherence to plastic microwells pre-coated with goat anti-mouse IgG. Non-adherent cells were removed by rinsing the wells and the radioactivity of the adhered cells was measured by liquid scintillation counting. The counts were corrected for the number of cells added to each well. This technique permits the phenotyping of a responder population without any preliminary separation of T cell subsets. The results (Table 2) indicate that both OKT 4^+ and OKT 8^+ cells proliferate in MLC whether the responder cells are newborn or adult, though there are slightly lower counts in the OKT 8^+ fraction in newborns.

Table 2. *Thymidine uptake by T cell subsets activated in mixed lymphocyte culture**

	Counts in cells adhered through:		
Responders (n)	OKT 4	OKT 8	4:8 ratio
Newborn (5)	1433 ± 160	384 ± 18	3.7
Adult (5)	1073 ± 314	764 ± 203	1.6

* Newborn or adult cells, 5×10^6, were cultured for 6 d with 5×10^6 3000r-irradiated single-donor stimulator cells. The cultures were pulsed for 2 h with tritiated thymidine, then incubated with OKT 4 or OKT 8 or control. Antibody coated cells adhered to microwells which were then rinsed free of nonadherent cells before counting on a liquid scintillation counter. Results are expressed as cpm/10^5 cells added to each well after subtraction with the nonantibody treated control cpm. The mean cpm in the latter controls was 96 ± 20. The *P* value for the difference in OKT 8^+ counts between newborn and adult responders is 0.05 for a two-tailed test; n, number of responders studied.

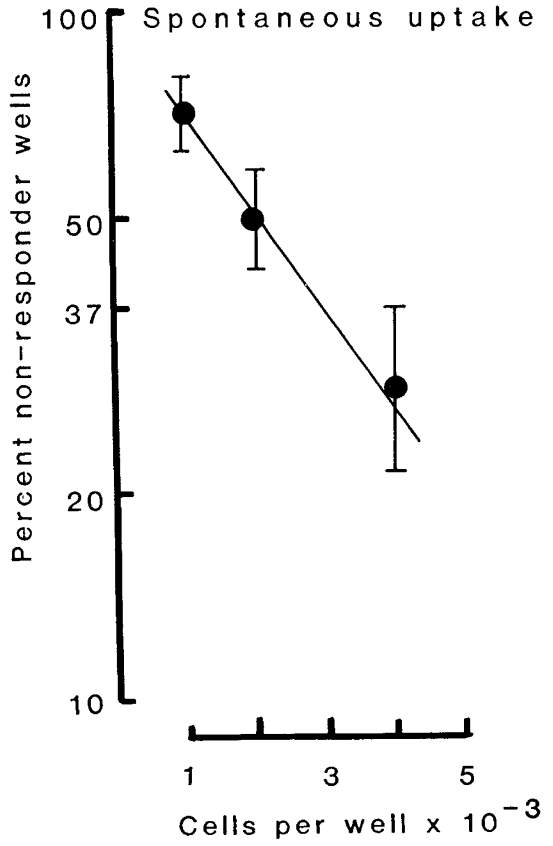

Fig. 2. Limiting dilution analysis of spontaneous thymidine uptake by newborn blood mononuclear cells. Results are the mean ± 1 SD for percentage non-responder wells for three samples

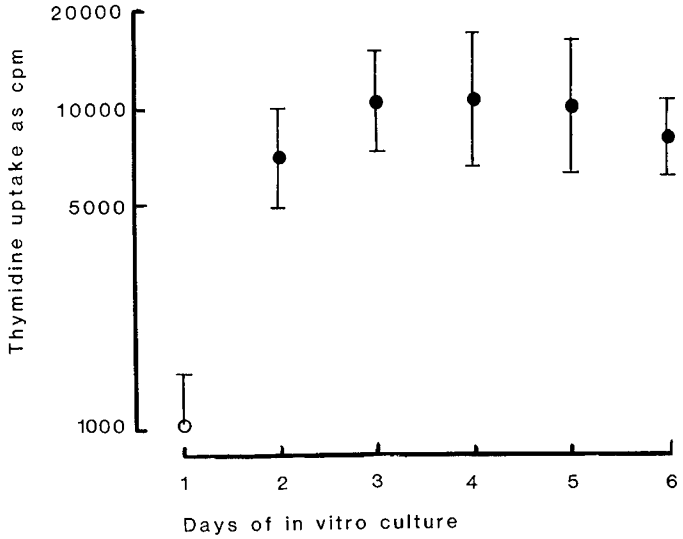

Fig. 3. Time course of spontaneous thymidine uptake by newborn mononuclear cells during *in vitro* culture. Results are the log mean ± SD for five samples of newborn blood.

Spontaneous thymidine uptake by newborn non-T cells. Spontaneous thymidine uptake by newborn MNC was detectable on the 1st d of culture and increased during the subsequent 4–5 d, after which it fell (Fig. 2). This property of newborn cells could interfere with an assessment of the newborn response in measurements involving thymidine uptake or karyotype analysis. we therefore determined the frequency of cells with spontaneous thymidine uptake in limiting dilution cultures of newborn and adult mononuclear cells. The results shown (Fig. 3) are from 6-d limiting dilution cultures as the responder frequency diminished after this time, in keeping with the time course found above. There is a linear relationship on the Poisson plot with about 1:3125 newborn MNC proliferating as judged by thymidine uptake. Insufficient wells containing adult unstimulated MNC incorporated tritiated thymidine to derive a precursor frequency.

Blood lymphocyte T cell subsets after exchange blood transfusion. Newborns with severe hyperbilirubinemia, such as follows rhesus isoimmunization, are treated by exchange blood transfusion. The donor blood contains lymphocytes and stimulates an immunologic response. As a parallel to our *in vitro* studies we determined blood T cell subsets by immunofluorescence at the start of exchange blood transfusion and 5–7 d later. This interval was selected on the basis of the Schechter *et al.* (23) finding of peak unstimulated thymidine incorporation by blood lymphocytes at this time. Our results (Table 3) indicate that the percentage of T cells in the MNC preparations rose after blood transfusion and that this was accompanied by a slight but consistent increase in the proportion of OKT 8^+ cells. It is difficult to obtain suitable controls for the hyperbilirubinemic patients, but leukocyte-free plasma infusions in two babies with hyperviscosity did not induce comparable increases in OKT 8^+ cells. A possible explanation for the changes in blood lymphocyte populations after exchange transfusion was that the donor lymphocytes persisted. We tested this possibility by examining metaphase spreads and interphase nuclei of phytohemagglutinin-stimulated blood lymphocytes from female babies transfused with male blood for fluorescent Y chromosome or Y bodies in

Table 3. *Blood T lymphocyte subsets in infants after exchange transfusion**

	Percentage cells stained for:			
	OKT 3	OKT 4	OKT 8	4/8 ratio
Pretransfusion	65.8 ± 6	52.2 ± 5	13.4 ± 3	3.9
Posttransfusion	76 ± 4	57.2 ± 4	19.4 ± 1	2.9
Hyperviscosity	78	64	16	4

* Five samples of lymphocytes from umbilical vein blood obtained at the start of exchange blood transfusion for rhesus isoimmunization, and from peripheral vein blood 5–7 d later, were characterized by immunofluorescence with monoclonal antibodies. Mean post-transfusion values from two comparable samples from infants receiving plasma exchanges for hyperviscosity are shown. The *P* value for the increase in percentage of OKT 3^+ and OKT 8^+ cells in the infants receiving blood is 0.05 for a two-tailed test.

Table 4. *Origin of dividing cells in newborn-adult mixed lymphocyte cultures as determined by karyotype.*

	Culture combination*					
	Unseparated		Separated		Irradiated, separated	
Expt.	†A	N	A	N	A	N
1	9	33	16	36	32	16
2	4	26	10	22	14	16
3	4	32	12	26	20	25
4	6	22	8	23	14	12
Mean % =	17	83	30	70	54	46

* In unseparated cultures, the adult and newborn mononuclear cells were cultured without preliminary separation into T- and non-T cells. The separated cultures comprised 4×10^6 each of newborn and adult T-cells separated by E-rosetting and 2×10^6 each of newborn and adult non-T cells. The irradiated separated cultures were identical except that the non-T cells were irradiated with 3000r before culture. All cultures were processed for metaphase spreads and the source of the dividing cell determined by the presence or absence of a fluorescent Y chromosome. The newborn was male in expts. 1–3 and female in expt. 4.

† A, adult and N, newborn where the numbers indicate the actual number of metaphases counted.

quinacrine-stained preparations. No lymphocytes of donor origin were found.

Origin of dividing cells in newborn-adult 2-way MLC. Our phenotypic results did not suggest that suppression was responsible for the previously reported predominance of newborn's mitoses in mixed newborn-adult 2-way MLC's (16). Newborn mononuclear cells are known to have a high rate of spontaneous thymidine uptake, and the cells responsible for this are in the non-T population. To test the possibility that division by newborn non-T cells might give an appearance of suppression, we compared the sex of dividing cells where the stimulator (non-T) cells were or were not irradiated. The responder cells in these cultures were mixtures of newborn and adult T cells isolated by E-rosetting. Unseparated cultures were included for comparison with Lawler *et al.* (16) results. Our results (Table 4) show that mitoses of the newborn karyotype predominate in 2-way MLC in which the stimulator cells are not irradiated. When the stimulator cells are irradiated, before addition to the culture, there is no predominance of newborn mitoses.

DISCUSSION

We (12) and others (21) have previously shown that human fetal lymphocytes can respond to MLC by mid-gestation. Asantila *et al.* (2) showed that this response had immunologic specificity. Although there is some disagreement in the literature as to whether human newborn's lymphocytes respond better (4) or less well (3) than adult cells in MLC, our present results suggest that the two populations have very similar precursor frequencies of cells which proliferate in MLC. Our own estimate of 1:150 responders in healthy adults is close to the 1:200–1:600 estimated by van Oers *et al.* (19) and to the 1:240–1:468 estimated by Singal (24). Accurate estimation of responder cell frequencies in man is made difficult by uncertainty as to the number of antigenic differences between the stimulator and responder. There is also a potential for error through recognition of the responder cells by T cells in the irradiated stimulator population or, where pooled stimulator cells are used, mutual recognition by T cells in the stimulator population. Either of these recognition events could result in IL 2 release by the stimulator cells and maintenance of proliferation by any pre-activated T cells which happened to be present in the responder population at the initiation of the cultures. We, and van Oers *et al.*, used T-depleted stimulator cells to avoid this type of error. As an additional check we determined the responder cell frequencies of newborn and adult cells to an irradiated AMoL cell line because these cells do not make IL 2. We obtained essentially similar responder cell frequencies with both types of stimulating cell, so it seems unlikely that IL 2 release by irradiated cells interfered with our results. The pooled stimulators we used were derived from four donors and so would be expected to be antigenetically more diverse than the AMoL cells and so give a higher responder cell frequency. Indeed, van Oers *et al.* (19) found that the MLC responder cell frequency was as high as 1:26 when the stimulator cells were prepared from a large pool of donors. It seems likely that other aspects of stimulator cell behavior contribute to the responder cell frequency which is observed. IL 1, for example, is required for a T cell response, and at low responder cell densities this IL 1 most likely comes from the stimulator cells. The AMoL cell line we used bears HLA-DR 4 and 7 and makes IL 1 constitutively (Giller *et al.*, in preparation), so it may be a more effective stimulator than T-depleted blood lymphocytes.

Our studies of adult MLC responder cell phenotypes by immunofluorescent staining of blasts resemble those of Engleman *et al.* (5), and they suggest that cells with both helper-inducer (OKT 4) and suppressor-cytotoxic (OKT 8) activity proliferate. This view is supported by our observation that both OKT 4^+ and OKT 8^+ cells recovered from MLC had incorporated tritiated thymidine. There was little difference between the responder cell phenotype of newborn and adult lymphocytes in MLC although the newborn cells tended to have less response in the OKT 8^+ subset than the adults. This difference might arise simply as a result of the lower frequency of OKT 8^+ cells in newborn blood (8). Our results give no phenotypic evidence for the generation of suppression in newborn T cell populations as a result of MLC stimulation. This extrapolation from phenotypic observations to function is based on our own (11) and Rodriguez *et al.* (22) functional studies in which newborn suppressor T cells did indeed bear the OKT 8^+ phenotype, though it should be remembered that OKT 8 binds to suppressor as well as cytotoxic T cells. The report by Lawler *et al.* (16) that newborn T cells were stimulated to become suppressor cells in MLC was based on their finding a higher frequency of dividing newborn cells (by karyotype analysis) in 2-way newborn-adult MLC and not on any conventional assay for suppression. We considered that their result might be due to proliferation by newborn non-T cells rather than suppression of adult T cells. Our finding that the predominance of newborn mitoses in 2-way adult-newborn MLC disappeared when the stimulator (non-T) cells were irradiated supports this view. The predominance of newborn metaphases in the control-separated (but unirradiated) 2-way MLC is less than in the unseparated cultures (Table 4). Although this result could suggest that a newborn suppressor cell had been lost during the E-rosetting procedure, we consider it more likely that the change results from an increased frequency of dividing adult T cells as a result of the separation procedure. An increase is to be

expected because the adult cells were exposed to fetal calf serum and sheep ethryocytes during the rosetting: this is known to activate adult T cells (14) or stimulate an autologous response (18). Both the 6-d peak (Fig. 2) and the frequency estimate (Fig. 3) of spontaneous thymidine uptake by newborn non-T cells suggest that division by these cells is responsible for predominance of newborn mitoses in unseparated 2-way newborn-adult MLC. Studies currently in progress indicate that the newborn non-T cells which incorporate thymidine lack T cell antigen (OKT 3, 4, 8) but are positive for surface Ia antigens. As would be predicted from our interpretation, Lawler *et al.* (16) did not find an excess of mitoses of one or other sex when newborn-newborn 2-way MLC were studied.

Our original finding that the percentage of OKT 8^+ cells was lower in newborn than adult blood (8) led us to speculate that antigen stimulus might normally play a role in the post natal expansion of the OKT 8^+ subset. It is difficult to test this possibility in *in vitro* cultures because little or no increase in thymidine uptake occurs in cultures of newborn's lymphocytes stimulated with soluble antigens. Our cultures with PPD under limiting dilution conditions were undertaken primarily to contrast with the positive responses to alloantigen, but they illustrate the difficulty in obtaining responses to microbial antigens by healthy newborn's lymphocytes. The increase in OKT 8^+ cells we found 1 wk after exchange blood transfusion was small. It could result from the alloantigen stimulus of the transfused lymphocytes although this is not securely established. Certainly the timing of the response would be compatible with the peak of spontaneous thymidine uptake by blood lymphocytes after transfusion described by Schechter *et al.* (23). None of the cells we recovered in the second sample had the karyotype of donor cells. Because graft *versus* host disease is exceptionally rare following exchange blood transfusion, it seems likely that newborn (and fetal) recipients of blood transfusions have some mechanism for eliminating donor cells. Our results, taken with previous demonstrations of specific cytotoxicity, are consistent with the view that the newborn response to alloantigen is mature before birth.

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28. Informed consent was obtained from the individuals who donated blood for this research.
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