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Research Article

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Characterization of the Suppressor Cell Activity in Human Cord Blood Lymphocytes

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ABSTRACT Newborn mononuclear cells are known to have increased suppressor activity when compared with adult cells. However, the precise phenotypic description of the suppressor cell subpopulation has not yet been reported. This study was designed to examine the surface markers on human cord blood cells as defined by monoclonal antibodies and to characterize cord blood mononuclear cells that suppress in vitro pokeweed mitogen-driven immunoglobulin production. The pattern of fluorescence with the 9,6-monoclonal antibody suggested either a decreased density or a partial blocking of E-receptors on cord blood lymphocytes. Otherwise, human cord and adult cells had similar proportions of T cell subpopulations. Different subsets of newborn cells isolated by a monoclonal antibody rosetting technique were tested for suppressor activity. Cord blood lymphocytes recognized by the OKT8 monoclonal antibody constituted the major functional suppressor cell subpopulation. These cells were present in both E-rosetting and non-E-rosetting populations. Removal of OKT8+ mononuclear cord blood cells abrogated most of the suppressor activity. Thus the major suppressor cell present in cord blood is an OKT8 positive lymphocyte.

INTRODUCTION

Human cord blood lymphocytes have been shown to include suppressor cells for in vitro polyclonal immunoglobulin production (1–10). However, the precise phenotypic definition of suppressor cells in cord blood has not yet been accomplished. For example, the suppressor cell has been identified by its ability to form sheep erythrocyte rosettes (E rosettes) (1–2, 11), its radiosensitivity (3–5, 7, 9–12) or by the presence of an Fc-receptor for IgG or IgM. The lack of clear phenotypic data concerning cord blood suppressor cells can be illustrated by reports of enrichment of suppressor activity in the Tγ (13), T-non-γ (4, 7) or even in both subpopulations (9). More recently, Hayward et al. (8) have also used monoclonal antibodies to further delineate the surface markers in human cord blood lymphocytes. The development of these antibodies recognizing specific markers in different subpopulations of lymphocytes has enabled a more precise phenotypic delineation of many functional subsets. Several monoclonal antibodies are available that identify common T cell antigens, OKT3 and OKT11 (14, 15); cytotoxic/suppressor T cells; OKT8 (16); inducer/helper T cells, OKT4 (17); antigens related to myelomonocyte origin, OKM1 (18); and a lymphocyte surface protein associated with the E-receptor termed 9.6 (19). A monoclonal antibody, 63D3, reacting with ~80% of adherent mononuclear cells has also been developed (20).

In this report we present a detailed analysis of the phenotypic profile of the surface markers present on cord blood mononuclear cells (CBC).[1] Furthermore, we also identify the major cord blood suppressor cell activity for pokeweed mitogen-induced immunoglobulin production in the OKT8+ subpopulation.

METHODS

Preparation of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood of healthy adult donors (ages 25 to 40 yr) on Ficoll-Hypaque gradients (21), washed two times in Hanks' balanced salt solution and incubated at 37°C for 45 min on glass petri dishes in RPMI 1640 with 20% fetal calf serum to remove adherent cells. After three additional washes in RPMI 1640, PBMC were then resuspended to 1×10^6 cells/ml in complete media (RPMI 1640 supplemented with 2 mM/ml L-glutamine, 200 IU/ml penicillin, 100 μg/ml streptomycin and 10% fetal calf serum).

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[1] *Abbreviations used in this paper*: CBC, human cord blood cells; PBMC, adult peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PWM, pokeweed mitogen; SRBC, sheep erythrocytes.

Fresh heparinized cord blood was obtained immediately after routine delivery from normal uncomplicated pregnancies. CBC were isolated and prepared as described above for PBMC. With the use of peroxidase stain the glass nonadherent cord cell population generally contained ~80% lymphocytes and 20% monocytes.

E-rosetting cord blood cells were obtained by incubation with neuraminidase-treated sheep erythrocytes (SRBC) followed by centrifugation over Ficoll-Hypaque for 30 min at 300 *g*. Rosetted cells contained an average of 95% E-rosette positive cells recovered before lysis of SRBC with Tris-NH_4Cl buffer. In some instances monocytes were depleted from PBMC by passage of the adult or cord blood cell suspensions through a Sephadex G-10 column as previously described (22).

Monoclonal antibodies. Monoclonal mouse hybridoma were obtained from Ortho Pharmaceutical Corporation (Raritan, N. J.). For characterization of the surface markers by immunofluorescence analysis the following antibodies were used: OKT3, OKT4, OKT8, OKM1, and OKT11. Monoclonal antibody 9.6 was purchased from New England Nuclear (Boston, Mass.). In addition, an antibody specific for human monocytes and macrophages, 63D3 (19), kindly donated by Dr. D. Capra (Department of Microbiology, Southwestern University, Dallas, Tex.), was used for monocyte enrichment or depletion in some experiments.

Immunofluorescence analysis of cord blood mononuclear cells. A cell pellet containing either 10^6 E-rosette positive cells or Sephadex G-10 nonadherent CBC was incubated with 50 μl of mouse monoclonal antibody (2.5–5 μg/ml) for 30 min on ice. The optimal dilutions of the monoclonal antibodies had been previously determined. The cells were washed twice in a refrigerated centrifuge with phosphate-buffered saline (PBS) pH 7.4 containing 0.01% sodium azide.

The pellet was further incubated for 30 min on ice with 50 μl of 1/10 dilution of fluorescein conjugated goat anti-mouse IgG (Meloy Laboratories, Springfield, Va.), previously adsorbed with human Cohn Fraction II insolubilized on Sepharose 4B. The cell pellet was washed twice, resuspended in 1 ml PBS-azide and analyzed with a fluorescence activated cell sorter (FACS III, Becton, Dickinson & Co., Inc., Sunnyvale, Calif.). The electronic amplifier and gain settings were such that the majority of cells in the control sample were in fluorescence channels 1 to 20. To exclude any binding of monoclonal antibodies to Fc receptors, several controls were included. These controls consisted of cells incubated with OKT6 (which reacts with thymocytes but not peripheral lymphocytes) (14) or mouse IgG and the second labeled antibody. Mouse IgG was isolated on D Sepharose 4B Protein A column. The number of positive cells reactive with these reagents was subtracted from the proportion of positive cells detected by the other monoclonal antibodies. The background reactivity detected by these controls never exceeded 1.0%.

Separation of cord blood subpopulations. Either glass-nonadherent cells or E rosette-positive lymphocytes were fractionated into different subpopulations by means of a monoclonal antibody rosetting technique (23). Briefly, a pellet of $10–20 \times 10^6$ cord cells was incubated at 4°C with 100 μl of the corresponding monoclonal antibody at a concentration of 2.5–5 μg/ml and washed twice in cold Hanks' balanced salt solution at 4°C. The cells were resuspended to $2–4 \times 10^6$/ml in complete media and mixed with an equal volume of 0.5% suspension of ox erythrocytes coated with purified goat anti-mouse IgG (Tago, Burlingame, Calif.). The goat antibody was coupled to ox erythrocytes by the $CrCl_3$ method. The mixture was centrifuged for 10 min at 200 *g*; incubated for 30 min at 4°C, and gently resuspended. Rosetting cells (more than three erythrocytes per lymphocyte) were counted to determine the relative proportion of positive cells in the sample and then separated from nonrosetting cells by centrifugation over Ficoll-Hypaque for 30 min at 300 *g*. The interface layer (nonrosetting population) was recovered and washed three times in RPMI 1640 before resuspension at 1×10^6 cells/ml in complete media. The purity of the negative selected fraction was tested by immunofluorescence analysis on the FACS with the same antibody used for the depletion. These fractions always contained <5% residual contaminating cells (2.6±1.8%, mean±SE). Positive fractions were tested by counting the number of rosettes in the pellet (81.4±4.2%, mean±SE). Ox erythrocytes were lysed with Tris-NH_4Cl after 5 min. The rosetting cells were then washed and resuspended as above. Viability of both fractions was >95% as tested by trypan blue dye exclusion.

Because monocyte contamination might be expected to influence functional studies, an attempt was made to study the nonadherent cells remaining after the adherence preparation step using the 63D3 monoclonal antibody, which recognizes antigens unique for blood monocytes, in parallel with peroxidase staining. The rosetting technique showed an average of 22±8% monocytes remaining even after glass adherence depletion; peroxidase staining indicated 17±3% positive residual cells.

Lymphocyte cultures. The cells in complete media were cultured in 12 × 75-mm plastic capped tubes (Falcon 2054, Becton, Dickinson & Co., Inc.) at 37°C, 5% CO_2 in a humidified incubator. In each experiment a culture with 1×10^6 fresh adult PBMC was set up to measure the base-line production of immunoglobulins.

Co-culture experiments were carried out by mixing 1×10^6 adult PBMC with 10, 30, or 50% of either unfractionated CBC or the specifically isolated lymphocyte subpopulations. In a simultaneous set of cultures 10, 30, or 50% of autologous adult PBMC were added as controls. Also 5×10^5 unseparated CBC or the different subpopulations corresponding to the maximal amount of cord cells added in the coculture experiments were cultured alone. Pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.) was added at the optimal dilution of 1:300 (1 μg/culture). The same lot (A495109) was used in all the experiments. The final volume per culture was 1.5 ml. The cultures were terminated by centrifugation on the 7th d and the supernatants harvested and stored at 4°C before testing.

Enzyme linked immunosorbant assay (ELISA) for measurement of IgG and IgM in the supernatants. Flat-bottom flexible microtitre plates (Cooke Laboratory Products Div., Laboratories, Inc., Alexandria, Va.) were used for the Elisa technique. 100 μl of purified IgG or IgM (20 μm/ml) in PBS was placed in the wells. Plates were incubated 1 h at 37°C and 16 h at 4°C. The wells were then washed once in PBS/Tween buffer 0.05% (Tween 20, Atlas Chemical Industries, Inc., Wilmington, Del.) and twice in PBS before being dipped in PBS/gelatin 0.3% for 2 h. The wells were washed a second time in PBS/Tween and PBS. A standard curve from 10 μg to 10 ng was prepared with purified IgG or IgM in complete media. 50 μl of either supernatant or standard plus 50 μl of a 1:1,000 dilution of peroxidase-labeled, goat anti-human IgG or anti-human IgM (N. L. Cappel Laboratories, Cochraneville, Pa.) were added to the wells and the plates were incubated 30 min at 37°C and 2 h at 4°C. Each experimental culture was tested in triplicate. After further washes with PBS/Tween and PBS, 100 μl of the substrate prepared with citric acid 3.2 g%, pH 4 (Eastman Kodak Co., Rochester, N. Y.), hydrogen peroxide (1/100) (J.T. Baker Co., Phillipsburg, N. J.) and 2.2¹-azino-Di-[3-äthyl-benzthiazolin-sulfonat (6)] (Boehringer & Sohne, Mannheim, West Germany), was placed in the wells

TABLE I
*Surface Markers on Adult and Cord Blood Cells**

	OKT3	OKT11	9.6	OKT4	OKT8	OKM1
E-rosetting cord blood cells	72 (±10)‡	ND	98 (±1)	53 (±7)	28 (±8)	29 (±9)
E-rosetting adult cells	88 (±6)‡	ND	96 (±2)	53 (±8)	34 (±8)	20 (±5)
Sephadex G-10 nonadherent CBC	70 (±15)§	76 (±13)	79 (±11)	52 (±13)	29 (±8)	24 (±11)
Sephadex G-10 nonadherent adult cells	71 (±4)§	79 (±2)	82 (±2)	43 (±8)	32 (±9)	36 (±12)

* Either fraction was treated with the corresponding monoclonal antibody and the percentage of stained (positive cells) was determined by FACS analysis.
‡ Means and SD of five experiments.
§ Means and SD of six experiments.

and the reaction stopped after 30 min with citric acid buffer, pH 2.8. The plates were read in a Microelisa Autoreader MR580 (Dynatech Lab, Inc., Alexandria, Va.) and optical densities translated to nanograms by plotting from the standard curve. Results were expressed either as nanograms per milliliter culture or as a percentage of inhibition (percentage of suppression) caused by CBC in the basal production of immunoglobulins by 1 million per control adult PBMC.

Human IgG was purified from human gammaglobulin Fraction II (Pentex Biochemical Co., Kankakee, Ill.) by DEAE chromatography. Human IgM was purified from pooled plasma obtained from five patients with Waldenstroms macroglobulinemia using an euglobulin precipitation method and DEAE chromatography (3). The purity of the immunoglobulin was tested by conventional immunodiffusion techniques.

Statistical analysis. Statistical significance was determined by using the unpaired Student *t* test.

RESULTS

Surface markers present on CBC and adult PBMC. FACS analyses were performed on both Sephadex G-10 nonadherent and E rosette-positive cells. Table I shows the relative proportions of each subpopulation in both cord blood and adult T lymphocytes isolated by the E-rosetting or in lymphocytes depleted of adherent monocytes by the Sephadex G-10 technique. No significant differences in proportions of OKT3+, OKT4+, OKT8+, or OKM1+ cells in either Sephadex G-10 nonadherent or E rosette-forming cord blood cells were noted as compared to the same fractions of adult cells (Table I). Analysis of OKM1+ cells present in E-rosetting CBC fractions did not suggest patterns typical of monocytes since these cells showed the same light scatter on the FACS as small lymphocytes. Furthermore, the percentages of peroxidase positive cells in cord blood and adult E-rosetting cells were only 7 and 2%, respectively.

When Sephadex G-10 nonadherent cells were studied, the proportions of CBC staining either with OKT11 or 9.6 were almost identical (Table I). However, when the intensity of CBC staining was compared to the adult control, it was evident that the relative fluorescence intensity of the determinants recognized by the OKT11 or 9.6 antibodies was lower in newborn lymphocytes (Fig. 1).

CBC suppress in vitro PWM-induced synthesis of immunoglobulins. 1×10^6 adult PBMC were cultured alone or with increasing concentrations of glass nonadherent CBC in a PWM-induced immunoglobulin production assay. The amounts of IgG and IgM in culture supernates were measured by an ELISA technique. As controls, 10, 30, or 50% autologous adult PBMC were added in a simultaneous set of cultures. As can be seen in Table II, a progressive inhibition in the synthesis of both IgG and IgM occurred with increasing concentrations of CBC. The addition of identical numbers of adult PBMC did not suppress

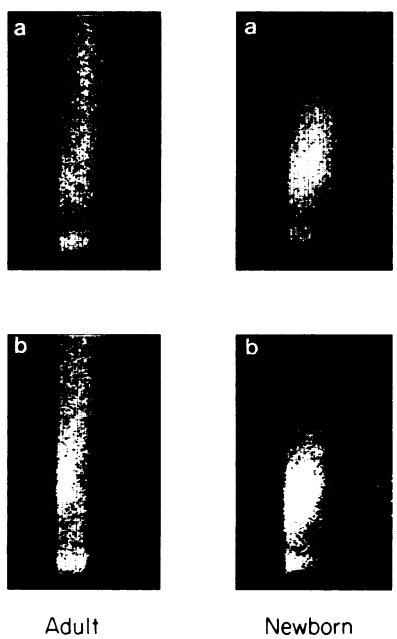

FIGURE 1 Comparison of OKT11 and 9.6 reactivity of Sephadex G-10 nonadherent cord blood or adult cells. Figures are photographs taken from the FACS oscilloscope screen. Vertical axis represents fluorescence intensity and horizontal axis, light scatter. Each cell is represented by a dot whose position on the oscilloscope is determined by the cell's light scatter (representative of cell size) and fluorescent intensity. a. OKT11 b. 9.6. Note the lower intensity of fluorescence on cord blood cells as compared with adults.

TABLE II

*Human CBC Suppress In Vitro Immunoglobulin Production in PWM-stimulated Cultures**

	Exp. 1		Exp. 2		Exp. 3		Exp. 4	
	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM
Adult PMC alone	2,500‡	5,600	2,700	1,300	2,300	2,200	4,600	1,700
Adult PBM + 10% CBC	$1,800^{28}$§	$2,700^{52}$	$1,800^{34}$	640^{51}	$1,100^{53}$	470^{79}	$1,700^{64}$	$1,300^{24}$
Adult PBMC + 30% CBC	$1,600^{36}$	$2,200^{61}$	$1,250^{54}$	220^{84}	156^{94}	390^{83}	$1,500^{68}$	$1,200^{30}$
Adult PBMC + 50% CBC	$1,300^{52}$	$1,000^{63}$	$1,000^{63}$	100^{83}	78^{87}	480^{80}	$1,500^{68}$	980^{43}
Adult PBMC + 10% Autologous PBMC	ND	ND	ND	ND	2,200	1,800	5,000	1,150
Adult PBMC + 30% Autologous PBMC	2,500	4,000	ND	ND	4,000	2,000	4,600	1,600
Adult PBMC + 50% Autologous PBMC	2,000	4,000	ND	ND	ND	ND	4,800	2,000

* Cultures were set up with 10^6 adult PBMC either alone or with 10, 30, or 50% of glass nonadherent CBC. As control 10, 30, or 50% of autologous PBMC were added in a different set of cultures, PWM was added at an optimal dilution (1:300). IgG and IgM were measured (ELISA technique) in the supernatants harvested the 7th d.

‡ Nanograms of IgC or IgM per milliliter culture.

§ Percentage of suppression is indicated by small suffix numbers.

immunoglobulin production. Only if >50% autologous adult PBMC were added to the culture was any decrease in immunoglobulin production observed. This decrease was not consistant and of much smaller magnitude than that observed with CBC. For that reason, to avoid results caused by overcrowding, 50% was set as the maximal number of cells added in all co-culture experiments. No differences in the viability of the cultures were found at the 7th d between the autologous (PBMC + autologous PBMC) and the allogeneic (PBMC + CBC) combinations. Although some differences in the degree of suppression between different CBC samples occurred, the strong inhibitory effect on both IgG and IgM production was always present.

Depletion of the OKT8+ subpopulation removes the suppressor activity in CBC. Glass nonadherent CBC were depleted of OKT8+ cells by the monoclonal antibody rosetting technique. As shown in Table III the removal of the OKT8+ fraction completely eliminated the cord blood suppressor activity of IgG production by adult PBMC.

Suppressor activity in human CBC is enriched in the OKT8+ population. Since the depletion of a given subpopulation could simply disrupt a critical balance in CBC necessary to express suppression without meaning that the removed subpopulation was in fact the actual suppressor effector cell, we then performed co-culture experiments with both the OKT8+ (positively selected) and the OKT8− (negatively selected) subpopulations. As can be seen in Table IV there was a marked enhancement of suppression in the OKT8+ fraction and conversely an abrogation when OKT8− CBC were added. Autologous PBMC had no significant effect when added as a control in a simultaneous set of cultures. The proportion of cells that were OKT8+ in these enriched suspensions was 81.4±4.2% and the number of monocytes identified by histochemical stain was 16.5±4.9%.

In view of the slight residual suppressor activity

TABLE III

*Abrogation of Suppressor Activity in CBC by Depletion of OKT8+ Population**

Exp.	Adult PBMC alone	Adult PBMC plus unseparated CBC			Adult PBMC plus OKT8-depleted CBC		
		10%	*30%*	*50%*	*10%*	*30%*	*50%*
1	4,200‡	3,100	3,500	2,700	4,200	4,000	3,900
2	9,000	ND	2,500	3,700	10,000	9,000	10,000
3	4,400	980	110	ND	5,200	3,300	3,400
4	10,000	7,200	2,100	625	10,000	9,000	9,200

* Cultures were prepared with 10^6 adult PBMC plus PMN (1:300) as base line. In the experimental cultures 10, 30, or 50% of either unseparated glass-nonadherent CBC or the OKT8-depleted population were added. IgG production was measured by the ELISA technique in supernatants harvested at the 7th d.

‡ Nanograms of IgG per milliliter culture.

TABLE IV
*Enrichment for Suppressor Activity in OKT8+ Cord Blood Lymphocytes**

Exp.	Adult PBMC alone	Adult PBMC plus						Adult PBM plus						CBC alone‡
		Autologous PBMC			Untreated CBC			OKT8+ CBC			OKT8− CBC			
		10%	*30%*	*50%*	*10%*	*30%*	*50%*	*10%*	*30%*	*50%*	*10%*	*30%*	*50%*	
1	4,800§	4,800	5,000	ND	4,800	3,500	2,700	1,000	90	80	5,000	3,800	3,500	80
2	2,500	2,200	2,500	2,000	1,800	1,700	1,300	1,700	1,100	1,100	2,200	2,200	2,300	420
3	2,700	2,900	4,000	2,000	1,650	1,200	1,400	ND	210	190	ND	2,300	1,850	ND
4	2,700	ND	ND	ND	1,800	1,250	1,000	ND	530	370	ND	1,200	1,000	300

* A set of cultures were set up with 10^6 adult PBMC as base line. In the experimental cultures 10%, 30% or 50% of either untreated glass nonadherent CBC, or each of the separated populations (OKT8+, OKT8− CBC) added at the beginning of the culture. As controls, 10, 30, or 50% autologous PBMC instead of CBC were added. All the cultures were stimulated with PWM 1:300. IgG production was measured by the ELISA technique in supernatants harvested the 7th d.
‡ 5×10^5 CBC were cultured separately.
§ Nanograms IgG per milliliter culture.

in the OKT8-depleted subpopulation of some donors and of the presence of residual peroxidase positive cells, we then used two different approaches to define the possible contribution of monocytes to suppressor activity. Since OKM1 is a monoclonal antibody reacting with monocytes and null lymphocytes (18), we compared the effect of either OKT8 or OKM1 enrichment to the functional suppressor activity of the same cord blood sample.

As shown in Fig. 2, the OKT8+ fraction showed significantly more suppressive activity than the OKM1+ subpopulation; moreover, abrogation of suppressor activity was only evident in the OKT8-depleted subpopulation but not in the OKM1-negative cell fraction. The experiments with OKM1 monoclonal antibody did not allow differentiation between the possible suppressive activity of classical monocytes or OKM1+ lymphocytes. For these reasons we used a monoclonal antibody 63D3 reacting with human monocytes in other experiments.

We isolated two fractions from a sample of glass nonadherent CBC using the rosetting technique with ox erythrocytes linked to rabbit anti-mouse IgG and preincubation of mononuclear cells with mouse hybridoma antibody, 63D3. One fraction was monocyte enriched (>90% peroxidase positive cells) and the other was monocyte depleted (<7% peroxidase positive). The monocyte depleted fraction was further separated into OKM1+ and OKM1− cells by the same rosetting technique and the effect of these fractions tested (Fig. 3). Depletion of monocytes did not abrogate the suppressor activity in cord blood. A monocyte fraction (>90% peroxidase positive) showed suppressive activity but not to the extent as OKT8+ cell enriched suspensions (Fig. 3). Furthermore the OKM1+ lymphocytes present in a monocyte-depleted subpopulation showed comparatively less suppressor activity than the whole monocyte-depeleted fraction. In summary, experiments shown in Fig. 3 indicate that even though highly enriched cord blood monocytes may have a suppressor effect on a PWM-driven lymphocyte immunoglobulin production under certain experimental conditions, the major cell responsible for such activity in human cord blood was an OKT8+ lymphocyte.

Suppressor activity in cord blood lymphocytes is present in both E-rosetting and non-E rosetting cell suspensions. Using the standard E-rosetting technique we found a significant decrease in the number of E-rosetting cells in cord blood as compared with adult (44±13 vs. 75±8, $P < 0.05$). Since we found a

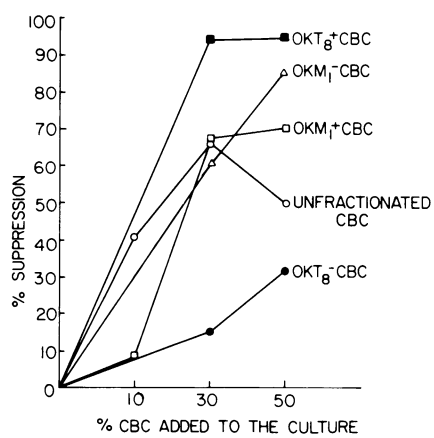

FIGURE 2 Preferential enrichment of the suppressor activity of CBC in the OKT8+ fraction as compared to the OKM1+ subpopulation. Depletion of the OKT8+ CBC but not of the OKM1+ CBC significantly decreased the suppressor effect of CBC (three experiments; standard error at each point = range, 4.1–5.8%). Results are expressed as percentage of suppression by 10, 30, or 50% CBC on the PWM-induced production of IgG by 10^6 adult PBMC.

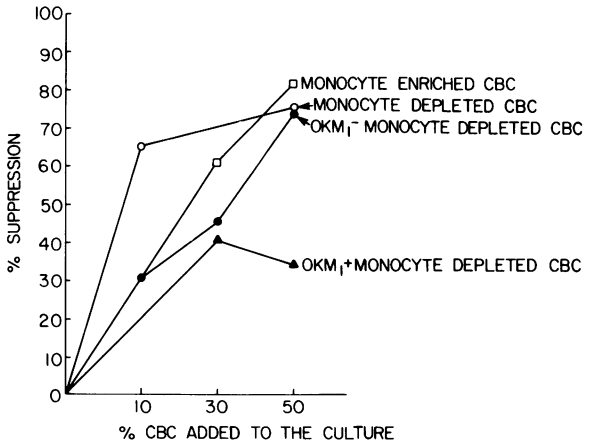

FIGURE 3 Persistence of suppression in monocyte-depleted CBC. Remaining OKM1+ lymphocytes have comparatively less suppressor activity. Results are expressed as percentage of suppression as indicated in the legend of Fig. 1 (two experiments; standard error each point = range, 0.5–5.7%).

normal proportion of lymphocyte cell surface markers that identify T cell lineage (OKT3, OKT4, OKT8, and OKT11, Table I) we questioned whether the E-rosetting technique actually defined a complete T cell population. This problem was approached by analyzing the phenotypic profile of both E-rosette positive and non-E-rosetting fractions with flow cytometry. In two experiments we found OKT8+ cells in both E rosette-positive and E rosette-negative fractions. We corroborated this finding in several experiments using the monoclonal antibody rosetting technique that directly demonstrated OKT8+ cells in both the E rosette-positive and the E rosette-negative fractions (Table V). As expected, both fractions were able to suppress in vitro IgG production in proportion to the number of OKT8+ cells present (Fig. 4).

DISCUSSION

We have identified the major cell responsible for suppression of in vitro immunoglobulin production as an OKT8+ lymphocyte. In addition, we have measured the phenotypic profile of CBS using flow cytometry and a panel of monoclonal antibodies defining various T cell subpopulations. No differences were found in the proportions of cord and adult mononuclear cell subsets. Finally, there exists a population of newborn cells with a low density of E receptors as defined by fluorescence analysis, which may account for the relatively low proportion of E-rosetting cells with SRBC found in cord blood

Human newborn lymphocytes that suppress the in vitro PWM-induced differentiation of adult B cells have been previously described by several different groups (1–10). We have previously demonstrated that this suppression is not mediated by allogeneic effects per se (3). In these studies significant suppression was not observed when allogeneic adult lymphocyte co-cultures were tested. On the contrary, enriched suppressor activity was demonstrated in cord blood when cultivated with adult cells. Most studies have characterized the cord blood suppressor lymphocyte as a radiosensitive cell that forms E rosettes. More conflicting results have arisen when differential Fc receptor binding has been used as a method for further delineation. In 1977, Oldstone et al. (13) reported that the suppressor activity of newborn lymphocytes on the mother's lymphocyte proliferation was present in a T cell subset bearing Fc receptors for IgG. Later, Hayward et al. (7) in studies corroborated by others (4) concluded just the opposite: only the T non-γ population of CBC could abrogate PWN induced in vitro immunoglobulin production. Finally, a different group of investigators found suppressor activity in both subpopulations (9).

Previous attempts to identify functional subsets of adult lymphocytes with monoclonal antibodies defining unique and distinct surface markers have been successful. Reinherz et al. have identified the cytotoxic/suppressor and the inducer/helper adult T cell in the OKT8+ and OKT4+ subpopulations, respectively (15, 17). No such information is currently available for human CBC. Mosier et al. (24) demonstrated that the neonatal suppressor T cell in mice has the Ly 1+, Ly 2+, TL+, Thy+ phenotype as opposed to the Ly 2+, 3+ characteristic of the adult subset. By using different monoclonal antibodies we have identified the major suppressor cell in CBC as an OKT8+ lymphocyte. OKT8+ CBC were found in the same relative proportions as the adult controls. This finding extends the

TABLE V
Distribution of OKT8+ Cells among E-Rosetting and E-Nonrosetting CBC

Exp.	E-Rosetting CBC	E-Nonrosetting CBC	% E-Rosettes
1	32‡	60‡	22
2	13‡	22‡	51
3	16‡	5‡	41
4	29§	9§	ND
5	18§	10§	ND

* CBC were separated by E-rosetting with neuraminidase-treated sheep erythrocytes then both fractions were treated with OKT8 monoclonal antibody (Methods) and the number of rosetting cells (OKT8+) counted. In experiments 4 and 5 the fractions were retested by FACS analysis.
‡ Figures are given as percentages of OKT8+ rosette forming cells.
§ Figures represent percentages of OKT8+ stained (positive) cells as tested by FACS.

previous observation by Reinherz et al. (25) in adult cells using the same system. The fact that newborn lymphocytes have increased suppressor activity when compared with adults, in the presence of similar expression of the OKT8 determinant suggests that such cells may somehow be activated in vivo by factors present in the pregnant sera. Lester et al. (26) demonstrated the role of alpha-fetoprotein during pregnancy in the activation of human suppressor cells. The influence of pregnancy in this activation is decisive; thus Hayward et al. demonstrated that the suppressor activity appeared as early as the 26th wk (8) and Miyawaki et al. (2) showed a gradual decrease until the 2nd yr of age. The nature of possible factors in pregnant sera involved in such activation, as well as the precise role of the OKT8 determinant in CBC remains to be elucidated. Previous attempts have been made to characterize the neonatal suppressor cell by using the differential Fc receptor binding. Several groups showed that most of the suppressive activity was present in the T non-γ subpopulation (4, 7). These reports are in agreement with our findings since Reinherz et al. (27) demonstrated that most of the OKT8+ cells were present in that fraction. However, a different laboratory also reported enrichment of suppressor activity in the Tγ cells (13). Tγ cells also include OKT8+ lymphocytes yet in a lower proportion than the T non-γ fraction. This could explain why Durandy et al. (9) found suppressor activity in both Tγ and T non-γ cells.

The participation of cord blood monocytes in the in vitro PWM-induced production of immunoglobulin has not previously been well defined. Weston et al. (28) found suppressed chemotaxis but normal phagocytosis and microbicidal activity in cord blood as compared to adult monocytes. Similarly, Milgrom et al. (29) reported normal activity of cord blood monocytes as effector cells in antibody-dependent cellular cytotoxicity. By enrichment or depletion of these cells we were able to demonstrate that monocytes do not have a major suppressor activity in the PWM-driven system as compared with OKT8+ lymphocytes. Furthermore, their presence is not necessary for a full expression of suppression by OKT8+ cells. Olding et al. (11) found that glass-adherent newborn cells did not inhibit the division of maternal lymphocytes; in addition, Hayward et al. (7) reported an increase of PWM-induced plaque-forming cells by adult cells when cocultured with non-E-rosetting CBC. In neither study was the actual number of monocytes involved given. It has been shown that monocytes can have a dual effect (helper or suppressor) on polyclonal B cell differentiation depending on the proportions present in the culture (30). It is conceivable that the suppressor activity in OKT8+ enriched preparations may have been due to some other cell populations besides OKT8+ cells and monocytes. However, this is unlikely since OKT8+ cells and monocytes comprise ~95% of this cell preparation. Moreover, confirming the results by Thomas et al. (31), we have found that OKT8− preparations are highly enriched for OKT4+ cells when tested by FACS analysis (unpublished data). OKT8− suspensions were not suppressive even at 50% concentration (Table III).

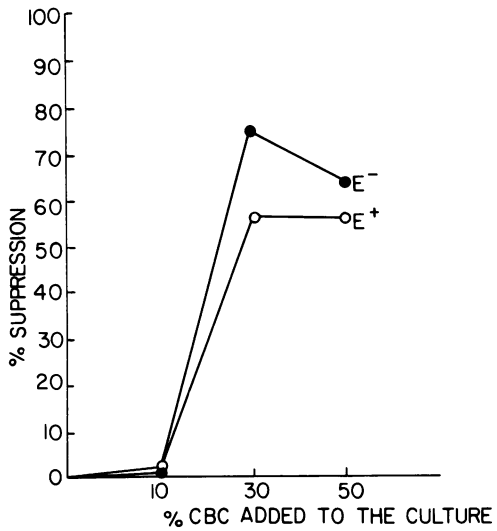

FIGURE 4 Suppressor activity is present in both E rosette+ and E rosette− cord blood mononuclear cells. 10, 30, or 50% of either E+ or E− CBC were added to 10^6 adult PBMC in a culture stimulated with PWM (1:300). Results representing three experiments are expressed as indicated in the legend of Fig. 1.

In both E rosette-positive and G-10 monocyte depleted CBC the proportion of OKT11 positive lymphocytes was comparable to normal adult controls. Similarly, the proportions of CBC staining with the hybridoma 9.6 antibody reacting with the E-receptor was not different from controls; however, the pattern of staining suggested a lower cell surface density of this determinant. Since this hydridoma recognized a protein identical or closely related to the E-receptor on T lymphocytes (20), the lower reactivity with the 9.6 antibody could account for the decreased number of E-rosetting cord blood cells that we observed in this and previous studies (32). In addition to the possibility that cord cells have a decreased density of E-receptors, it is also conceivable that the reduced numbers could be accounted for by less affinity for the ligand. Another alternative explanation is that despite a comparable number of E receptors in both adult and CBC, the presence of blocking factors in newborn sera might interfere with their normal function in CBC rendering them less susceptible to separation by this technique. Several such factors capable of interaction with CBC or maternal lymphocytes have already been

described (33–35). Stimson et al. (34) described a pregnancy associated α-macroglobulin that significantly reduced the number of lymphocytes binding sheep erythrocytes in the spontaneous rosette test. The similar proportions in cord blood and adult lymphocytes of cells reacting with four monoclonal antibodies known to recognize T cell antigens suggest an already fully developed phenotypic profile in lymphocytes at birth. In fact, OKT3, OKT8, OKT4, and OKT11 were present in comparable numbers in CBC as has been shown by Janossy et al. studying thymus and bone marrow from infants (15). We also found a normal OKT4/OKT8 ratio in CBC. These results sharply contrast with the report by Hayward et al. (8) of an increase of the OKT4+ and, notably, a decrease in the OKT8+ subpopulations in cord blood. A possible explanation for this discrepancy is that these authors only tested E rosetting CBC, which as we pointed out in the results, does not necessarily select the total proportions of cord blood lymphocytes bearing this determinant.

In summary, we have characterized the major suppressor cell in cord blood as one bearing OKT8 determinants, thus providing further insight into the nature of suppressor cells in newborn infants. Possible differences between intrinsic suppressor activity of OKT8+ lymphocytes in cord blood and adult blood remain to be defined and may ultimately reveal important features inherent to suppressor cell activation.

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