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Nonimmune rosette formation: A measure of the newborn infant's cellular immune response

Nonimmune rosette formation, an in vitro measure of cellular immunity, was evaluated in normal newborn infants. Active rosette formation in 14 specimens of cord blood was 18.9 ± 4.8% compared to 28.1 ± 5.2% in 15 adult control samples (p < 0.05). Total rosette formation in 13 cord blood samples was 33.3 ± 7.6% compared to 55.1 ± 6.5% in 15 adult control specimens (p < 0.05). Tritiated thymidine uptake from phytohemagglutinin stimulation was comparable in cord blood and adult control lymphocytes. The importance of these findings is discussed in light of other recent reports suggesting that cord blood thymic derived T lymphocytes may have reduced immune capability when compared to adult lymphocytes.

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THE NEWBORN INFANT has unique susceptibility to fungal, viral, protozoan, and certain bacterial infections.[1, 2] These organisms require an intact cell-mediated pathway of the immune system for effective killing to occur.[3] This pathway involves antigen recognition, thymic-derived lymphocyte proliferation, lymphokine release, macrophage activation, and nonimmunologic inflammatory factors.[4] The newborn infant's cellular immunity has been evaluated for several of these factors. Cord blood lymphocytes can respond to several ubiquitous mitogens and antigens with a good proliferative response.[5, 6] In fact, high spontaneous blast transformation occurs in these lymphocytes, as compared to adult cells.[6] Several recent studies, however, suggest that lymphokine release[7] and direct lymphocyte-killing ability[8] may be significantly less active in the lymphocytes of cord blood. In vivo testing, such as development of cutaneous-delayed hypersensitivity to dinitrochlorobenzene,[9] cutaneous hypersensitivity following the transfer of sensitized maternal lymphocytes (to purified protein derivative),[10] and graft rejection,[11] reveals a diminished response in the newborn infant compared to the adult. One cannot conclude from these latter studies, however, that immunologic factors are abnormal, since these results could easily reflect the known reduced inflammatory capability of the newborn infant's skin.[12]

Recently, techniques utilizing cell surface markers have been developed to characterize the thymic-derived lymphocyte[13, 14] and bursal-derived lymphocyte.[15] The purpose of this study is to investigate the newborn infant's cellular immune response utilizing the technique of non-immune rosette formation, which is believed to correlate with T lymphocyte function.[13] The E rosette is formed when three or more sheep red blood cells attach to a T lymphocyte. This occurs without prior sensitization or utilization of complement. E rosettes have been classified by Wybran and associates[13] into active and total categories. Active rosettes are those rapid rosette formers read

Abbreviations used	
PHA:	phytohemagglutinin
T:	thymic derived
B:	bursal derived
E:	nonimmune

following a short incubation. Decreases in rosette formation seen with some viral and neoplastic diseases appear to involve primarily active rosettes.[16] Total rosettes are read after a longer incubation period and are thought to reflect the total number of peripheral T lymphocytes.[13, 14] In this study we are reporting both active and total rosette formation in cord blood lymphocytes.

MATERIALS AND METHODS

Ten to 15 ml of cord blood and a control specimen from a healthy adult are collected in heparinized containers

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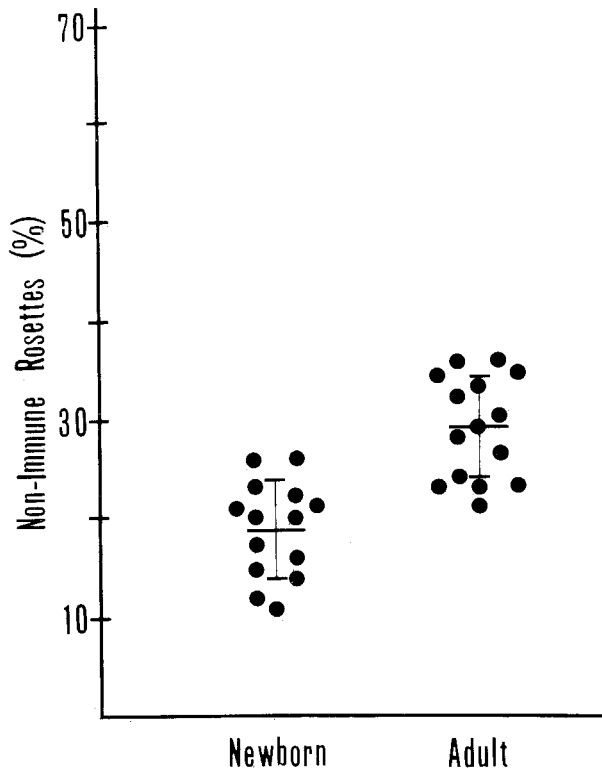
Fig. 1. Percentage of active E rosettes is shown on the ordinate and newborn and adult populations are separated on the abscissa. The mean and 1 SD are shown (‡).
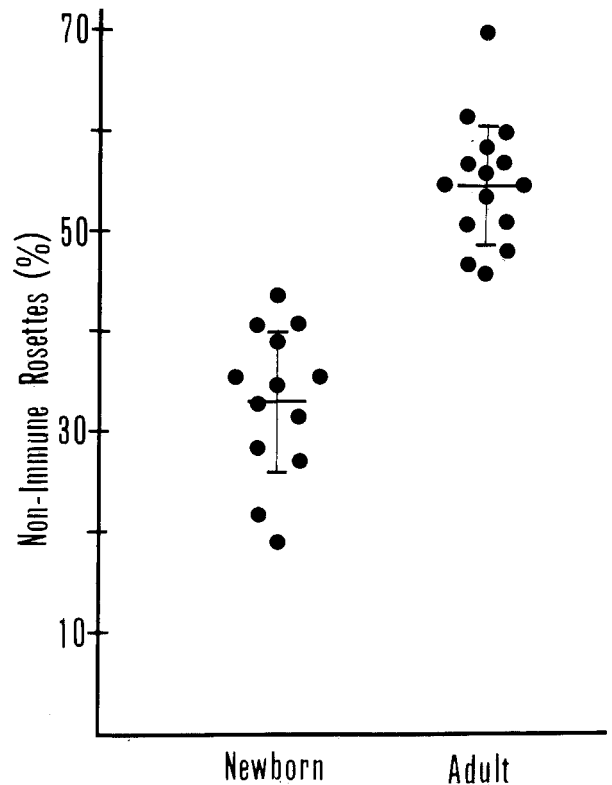

Fig. 2. Percentage of total E rosettes is shown on the ordinate and newborn adult populations are separated on the abscissa. The mean and 1 SD are shown (‡).

(100 units sodium heparin/ml blood). The blood is diluted to 35 ml with phosphate-buffered saline and then underlayed with 15 ml of Ficoll-Hypaque. This mixture is centrifuged for 40 minutes at 100 g. The lymphocytes separate at the saline Ficoll-Hypaque interface and are removed by pipette. They are washed three times in phosphate-buffered saline and resuspended to a final concentration of 1.0 × 10⁷ cells/ml. Fetal calf serum is adsorbed against sheep red blood cells and incubated for one hour at 56°C.

Active E rosettes are formed by adding 0.05 ml of washed lymphocytes (1.0 × 10⁷ cells/ml) to 0.05 ml of fetal calf serum and incubated in 5% CO_2 at 37°C for one hour. Following this 0.40 ml of washed sheep red blood cells (1.0 × 10⁷ cells/ml) are added and the mixture is centrifuged for 5 minutes at 100 g. The pellet is gently resuspended and the active rosettes are counted. Rosettes are defined as lymphocytes with three or more sheep red blood cells attached to their surface and are expressed as a percentage of the total number of lymphocytes. Total E rosettes are formed by adding 0.05 ml of lymphocytes, 0.05 ml of fetal calf serum and 0.40 ml of sheep red blood cells simultaneously, centrifuging for 5 minutes at 100 g

and leaving at room temperature overnight (16 to 20 hours). The next day the total rosettes are counted.

Mean values and standard deviations were determined for active and total rosettes in both cord blood and adult control specimens. The t test for the difference between two means was used to compare adult and cord blood E rosette formation.[17]

Phytohemagglutinin stimulation of peripheral blood lymphocytes was performed in the usual manner[5] for comparative purposes with rosette formation. Highly purified Burroughs Wellcome PHA at 0.5 μg/ml was used. Trypan blue exclusion was used to evaluate cell viability of the cord blood.

RESULTS

Active rosette formation in 14 cord blood specimens with a mean value of 18.9 ± 4.8% was significantly less than the control result of 28.1 ± 5.2% in 15 adult subjects (p < 0.05). As can be seen in Fig. 1, the adult and newborn active rosette populations clearly fall in two distinct groups. Total rosette formation in 13 specimens of cord blood had an average value of 33.3 ± 7.6% (Fig. 1) and the control adult mean value in 15 samples was

51.0 ± 6.5% (Fig. 2). The p value for this difference was also less than 0.05. There is no overlap of adult and neonatal total rosette percentages.

Result of PHA stimulation (Table I) revealed comparable tritiated thymidine uptake in cord blood and adult control lymphocytes. The lower stimulation indices seen in the cord blood reflect the common observation that these unstimulated cells have high spontaneous incorporation. Finally, trypan blue exclusion revealed > 99% viability of both cord blood and adult control cells.

DISCUSSION

We have found a significantly lower percentage of active and total rosette formers in the lymphocytes of cord blood compared to that of the adult control specimens. If total E rosettes generally reflect the percent of T lymphocytes in the peripheral blood,[13, 14] our findings suggest that the neonate has a relative decrease in circulating T lymphocytes. This could be accounted for by a dilutional effect of increased numbers of B lymphocytes or of "null" cells,[18] or by immaturity of the sheep red blood cell receptors on the T lymphocyte. The rapid forming active rosettes, which may be a subpopulation of T lymphocytes, are thought by some to be more indicative of T lymphocyte competence than the total rosette population.[13, 19] This was also significantly reduced in the lymphocytes of cord blood.

Ferguson and associates[20] reported that normal neonates, 1 to 10 days of age, had total rosette formation which did not differ significantly from adult controls. However, these data are not comparable to studying only lymphocytes of cord blood as in the present study. Smith[21] recently described statistically significant lower total rosettes in cord blood when compared to young adults.

Our observed decreases in percent of E rosette formation is not in agreement with the neonate's normal response to PHA stimulation.[5] We have also found a response comparable to adult controls in lymphocytes of cord blood; however, heterogeneity of lymphocyte response is not unusual[22] In addition, the findings of essentially normal PHA responses in Ferguson and associates'[20] report in patients with abnormal delayed cutaneous hypersensitivity and diminished rosette formation suggest that E rosette formation may be a more sensitive indicator of cell-mediated immunity. Evidence that lymphotoxin release may be defective in cord blood lymphocytes with normal PHA response[7] supports this contention.

In vivo studies of cell-mediated immunity in the neonate previously reported have been hampered by their diminished inflammatory response in this age group.[12]

Table I. Phytohemagglutinin (PHA) stimulation

	Tritiated thymidine uptake (mean counts per minute)		Stimulation index PHA + /
	PHA −	*PHA* +	*PHA*−
Adult controls			
1	1,440	36,604	25.6
2	1,067	40,268	37.8
3	375	35,581	94.9
Cord blood			
1	2,628	37,380	14.2
2	4,045	63,860	15.8
3	3,421	38,523	11.3

Thus, in vitro stimulation of cord blood lymphocytes with PHA has been used to evaluate cell-mediated immunity in the newborn infant. It is becoming increasingly clear, however, that the proliferative response to PHA, although valuable in the general assessment of T lymphocyte function, may give little information about the effector capability of the T cell.[23] Perhaps E rosette formation will provide better correlation with T effector function.

Our study gives further evidence that in addition to a diminished inflammatory response, the neonate may actually have reduced capacity for cell-mediated immune responses. Further clarification will necessitate correlating E rosette formation with T effector cell function, particularly lymphokine release, in cord blood lymphocytes as well as in lymphocytes of the older neonate.

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