COMPARATIVE STUDY OF THE HETEROGENEITY OF CD4 AND CD8 LYMPHOCYTE SUBPOPULATIONS IN NEONATES AND ADULTS BY TWO-COLOR CYTOFLUOROMETRY

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UDC 616.155.32-076.5:543.426

KEY WORDS: lymphocyte subpopulations; neonates; flow cytofluorometry.

The functional properties of lymphocytes in neonates differ radically from those in adults. Newborn infants are characterized by high suppressor activity of their lymphocytes, which is associated with $CD4^+$-cells, whereas in adults lymphocytes possessing suppressor activity mainly have the $CD8^+$ phenotype [9, 12]. It has been shown in recent years that the subpopulation of $CD4^+$-lymphocytes is not homogeneous and includes inducers of both suppressor and helper cells. The use of monoclonal antibodies has shown that suppressor inducers have the $CD4^+CD45RA^+$ phenotype [6], whereas cells with a helper function during differentiation of B lymphocytes into immunoglobulin-synthesizing cells, have the $CD4^+Leu8^-$ phenotype [2]. The subpopulation of $CD8^+$-lymphocytes, which includes both precursors and effectors of allocytotoxic T lymphocytes with the $CD8^+CD11b^-$ phenotype and suppressor-effectors with the $CD8^+CD11b^+$ phenotype, also is functionally heterogeneous [3].

The aim of this investigation was to study, with the aid of monoclonal antibodies and, by the method of two-color flow cytofluorometry, the patterns of distribution of lymphocytes with differing functional activity within the $CD4^+$ and $CD8^+$ subpopulations of cells in neonates.

EXPERIMENTAL METHOD

Altogether 15 healthy neonates on the 5th day after birth and 20 healthy individuals aged 25-40 years were studied. Lymphocytes, isolated in a Ficoll—Verografin density gradient, were conjugated with FITC or with phycoerythrin. Anti-Leu3a (CD4), anti-Leu18 (CD45RA), anti-Leu8, anti-Leu2A (CD8), and anti-Leu15 (CD11b) monoclonal antibodies (Becton Dickinson) were used. The source of exciting light was an argon laser with a power of 15 mW and wavelength 488 nm; fluorescence of FITC was recorded at 520-540 nm and of phycoerythrin at 563-587 nm. Emission of FITC in the phycoerythrin channel was compensated by means of an analog-differential subtracter at the preamplification level and standardized by the use of samples labeled with antibodies conjugated with one of the fluorochromes. Granulocytes and monocytes were excluded from analysis by isolating a subpopulation of lymphocytes by using a discrimination window. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

We found a significantly higher content of $CD4^+$-lymphocytes and a lower content of $CD8^+$-lymphocytes in neonatal blood, in agreement with the findings of most investigators [4, 10, 11]. However, as Table 1 shows, most $CD4^+$-lymphocytes of neonates have the $CD4^+CD45RA^+$ phenotype, i.e., they are inducers of suppressors, whereas the level of $CD4^+$-lymphocytes, transmitting helper function ($CD4^+Leu8^-$) is 7 times higher in adults than in neonates. Neonatal $CD4^+$-lymphocytes are known to suppress proliferation and immunoglobulin synthesis of adult lymphocytes, but cannot give a helper effect when cultured with adult B lymphocytes [1]. These observations, made during a study of the

Laboratory of Immunology, Kiev Research Institute of Pediatrics, Obstetrics, and Gynecology, Ministry of Health of the Ukrainian SSR, Kiev. (Presented by Academician E. D. Luk'yanova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 10, pp. 405-407, October, 1991. Original article submitted February 18, 1991.

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TABLE 1. Content (in %) of Lymphocytes Expressing CD4- and CD8-Markers in Neonatal and Adult Peripheral Blood (M ± m)

Subpopulation of lymphocytes	Neonatal (n = 20)	Adult (n = 20)	p
$CD4^+$	54,11±1,74	41,24±2,03	<0,001
$CD8^+$	16,64±1,39	26,94±1,86	<0,01
$CD4^+CD45RA^+$	40,44±2,24	13,58±1,24	<0,0001
$CD4^+Leu8^-$	1,02±0,17	7,03±0,95	<0,0001
$CD8^+CD11b^+$	4,59±0,64	8,69±1,32	<0,01
$CD8^+CD11b^-$	12,05±1,36	18,26±1,99	<0,01

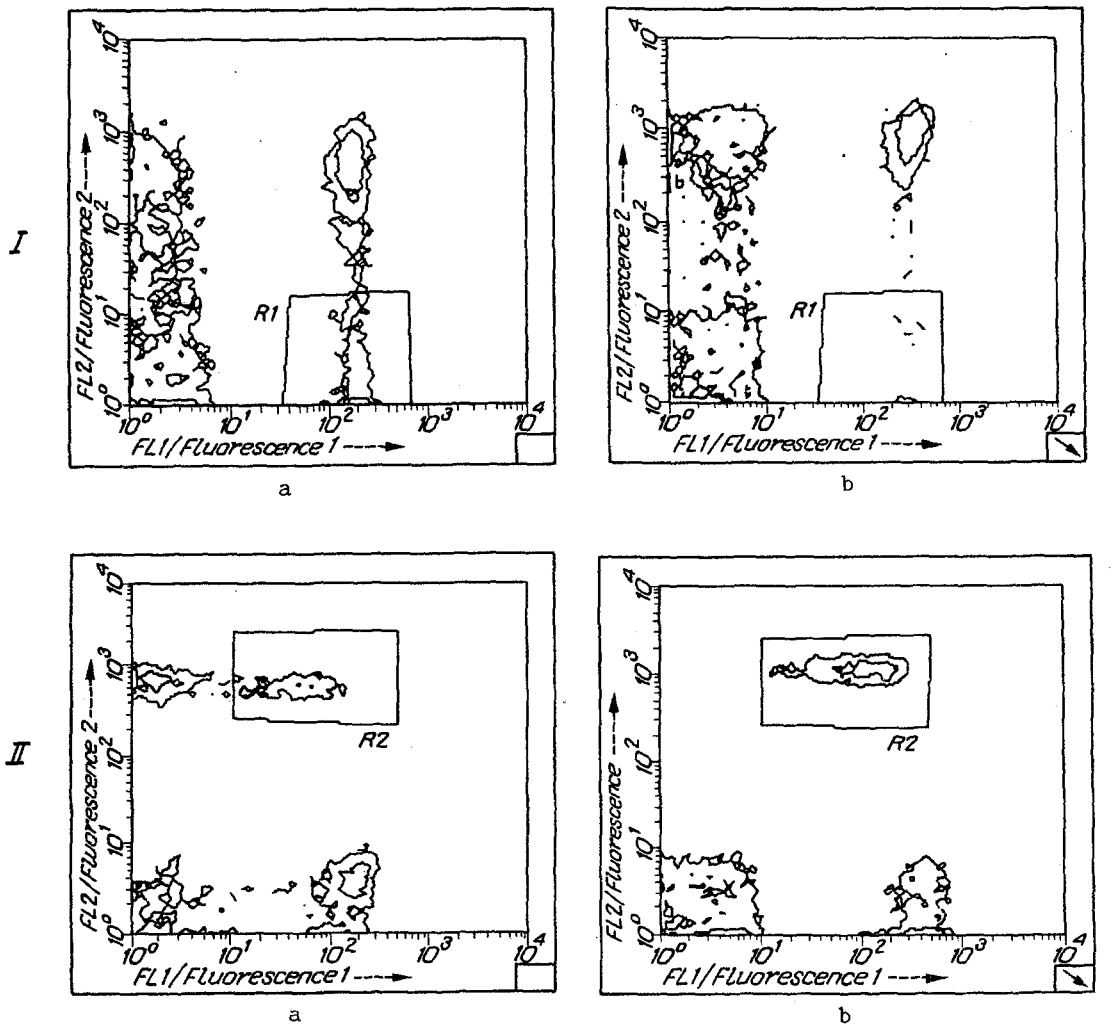

Fig. 1. Two-color cytofluorometric analysis of adult (a) and neonatal (b) lymphocytes. 1) Lymphocytes labeled with FITC-anti-Leu3a (FL1) and phycoerythrin-anti-Leu8 (FL2) antibodies; R1) Leu3a + Leu8-cells. II) Lymphocytes were labeled with FITC-anti-Leu18 (FL1) and phycoerythrin-anti-Leu3a (FL2) antibodies. R2) Leu3a + Leu18-cells.

functional properties of $CD4^+$-lymphocytes can be explained on the basis of the phenotypic distinguishing features of this lymphocyte subpopulation which we discovered: neonatal $CD4^+$-lymphocytes are mainly suppressor inducers (79.3% of neonatal $CD4^+$-lymphocytes have the CD45RA marker, compared with only 25.2% in adults), among neonatal $CD4^+$-cells,

helpers (Leu8-cells) account for not more than 3%, in adults 19% of $CD4^+$-lymphocytes do not carry the Leu8 marker. A typical example of analysis of the distribution of CD45RA and Leu8 markers on neonatal and adult $CD4^+$-lymphocytes by the method of two-color cytofluorometric analysis, using the "lysis" program (Becton Dickinson), is shown in Fig. 1.

Meanwhile it was found that suppressor activity mediated by $CD8^+$-lymphocytes is low in neonates. This is shown by the low level of activity of con A-induced suppressors in the neonatal period [5, 8]. In neonates the level of $CD8^+CD11b^+$, i.e., suppressor-effectors, is significantly lower than in adults (Table 1), indicating a link between low suppressor activity of $CD8^+$-lymphocytes and a low level of the cells mediating this activity. We also found a lower level of precursors and effectors of cytotoxic lymphocytes ($CD8^+CD11$) in neonates than in adults. Low activity of cytotoxic lymphocytes in neonates [7] may therefore be linked to a certain degree with the low level of cytotoxic cells in the neonatal period.

Thus in neonates we found a high content of cells in the $CD4^+$-subpopulation with the phenotype of inducer-suppressors ($CD4^+CD45RA^+$) and a low content of cells mediating a helper effect ($CD4^+Leu8^-$). Consequently, during assessment of the neonatal immune status, the $CD4^+$ lymphocyte subpopulation must not be described as helpers, nor must the pathogenetic mechanisms of immunopathological states in the neonatal period be interpreted on the basis of these propositions. The high level of suppressor-inducers associated with a low level of suppressor-effectors in neonates may be one of the factors responsible for increased susceptibility of neonates to infection associated with inability to respond adequately to an antigenic stimulus. Another factor in the increased sensitivity to infection in the neonatal period may be the low level of precursors and effectors of cytotoxic T lymphocytes ($CD8^+CD11b^-$) in neonates.

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