Characterization of Immature T Cell Subpopulations in Neonatal Blood

By Sian Griffiths-Chu, Jennifer A.K. Patterson, Carole L. Berger, Richard L. Edelson, and Anthony C. Chu

A series of monoclonal antibodies directed against T cell differentiation antigens was used to identify circulating T cells in normal human neonates. Twenty-five cord blood samples, taken after cesarean or vaginal delivery, and 16 venous blood samples from normal adult controls were examined using monoclonal antibodies in an indirect immunofluorescence technique. The percentage of circulating OKT3 positive (pan-T cell) cells was significantly lower in the neonatal blood (52.8%) compared with the adult controls (74.9%) \( (P < .001) \). Of the cord mononuclear cells, 58% showed reactivity with OKT10 (common thymocyte antigen) compared with only 7% in adult controls \( (P < .001) \). The helper:suppressor T cell ratio was lower in cord blood \( (1:2) \) as compared with 1:3 for adult blood \( (P < .005) \) blood OKT4+ cells, however, showed strong suppressor activity, equivalent to OKT8+ cells in adult blood.

We used a large series of monoclonal antibodies generated against T cell differentiation antigens,\(^4,5,7-9\) and our results showed marked variance with those of Yachie et al.\(^6\) We identified a population of phenotypically immature T cells in human cord blood. These cells demonstrate significant reactivity with OKT10 (usually present on less than 5% of adult peripheral T cells)\(^9\) and OKT6 (not present in adult peripheral blood),\(^4\) and a moderate decrease in reactivity with OKT11 (present on all E rosette-positive adult peripheral blood cells).\(^9,10\) In addition, there was a decreased ratio of helper:suppressor cells, which reflected a population of cells simultaneously reactive with OKT4 and OKT8. The total OKT4+ population in cord blood was lower than in adult blood, and the OKT8+ population was higher than in adult blood. We also describe a previously unrecognized phenotype of OKT3+, OKT6+ cells circulating in the neonate.

**MATERIALS AND METHODS**

**Blood Specimens**

Cord blood was collected from the placental portion of the umbilical vein of 25 full-term infants shortly after birth. Heparinized samples were collected from 15 neonates delivered by elective cesarian section and from 10 newborns delivered vaginally. Fifteen control samples were obtained from healthy adult volunteers aged 20–45 years. Prior to lymphocyte separation, the total white cell count in 10 cord samples was determined using a Coulter counter (model ZF-4174, Coulter Electronics Inc, Hialeah, Fla). Differential white cell counts were performed in Wright-stained whole blood smears.

**Separation of Mononuclear Cells**

Mononuclear cells were separated using velocity sedimentation of Ficoll-Hypaque, as previously described.\(^11\) In most cord blood sam-

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suspension was mounted under a coverslip prior to examination. Antibody diluted...

...cells, necessitating lysis by 0.15 mol/L NH₄Cl and 0.01 mol/L KHCO₃. Such lysis does not alter antigen expression, as shown in studies of 100 control adult blood samples over a period of 1 year in our laboratory (unpublished data); this is a routine procedure in cytofluorographic analysis of whole blood. Prior to their use in the assay system, the mononuclear cells were washed twice and then resuspended at 10 x 10⁶ cells/mL in RPMI (GIBCO, Grand Island, NY).

Monoclonal Antibodies

The monoclonal antibodies used were obtained from Ortho Pharmaceutical Corp, Raritan, NJ. Their production and specificities have been previously reported. Briefly, OKT3 reacts with mature T cells in the adult peripheral circulation and is also reactive with 10% of thymocytes; OKT4 reacts with the majority of thymocytes and with 55% of adult peripheral T cells; it defines the “helper or inducer” T cell subset; OKT6 was produced using human thymocytes as the immunogen and is reactive with 70% of these cells but not with normal adult B cells and is also present on epidermal Langerhans cells. OKT8 reacts with the majority of thymocytes, with 30% of adult peripheral T cells, and defines the “suppressor” T cell subset; OKT10 reacts with all thymocytes and with <5% of normal adult circulating T cells. Because OKT10 is found in bone marrow cells as well, it is considered an early differentiation antigen. OKT11 is a pan-T cell antibody, which reacts with all thymocytes and mature T cells. It apparently recognizes the sheep blood cell (E rosette) receptor on T cells.

Indirect Immunofluorescence

Mononuclear cells were examined using the monoclonal antibodies in an indirect immunofluorescence technique, as described previously. Briefly, 10⁶ cells were added per well to microtiter plates, and the cells were initially incubated with 100 μL of each monoclonal antibody diluted 1:200 with phosphate-buffered saline (PBS) at 4°C for 30 minutes. After three washes in PBS containing 0.02% sodium azide, the cells were incubated for an additional 30 minutes with fluorescein-labeled goat anti-mouse IgG (Meloy Laboratories, Springfield, Va) at 4°C. After three washes in PBS/azide, the cells were resuspended in 50 μL of 50% glycerine in PBS. A drop of each suspension was mounted under a coverslip prior to examination under oil immersion using a Leitz Ortho-lux 11 microscope with a fluorescein vertical illuminator (E. Leitz, Inc, Rockleigh, NJ). One hundred consecutive cells were counted in each specimen, and the percentage of fluorescein cells determined.

Double-labeling

Cells were indirectly labeled with OKT3, OKT6, or OKT8 (Ortho Pharmaceutical Corp) and rhodamine-conjugated goat anti-mouse IgG (Cappel Labs, Cochranville, Pa) and were then directly labeled with a reciprocal antibody (OKT3, OKT4, or OKT11) having first blocked any free binding sites on the goat anti-mouse IgG with mouse serum. These cells were examined using a Leitz Ortho-lux 11 microscope, using rhodamine or fluorescein filters to permit visualization of the appropriate reagent.

Table 1. T Cell Subpopulations in Cord Blood and Adult Blood Specimens Defined by Monoclonal Antibodies

<table>
<thead>
<tr>
<th></th>
<th>OKT3</th>
<th>OKT4</th>
<th>OKT6</th>
<th>OKT8</th>
<th>OKT10</th>
<th>OKT11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord</td>
<td>52.8 ± 6</td>
<td>49.6 ± 7</td>
<td>24.2 ± 8</td>
<td>27.0 ± 9</td>
<td>58.2 ± 21</td>
<td>60.8 ± 9</td>
</tr>
<tr>
<td>(n = 25)</td>
<td></td>
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<tr>
<td>Adult</td>
<td>74.9 ± 7</td>
<td>58.0 ± 9</td>
<td>2.0 ± 3</td>
<td>20.8 ± 5</td>
<td>7.0 ± 5</td>
<td>76.0 ± 7</td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.768</td>
</tr>
</tbody>
</table>

Statistical Method

A nonparametric statistical test, the Kruskal-Wallis one-way analysis of variance by ranks, was used to analyze the data.

RESULTS

Examination of Whole Blood

The mean circulating absolute white cell population in ten samples of neonatal blood was 15.1 x 10⁶/mL. Examination of Wright-stained smears in eight of these preparations revealed that a mean of 38% of these cells were lymphocytes, indicating an increased total number of lymphocytes in neonatal blood (mean 5.74 x 10⁶/mL). The normal adult white blood cell count ranges from 5 to 10 x 10⁶/mL, 25%–35% of which are lymphocytes (1.25–3.5 x 10⁶/mL).

Comparison of Neonatal and Adult T Cell Subpopulations Using Monoclonal Antibodies

Some 52.8% ± 6% of circulating neonatal mononuclear cells reacted with the pan-T cell marker OKT3, a significantly lower percentage (P < .001) than that seen in normal adult controls (74.9% ± 7%) (Table 1). Parallel studies with the mature T cell marker, OKT11, revealed that 60.8% ± 9% of the neonatal mononuclear cells were positive, a highly significant discordance with OKT3 (P < .001). Of the normal adult control mononuclear cells, 74.0% ± 7% reacted with monoclonal OKT11. Of the cord mononuclear cells expressing OKT11 reactivity, 17% ± 11% were OKT3-negative, as shown in double-labeling studies (Table 2). All of the OKT3-positive cells were also reactive with OKT11.

OKT4-positive (helper/inducer) cells were significantly less common in cord blood (49.6% ± 7%) as compared with control blood (58.0% ± 9%) (P < .02). Conversely, OKT8-reactive (suppressor) cells were increased in cord blood: 27% ± 5% compared with 20.8% ± 5% in adult controls (P < .01).

Unlike adult control samples in which the sum of percentages of OKT4-reactive cells plus OKT8-reactive cells (79%) was approximately equal to the number of OKT3-reactive cells (75%), the sum of OKT4-positive and OKT8-positive cells in cord blood exceeded the percentage of OKT3 + cells by a mean of 22% (range 44%–0%). This suggested that, although
the ratio of OKT4:OKT8 was lower (1:2) for cord samples compared with 1:3 for the adult control group observed in this study \((P < .005)\), this may be the consequence of the presence of a population of immature cells simultaneously expressing both OKT4 and OKT8 antigens. To study this further, double-labeling experiments were carried out on cord blood samples; results obtained are shown in Table 2. In the study of OKT4 and OKT8 reactivity, 25% ± 17% of the cells reacted with both OKT4 and OKT8. The helper:suppressor ratio of the cells reactive with only OKT4 or only OKT8 was 3:1, which was the same as in our control group.

OKT10 reactivity was present on 58.2% ± 21% of cord mononuclear cells, but only on 7.0% ± 5% of the normal adult control cells \((P < .001)\). Of the neonatal mononuclear cells, 24.2% ± 8% expressed OKT6 reactivity as compared with 5.0% ± 3% reactivity in control adult blood \((P < .001)\). Double-labeling studies were performed on a population of cells reactive with OKT3 or OKT6: 35% were OKT3-positive and OKT6-negative (a mature T cell phenotype); 15% were OKT3-negative and OKT6-positive (a cortical thymocyte phenotype); and 43% were simultaneously OKT3- and OKT6-positive (a previously undescribed phenotype).

The results obtained for all samples are summarized in Fig 1.

**DISCUSSION**

The possible presence of immature T cells in neonatal blood has been suggested by previous immunologic investigations.

Diaz-Jouanen et al\(^{17}\) demonstrated that the percentage of T cells in cord blood, detected using an anti-human fetal thymocyte heteroantisierum, was within the normal range found in adult blood, despite a significantly lower number of E rosette-forming cells in these blood samples as compared to the adult controls.\(^{18}\) This dissociation of membrane markers, they suggested, was due to a relative immaturity of the E rosette receptors present on a percentage of the cord blood T cells. Ben-Zwi et al\(^{18}\) also suggested that immature T cells may be present in cord blood, as they observed that 5% of T cells in cord blood formed heat-stable E rosettes, which are formed by the majority of thymocytes but are not a characteristic feature of mature T cells.\(^{19}\) However, because some T cells, activated in mixed lymphocyte cultures, will also form heat-stable E rosettes and because a high rate of spontaneous DNA synthesis has been noted in cord blood,\(^{1,20-22}\) possibly in response to maternal antigens,\(^{22}\) it is also possible that the findings in cord blood reflect the presence of activated rather than immature T cells.

In this study, using monoclonal antibodies, we have demonstrated that a phenotypically immature population of T cells is present in the blood of normal human neonates. These cells react with OKT6 and OKT10, markers of intrathymic T cells, and some also simultaneously express phenotypic markers characteristic of mature helper and suppressor T cells.

Reinherz et al suggested that normal T cell differentiation involves three main stages.\(^{7}\) Initially, the early thymocyte is OKT9-positive and OKT10-positive. In the second stage, the common thymocyte in the thymic cortex expressed OKT4, OKT6, OKT8, and OKT10 differentiation antigens. However, in the third stage of the mature thymocyte within the thymic medulla, all cells display the OKT3 antigen, lose the OKT6 antigen, but maintain the OKT10 antigen. In addition, these cells may be divided into two distinct subsets—precursors of helper/inducer cells, which are OKT4-positive and OKT8-negative, and precursors of suppressor/cytotoxic cells, which are OKT8-positive and OKT4-negative. Upon release of thymocytes into the peripheral circulation in normal adults, OKT10 antigen is retained by <5% of the circulating T cells. The results from our study reveal blood lymphocyte phenotypes in the neonatal T cell population similar to those found in the thymus during early development of the T cell.

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**Table 2. Double-Labeling Study on Cord Blood Cells, Comparing Reactivity of OKT3 and OKT6, OKT3 and OKT11, OKT4 and OKT8**

<table>
<thead>
<tr>
<th>Number of Specimens Examined</th>
<th>OKT3 Only</th>
<th>OKT6 Only</th>
<th>OKT3 + OKT6</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>35 ± 19</td>
<td>15 ± 13</td>
<td>43 ± 20</td>
</tr>
<tr>
<td>OKT3 Only</td>
<td>0%</td>
<td>17 ± 11</td>
<td>85 ± 21</td>
</tr>
<tr>
<td>OKT4 Only</td>
<td>31 ± 12</td>
<td>10 ± 8</td>
<td>25 ± 17</td>
</tr>
</tbody>
</table>

**Fig 1. T cell subpopulations in human adult and cord bloods, characterized by monoclonal antibodies. C - cord; A - adult.**
Our findings support the suggestion of Yokoi et al.\(^2\) that a functionally immature T cell is present in human cord blood. They showed that, in both cord blood and adult blood, <5% T cells expressed Ia and Tac antigens, which are both activation antigens.\(^3\) However, cord blood T cells showed reduced expression of Ia compared with adult blood T cells following pokeweed mitogen or allogeneic stimulation. In addition, cord blood cells and adult blood cells grown with T cell growth factor both showed the same degree of expression of Tac antigen, but Ia expression was markedly reduced in cord blood cells. This inability to express Ia on activation is a characteristic of T cells in early human ontogeny and suggests a functional immaturity of cord blood T cells. In addition, our study demonstrated a population of cells that is simultaneously OKT3- and OKT6-positive; this presumably represents an additional step in normal T cell differentiation that was not previously identified from studies limited to intrathymic populations.

The exact nature and fate of these immature T cells is unclear. They may represent an immature set of T cells produced by a rapid division of thymocytes in the neonate and exported to the periphery before they are able to complete normal maturation. An analogous situation is encountered in pathologic states in which cell division is accelerated. For example, in the case of severe blood loss, a compensatory increase in red blood cell production leads to the presence of immature nucleated red blood cells in the circulation. If this were the mechanism, the cells could follow one of two pathways. They could either complete their maturation in peripheral tissues to become functional mature T cells, or they could be sequestered in the lymphoreticular system and destroyed.

A more intriguing explanation for the presence of these cells in neonatal blood is that they may represent, at least in part, the pool of immunoincompetent (early post-thymic or precursor) cells, which, in the mouse, differentiate in an extrathymic site and eventually migrate to the spleen, bone marrow, and other sites in the lymphoreticular system.\(^4\) Postthymic precursor cells are immunologically incompetent, but are committed to differentiation under thymic influence into one of the functionally competent T cell subpopulations. They also represent a possible source of T cell regeneration within the extrathymic compartment of the body.\(^5\) This mechanism may explain the high rate of spontaneous cell division in neonatal blood\(^6\) and also the ease with which cultures of neonatal lymphocytes are established.\(^7\)

Recent studies have suggested the possibility that an immature neonatal T cell population may be transformed by adult T cell leukemia virus (ATLV).\(^8\) Therefore, further study of this population of neonatal cells could provide more insight into the pathogenesis of ATLV- and HTLV-induced disease,\(^9\) as these viruses are either identical or closely related,\(^10\) both causing variants of cutaneous T cell lymphoma.

ACKNOWLEDGMENT

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REFERENCES


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