Expression of P2X Receptor Subtypes on CD34$^+$ Cells and c-kit$^+$ Cells of Human Umbilical Blood

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The presence of several subtypes of P2X receptors on early hemopoietic precursors (CD34$^+$) from human umbilical blood was detected by flow cytometry. The expression of P2X receptors on umbilical blood lymphocytes was an order of magnitude higher than that on adult human blood cells. Our results attest to early involvement of P2X receptors in differentiation of human hemopoietic cells.

Key Words: *CD34$^+$ cells; P2 receptors; umbilical blood*

Extracellular nucleotides (ATP, ADP, and uridine triphosphoric acid) serve as P2 receptor antagonists. These receptors were found on the cell surface in various human and animal tissues, including peripheral blood cells. Published data show that P2 receptors are involved in the inflammatory response [3]. It was hypothesized that P2 receptors can be identified at the earliest stages of tissue development (stage of stem cells) and play a role in cell differentiation and maturation. The membrane phosphoglycoprotein CD34 is one of the markers for stem cells. Tyrosine-protein kinase transmembrane receptor c-kit is another reliable marker of stem cells.

Previous studies showed that stimulation of CD34$^+$ cells from the peripheral blood and bone marrow with extracellular nucleotides is followed by a rapid release of calcium from the intracellular stores, activation of ion flux through the plasma membrane of these cells, and increase in cell proliferation [4,5]. Moreover, mRNA for P2X and P2Y receptors is expressed in human monocytes, lymphocytes, and bone marrow CD34$^+$ cells [6]. However, there are no data on the presence of certain subtypes of P2 receptors on CD34$^+$ hemopoietic precursor cells (*e.g.*, on human umbilical blood cells).

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This work was designed to reveal P2X receptor subtypes on CD34$^+$ cells from human umbilical blood.

MATERIALS AND METHODS

The umbilical blood was sampled during normal delivery. Informed consent form was obtained from each woman. The study was approved by the Ethics Committee of the Kazan State Medical University.

The blood was collected into tubes with sodium citrate. Mononuclear cells (monocytes, lymphocytes, and CD34$^+$ cells) were isolated by centrifugation on a Ficoll-Paque density gradient (Sigma). Immunomagnetic separation of CD34$^+$ cells from the mononuclear cell fraction was performed on a magnetic particle concentrator (Dynal MPS) according to the instructions for magnetic antibodies (Invitrogen).

CD34$^+$ cells were stained with allophycocyanin-conjugated mouse monoclonal antibodies (Abcam). The presence of CD34$^+$ cells was estimated from allophycocyanin fluorescence. P2X receptor subtypes were evaluated in the indirect immunofluorescence reaction with rabbit antibodies against $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$ receptors (Alomone, Abcam). Sheep secondary antibodies were conjugated with FITC (Sigma). c-kit receptor was verified with phycoerythrin-conjugated mouse monoclonal antibodies (Abcam). The expression of P2X receptors and

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TABLE 1. Relative Number of Human Umbilical Blood Cells Expressing P2X Receptors (%, *M*±*m*)

Receptor subtype	$CD34^+$ cells (*n*=11)	$CD34^+$/c-kit^+ cells (*n*=4)	Lymphocytes (*n*=3)	Monocytes (*n*=3)
$P2X_2$	12.8±5.1	13.2±7.8	32.8±15.0	16.8±10.4
$P2X_3$	17.0±5.9	15.2±4.7	27.0±8.5	11.1±8.2
$P2X_4$	18.3±7.1	15.4±7.3	36.3±6.2	11.5±6.5
$P2X_5$	17.1±6.5	15.2±5.8	42.0±8.3*	13.7±6.6
$P2X_6$	18.0±6.2	16.8±5.9	44.8±6.7*	17.8±10.4
$P2X_7$	16.7±6.5	16.6±5.9	40.8±4.8*	9.3±3.9

Note. *n*, number of samples. *$p<0.05$ compared to $CD34^+$ cells.

c-kit on $CD34^+$ and CD34 cells was studied by dual- and triple-color labeling.

The results of immunofluorescence study were recorded on a FACSCanto II flow cytometer (Becton Dickinson) with FACSDiva software. The results were analyzed by paired Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

Statistically significant data on the expression of P2X receptor subtypes were not obtained due to a low content of $CD34^+$ cells in the umbilical blood (1-2%). Further studies of P2X receptor expression were conducted on $CD34^+$ cells isolated from the mononuclear cell fraction by the method of immunomagnetic separation (Fig. 1).

The fraction of $CD34^+$ cells (at least 90% cells) and fraction of CD34 cells, lymphocytes, and monocytes were isolated from the mononuclear cell fraction by means of immunomagnetic separation. The presence of c-kit and P2X receptor subtypes on $CD34^+$ cells was estimated by dual- and triple-color flow cytometry. $CD34^+$ cells from human umbilical blood were shown to express all subtypes of P2X receptors (Table 1; Fig. 2).

Differences were found in the expression of P2X receptors on umbilical blood $CD34^+$ cells. $P2X_2$ receptors were expressed on 59 and 6% $CD34^+$ cells from two samples of the blood, respectively. The mean expression of $P2X_2$ receptors was 12.8±5.1% (Fig. 3).

Staining of $CD34^+$ cells by anti-c-kit antibodies showed that the majority of these cells contain 65.6-91.7% c-kit (75.7±3.9%, *n*=4). No significant differences were revealed in the relative content of $CD34^+$/c-kit^+ cells and $CD34^+$ cells that express the certain subtypes of P2X receptors (Table 1).

P2X receptor subtypes were also found on human umbilical blood lymphocytes. Their content on these

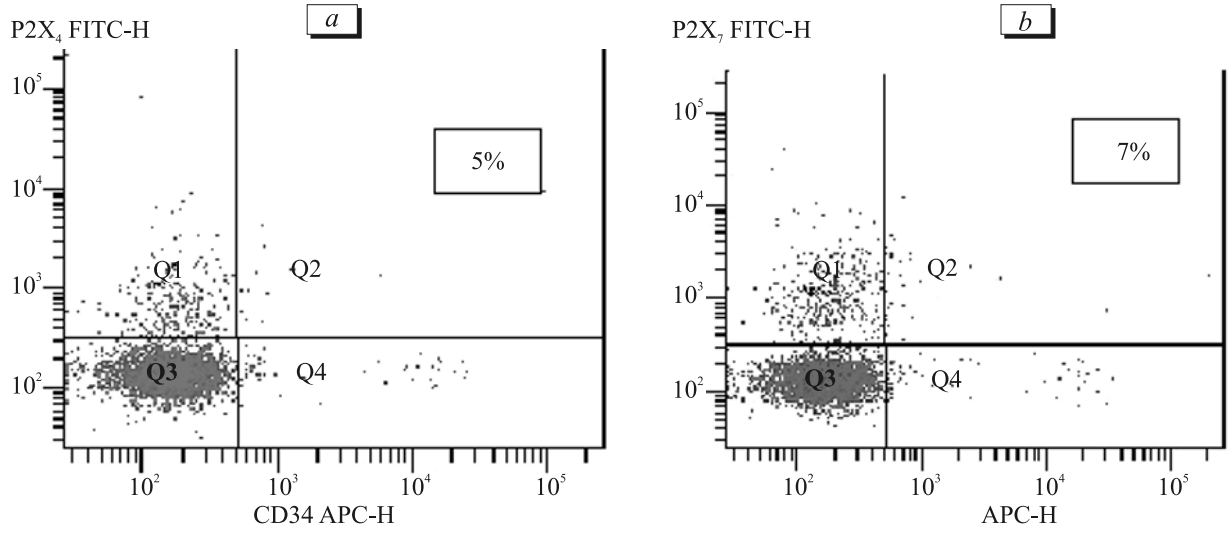

Fig. 1. Expression of P2X receptor subtypes (%) on umbilical blood $CD34^+$ cells before magnetic separation. Abscissa: fluorescence of anti-CD34 monoclonal antibodies conjugated with allophycocyanin (APC); ordinate: fluorescence of FITC-conjugated antibodies to $P2X_4$ (*a*) and $P2X_7$ receptors (*b*). Here and in Fig. 2: Q1, $P2X^+$/CD34 cells; Q2, $P2X^+$/$CD34^+$ cells; Q3, $P2X^-$/CD34 cells; Q4, $P2X^-$/$CD34^+$ cells.

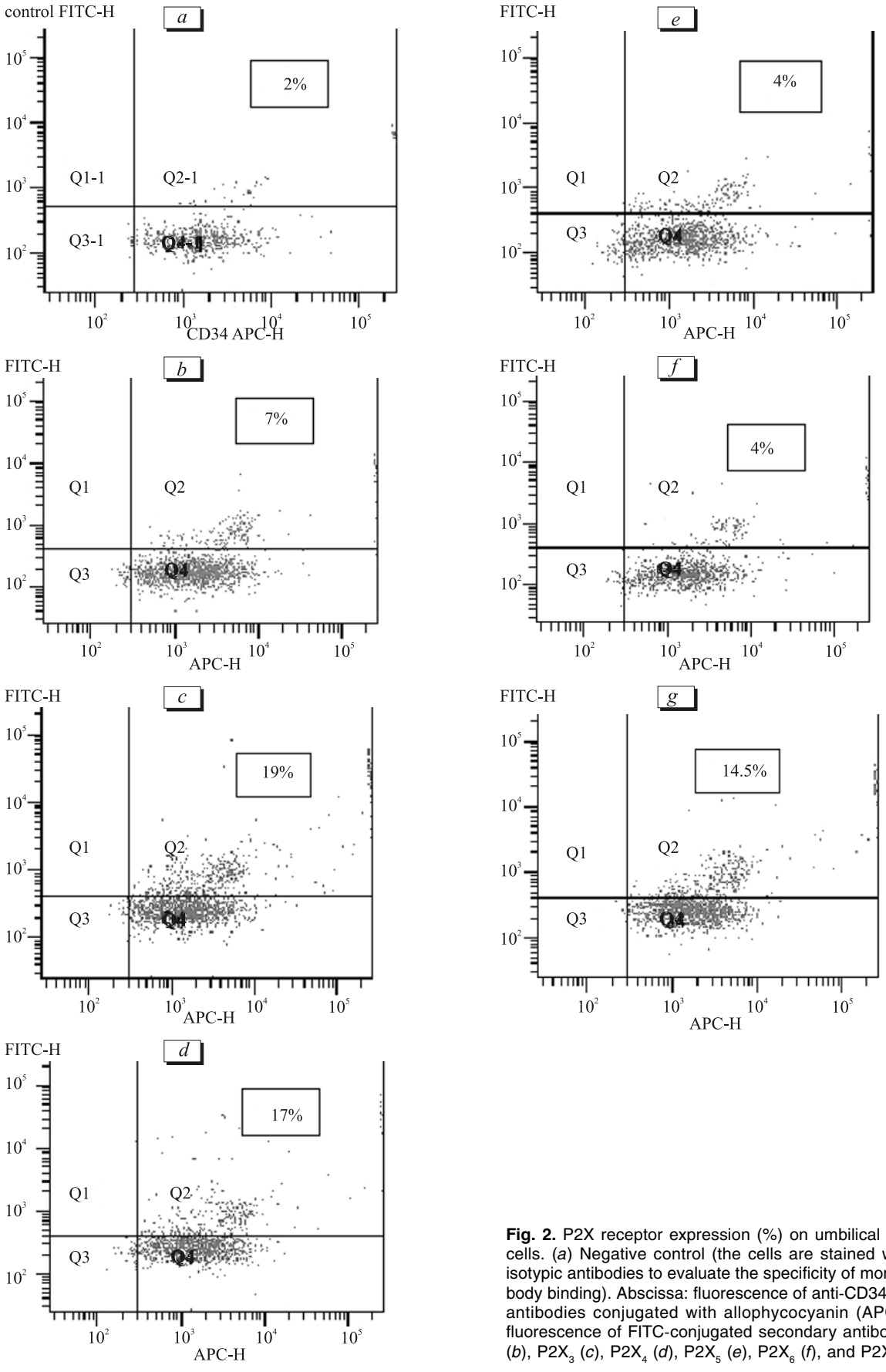

Fig. 2. P2X receptor expression (%) on umbilical blood $CD34^+$ cells. (*a*) Negative control (the cells are stained with negative isotypic antibodies to evaluate the specificity of monoclonal antibody binding). Abscissa: fluorescence of anti-$CD34^+$ monoclonal antibodies conjugated with allophycocyanin (APC); ordinate: fluorescence of FITC-conjugated secondary antibodies to $P2X_2$ (*b*), $P2X_3$ (*c*), $P2X_4$ (*d*), $P2X_5$ (*e*), $P2X_6$ (*f*), and $P2X_7$ (*g*).

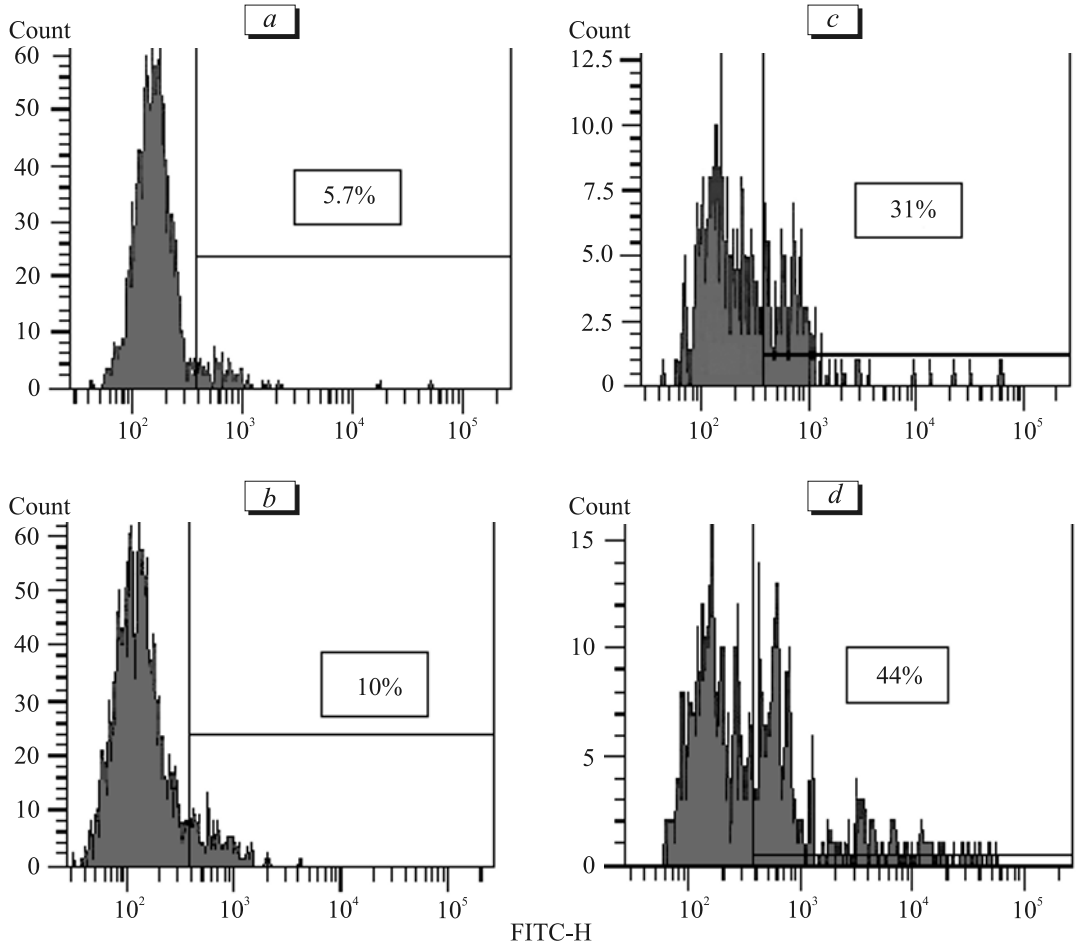

Fig. 3. $P2X_2$ receptor expression on umbilical blood $CD34^+$ cells. Marker (vertical line): $CD34^+$ cells expressing $P2X_2$ receptors (%), 4 samples of the umbilical blood (*a-d*). Abscissa: fluorescence of FITC-conjugated antibodies to $P2X_2$ receptors; ordinate: number of events.

cells was 2-3-fold higher than on $CD34^+$ cells. P2X receptor subtypes were identified on monocytes from the umbilical and peripheral blood. The expression of receptors on umbilical blood monocytes was shown to vary from 0 to 70%. However, we revealed no specific features of receptor expression (Tables 1 and 2). Various subtypes of P2X receptors were expressed on a lower number of peripheral blood lymphocytes from healthy donors (as compared to that on umbilical blood lymphocytes; Table 2).

Analysis of stem cells by only one parameter is not considered as an appropriate method. Therefore, we assayed the c-kit receptor for stem cell growth factor that plays a role in hemopoiesis. The results of quantitative measurements of c-kit on $CD34^+$ cells are consistent with published data [1].

Much attention was paid to studying of stem cells. However, the phenotype of true stem cells and the role of various populations in hemopoiesis are poorly understood. ATP plays a unique role in ontogeny [2]. It is important to evaluate the expression of P2X receptors on $CD34^+$ cells that serve as hemopoietic cells (*i.e.*, precursors of blood cells). This is of considerable fundamental and clinical importance, since umbilical blood serves as a promising source of the earliest stem cells.

These data show that the majority of umbilical blood $CD34^+$ cells (the one-fifth of cells) have a certain subtype of the P2X receptor. It remains unclear whether all subtypes of P2X receptors are present on one or several cells that express these subtypes. Multicolor fluorescence study with fluorescent-labeled antibodies is required to answer this question. The mean expression of P2X receptors in the population of $CD34^+$ cells is lower than in umbilical blood lymphocytes. The question arises whether the formation of P2X receptors on umbilical blood $CD34^+$ cells is a primary process, or they appear due to the interaction with an antigen for further integration? The expression of P2X receptor subtypes on umbilical blood $CD34^+$ cells is high, which suggests that these receptors play a role in the maturation of blood cells and whole body. ATP affects various P2X receptors, which probably modulates the direction of blood cell differentiation. This assumption is confirmed by the fact that ATP

TABLE 2. Relative Number of P2X Receptor-Expressing Lymphocytes and Monocytes in the Peripheral Blood from Donors (%, *M*±*m*)

Receptor subtype	Lymphocytes (*n*=4)	Monocytes (*n*=4)
$P2X_2$	3.90±1.10	2.47±1.00
$P2X_3$	6.40±0.70	19.40±16.90
$P2X_4$	3.30±1.00	7.57±6.85
$P2X_5$	1.20±0.80	25.20±18.50
$P2X_6$	2.30±0.90	37.80±21.30
$P2X_7$	1.80±0.50	24.00±18.90

serves as a universal agonist of nearly all subtypes of P2X receptors on blood cells.

Interestingly, in adult donors the number of peripheral blood lymphocytes carrying P2X receptor subtypes is an order of magnitude lower than the number of umbilical blood lymphocytes with P2 receptors. The appearance of P2X receptors on umbilical blood lymphocytes is probably a secondary process, which serves for additional signaling. These data confirm the notion that P2 receptors play a greater role in the developing body than in the mature organism. Published data show that P2X receptor expression in the myometrium increases with increasing the duration of pregnancy [2].

Blood $CD34^+$ cells can maturate into blood cells and endothelial cells. Moreover, c-kit serves as a marker of hemopoietic cells. Hence, the identification of c-kit on $CD34^+$ cells confirms the hemopoietic pathway of $CD34^+$ cell development.

Our results form a basis for studying the role of P2X receptors in differentiation and proliferation of hemopoietic stem cells.

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