

Original article

Isolation of transcriptionally active umbilical cord blood-derived basophils expressing FcεRI, HLA-DR and CD203c

Background: Basophils are inflammatory cells associated with allergy and parasite infections. Investigation of their true biological function has long been hampered by the difficulty in obtaining sufficient amounts of pure basophils and by the lack of phenotypic markers. Moreover, it has been very difficult to clone and identify basophil-specific granule proteins, partially because of an almost complete lack of mRNA in mature circulating basophils.

Methods: To obtain transcriptionally active immature basophils, umbilical cord blood cells were cultured in the presence of interleukin (IL)-3. The cells were analysed by flow cytometry and by histological staining.

Results: The continuous presence of IL-3 in cord blood cultures resulted in the expansion of basophil precursors co-expressing FcεRI and the recently described mast cell/basophil marker, 97A6 (CD203c). Several nonbasophil markers (i.e. CD3, CD14, CD15, CD16, CD19 and CD21) were absent on the cultured basophils. However, we show that in early cultures, almost 60% of the CD203c⁺ cells co-express human leukocyte antigen (HLA)-DR, a marker that is absent on mature circulating basophils. The presence of HLA-DR on basophil precursors may explain the low recovery ($24 \pm 5.2\%$) obtained after isolation of cultured basophils, when using a conventional basophil isolation kit that removes HLA-DR⁺ cells. A novel purification method was developed, including a two-step cocktail of antibodies against selected markers, which resulted in both high purity ($95 \pm 0.5\%$) and recovery ($59 \pm 1.5\%$) of cultured basophils.

Conclusions: We here establish cord blood cultures as a source from which transcriptionally active basophil precursors can be isolated in reasonable quantities for thorough biochemical characterization.

**J. M. Reimer¹, S. Magnusson¹,
M. Juremalm², G. Nilsson^{2, 3},
L. Hellman¹, S. Wernersson¹**

Departments of ¹Cell and Molecular Biology and ²Genetics and Pathology, Uppsala University, Uppsala, Sweden; ³Department of Medicine, Karolinska Institute, Stockholm, Sweden

Key words: basophil; facs analyses; IgE.

Sara Wernersson
Department of Molecular Biosciences
Section of Medical Biochemistry
Swedish University of Agricultural Sciences
Biomedical Center
Box 575
SE-751 23 Uppsala
Sweden
Present address: Mikael Juremalm, Gyros AB,
Uppsala, Sweden

Present address: Sara Wernersson, Department
of Molecular Biosciences, Swedish University of
Agricultural Sciences, Uppsala, Sweden

Accepted for publication 29 March 2006

Basophils share many functional and structural features with both mast cells and eosinophils, and all three are important effector cells in allergic responses [as reviewed in Refs. (1, 2)]. The relative contribution of basophils to human diseases has been difficult to estimate because of the lack of specific means of distinguishing these cells, and their effector molecules, from that of other cell types. However, antibodies binding specifically to the granules (3, 4) or to the surface (5, 6) of human basophils have been reported. This has provided new tools in the search for basophil function during allergic diseases, and has demonstrated the presence of basophils in late phase reactions of the nose (7), skin (8, 9) and lung (10). Yet, none of the antigens bound by these basophil-specific antibodies have so far been identified.

Interestingly, the generation of antibodies binding specifically to basophilic granules (3, 4, 11) indicates the

existence of unique still unidentified granule proteins in these cells. Moreover, we have previously described a serine protease denoted mMCP-8 that is specifically found in the granules of murine basophils (12). This was the first basophil-specific marker cloned in any species. However, the human counterpart for this protease has not yet been identified and a corresponding gene cannot be found in the human genome (M. Gallwitz and L. Hellman, unpubl. obs.).

A major obstacle in the identification of new basophil-specific proteins has been the difficulty in obtaining sufficient numbers of pure basophils in combination with an almost complete absence of functional mRNA in peripheral blood basophils (M. Poorafshar and L. Hellman, unpubl. obs.). A feasible explanation for the extremely low mRNA levels is that, like certain other leukocytes, i.e. neutrophils and eosinophils, basophils mature in the bone marrow and enter the circulation as terminally differentiated cells with very little ongoing protein synthesis. The cloning of novel lineage-specific genes from other granulocytes have, therefore, almost exclusively come from the use of immortalized tumour

Abbreviations: FcεRI, high-affinity Fc receptor for IgE; HLA, human leukocyte antigen; IL, interleukin; MACS, magnetic-activated cell sorting.

cell lines (13–15) or *in vitro* cultivated cells (16), which are actively dividing and contain large amounts of mRNA. Two tumour cell lines with basophil origin have also been described, KU812 and LAMA84 (17–19), but they display characteristics of multiple lineages (18) and have a very low degree of granulation, which means that they are not optimal for studies of lineage-specific granule proteins. An alternative is, therefore, to use cultured basophil precursors. It is well established that, in cord blood or bone marrow cultures, IL-3 promotes the expansion of a cell type closely resembling basophils as demonstrated by cell surface expression, staining properties and functional characteristics (20–22).

In the present study, we analyse the kinetics and surface marker expression on cord blood-derived basophils. We confirm the findings of Kepley et al. (22), that pulsing with IL-3 generates the most mature basophil phenotype. However, a continuous stimulation with IL-3 was optimal to obtain large quantities of basophil precursors. We identify the co-expression of FcεRI and the recently described mast cell/basophil marker 97A6 (CD203c) (23) on cultured basophils and show that a large proportion of CD203c⁺ cells express HLA-DR. Finally, we present a novel purification method for optimal recovery and purity of *in vitro* derived basophil precursors.

Materials and methods

Culture of umbilical cord blood basophils

After informed consent and after approval from the local Ethical Review Board at the University Hospital, Uppsala, umbilical cord blood was collected from normal, full-term deliveries. Mononuclear cells were isolated from heparinized cord blood by Ficoll Hypaque® (Amersham Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation, according to manufacturer's instructions. Cells were washed in phosphate buffer saline (PBS) and re-suspended at a concentration of 1×10^6 cells/ml in RPMI 1640 medium supplemented with 10% foetal calf serum, 50 µM 2-mercaptoethanol, 10 mM Hepes, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 IU/ml penicillin, and 50 µg/ml streptomycin (Life Technologies, Renfrewshire, UK). In addition, medium was supplemented with 10 ng/ml recombinant IL-3 (R&D Systems, Abingdon, UK) and in selected experiments with 10% of IgE-containing culture supernatant derived from the myeloma cell line U266 (24) (kindly provided by Helena Jernberg Wiklund, Department of Genetics and Pathology, Uppsala University, Sweden). Cells were maintained at 37°C and 5% CO₂ for up to 26 days. Medium was changed after the first 7 days and thereafter every third day.

Histological staining

Cord blood cells or purified basophils were cytocentrifuged onto slides and stained with May-Grünwald and Giemsa (Merck, Darmstadt, Germany). Metachromatic staining of granules was performed by incubating slides in 1% Alcian blue 8 GX (Sigma, St Louis, MO, USA), diluted in 60% ethanol and 3.7% hydrochloric acid, for 15 min. To stain cell nuclei, slides were incubated for 5 min with nuclear fast red counterstain (Apoteksbolaget, Södersjukhuset,

Stockholm, Sweden). After staining, slides were rinsed for 2 s in dH₂O, air-dried and mounted.

Flow cytometry

To analyse the expression of selected surface markers on cultured basophils, samples of cells were collected at different time points. Cells were washed in PBS containing 0.5% FCS. After staining of cells (10^5 – 10^6 per sample) for 30 min with monoclonal mouse IgG1 anti-human IgE (CIA-E-7.12., DAKO A/S, Glostrup, Denmark) or an unspecific control antibody, mouse IgG1 (BD Pharmingen, San Diego, CA, USA), cells were washed twice and incubated for 30 min with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG1 (BD Pharmingen). Alternatively, cells were stained with FITC-labelled anti-CD3, anti-CD16, anti-CD19, anti-CD21 (DAKO A/S), anti-CD15 (BD Pharmingen), or their isotype-matched controls. After washing twice, cells were stained with R-phycoerythrin (PE)-conjugated anti-CD203c (97A6, Immunotech, Marseille, France), anti-CD14 (BD Pharmingen) or with their isotype-matched controls. To avoid cell activation and apoptosis, cells were kept on ice, washed with ice-cold buffer and centrifuged at 4°C. After staining, cells were washed and re-suspended in 0.5 ml buffer and, in the final step, 1 µg/ml propidium iodide was added. Data from 10 000 cells per sample were collected and analysed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and the CELLQuest™ 3.1 software. To ignore dead propidium iodide-stained cells, a FL-3 gate was used. Additionally, the leukocyte population was gated in a forward scatter/side scatter plot to ignore erythrocytes and auto-fluorescent large blast cells.

Isolation of basophils

Cultured basophils were purified by magnetic-activated cell sorting (MACS) using either the Basophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to manufacturers description or using a one- or two-step cocktail described below. Remaining erythrocytes in cultures were initially removed by Ficoll Hypaque® separation (Amersham Pharmacia Biotech). In the one-step cocktail, cells were stained in $100 \mu\text{l}/10^7$ cells with 6 µl anti-CD14, 6 µl anti-CD3, 3 µl anti-CD15, 1 µl anti-CD16 and 4 µl anti-CD19 Microbeads (Miltenyi Biotec) in MACS-buffer (PBS, 2 mM EDTA, pH 7.2, 0.5% BSA) for 30 min on ice, followed by washing and re-suspension in $500 \mu\text{l}$ MACS-buffer/ 10^8 cells. In the two-step cocktail procedure, cells were primary stained by adding, to a washed cell pellet, per 10^7 cells: 5 µl FcR-Blocking Reagent (Miltenyi Biotec), 20 µl IgG1 anti-CD3 (0.1 µg/µl, DAKO A/S), 10 µl IgG1 anti-CD16 (0.1 µg/µl, DAKO A/S), 20 µl IgG1 anti-CD36 (0.04 µg/µl, Immunotech), and 35 µl IgG1 anti-CD45RA (0.05 µg/µl, Immunotech). Cells were then washed in MACS-buffer and labelled in $100 \mu\text{l}/10^7$ cells with 20 µl anti-mouse IgG1, 10 µl anti-CD14 and 10 µl anti-CD15 Microbeads (Miltenyi Biotec). Labellings were performed on ice for 30 min with gentle shaking. MACS-buffer corresponding to 20 times the secondary labelling volume was added before separation. Cells were magnetically separated on CS Columns according to manufacturers' instructions.

Results

Expansion of basophils in cord blood cultures

To study the kinetics of basophil differentiation *in vitro*, mononuclear cells were isolated from umbilical cord blood and cultured under the influence of IL-3. Cells were

either cultured in the continuous presence of 10 ng/ml IL-3 or pulsed with 20 ng/ml IL-3 for 3.5 h or 7 days. The number of basophils in cultures was determined by metachromatic staining of basophilic cells. All three culture conditions induced the expansion of basophils although the highest number of total basophils was obtained in the constant presence of IL-3 (Fig. 1). While promoting the lowest number of total basophils, IL-3 pulsing for 3.5 h resulted in a higher fraction of basophils (88% on day 21) when compared with the continuous IL-3 stimulation (61% on day 17) (Fig. 1A). Basophils stimulated with a continuous dose of IL-3 displayed a more immature phenotype characterized by large round nuclei instead of the lobed nuclei seen for pulsed basophils (not shown).

FcεRI and CD203c are co-expressed on cultured basophils

A common surface marker on human basophils, mast cells and their CD34⁺ progenitors was recently described and designated 97A6 or CD203c (23). This surface marker could become a very useful marker in allergy diagnosis as activated basophils upregulate their surface expression of CD203c [as reviewed in Ref. (25)]. The expression of CD203c on cord blood-derived basophils has not been described previously but may provide an excellent tool for detailed studies of early basophil differentiation. The presence of IgE is known to increase the expression of surface FcεRI on human basophils [as

reviewed in Ref. (26)]. However, in our culture system the presence of an IgE-containing culture supernatant derived from the myeloma cell line U266 (24) did not have a significant effect on either the basophil yield or the expression of FcεRI and CD203c (data not shown). To follow the early basophil differentiation, IL-3 driven umbilical cord blood cells were analysed for the expression of CD203c and FcεRI. Although the presence of IgE did not upregulate the FcεRI expression, cells were cultured in the presence of human IgE to allow for its binding to FcεRI, thus facilitating the staining procedure. Co-expression of FcεRI and CD203c was observed on the expanding basophil population days 4–19 (Fig. 2). A population of CD203c⁺FcεRI[−] cells was detected after 10 days in culture, probably reflecting the presence of a very early basophil precursor. Moreover, a small population of CD203c[−]FcεRI⁺ cells was seen by day 19 (Fig. 2).

Characterization of cultured basophils

Based on the kinetic data from previous experiments (Figs 1 and 2), we assumed that umbilical cord blood cells cultured in the presence of IL-3 would contain an adequate number of proliferating immature basophils in the second week of culture. Basophils cultured for 9 or 12 days were isolated by negative selection using a commercially available kit (MACS Basophil Isolation kit) that has been optimized for the purification of peripheral blood basophils. The purity of the isolated cells was 97% and 98% but the yield was only 14% and 32%, respectively, as determined by counting the fraction of Alcian blue positive cells on slides (Fig. 3A and Table 1, donor 1). The cells were analysed by May-Grünwald–Giemsa staining, resulting in a typical dark staining of basophilic granules (Fig. 3B). Flow cytometry analyses showed that by day 9, 77% of the purified cells expressed CD203c and of these 56% also expressed FcεRI (Fig. 3C). Notably, 23% of the purified Alcian blue-positive cells lacked expression of both CD203c and FcεRI (Fig. 3C). By day 12, 49% of the purified basophils expressed the FcεRI, and 53% expressed CD203c (Fig. 3D and E). The reduced level of CD203c and increased level of FcεRI at this time point may reflect the presence of more mature CD203c^{low}FcεRI^{high} basophils among the Alcian blue-stained cells.

Early expression of HLA-DR on cultured basophils

The low yield of basophils obtained when using the Basophil isolation kit suggested that immature basophils express surface markers not present on normal blood basophils. Therefore, the expression of different CD markers on cord blood derived basophils was analysed. This screening resulted in the detection of HLA-DR on a large proportion (59%) of the CD203c⁺ cells by day 10 (Fig. 4A). However, the HLA-DR expression was down-

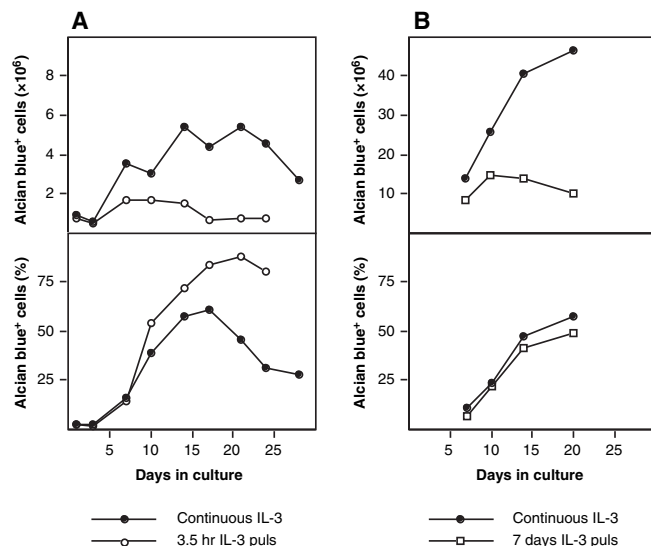


Figure 1. The expansion of basophils in umbilical cord blood cultures stimulated with a continuous presence of 10 ng/ml IL-3 (filled circles) or pulsed with 20 ng/ml IL-3 for 3.5 h (open circle) or 7 days (open square). Within each experiment, cells obtained from the same donor were split into two cultures each containing 10×10^6 (A) or 25×10^6 (B) leukocytes. The numbers of basophils were determined by Alcian blue staining. Each value was based on a total of at least 200 counted cells.

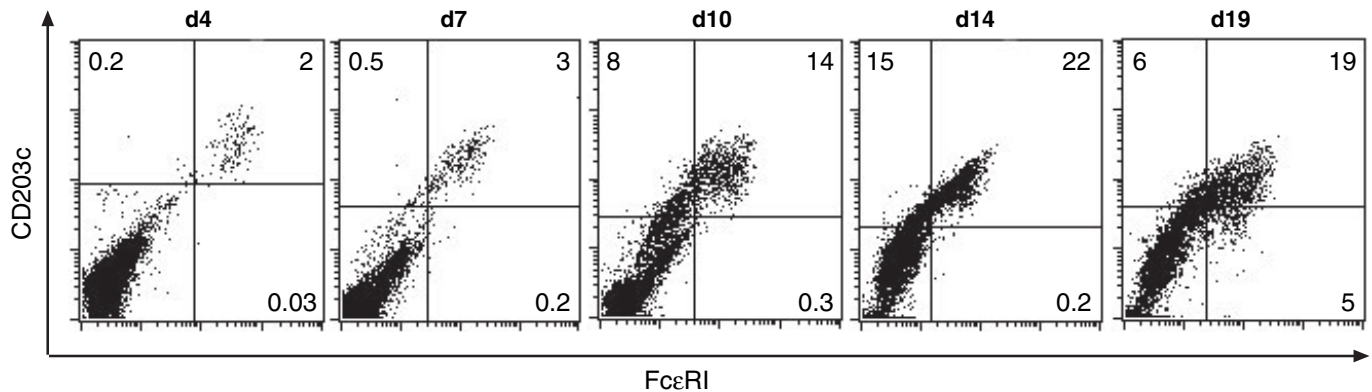


Figure 2. Expression of FcεRI and CD203c on cultured umbilical cord blood cells. Cord blood cells cultured in the presence of 10 ng/ml IL-3 and 10% IgE supernatant, were collected at the indicated time points and analysed by flow cytometry for the expression of FcεRI (staining with IgG1 anti-IgE followed by anti-IgG1-FITC) and CD203c (97A6). The viable leukocyte population was gated to exclude large blasts, dead cells and erythrocytes. Quadrant gates were set individually for each sample to assure that < 1% false positives were detected in each quadrant of control samples (stained with isotype-matched antibodies). Numbers of events are given as the percent in each quadrant of total events in each sample. Staining is expressed as log₁₀ fluorescence intensity. The results are representatives of at least two experiments for each time point.

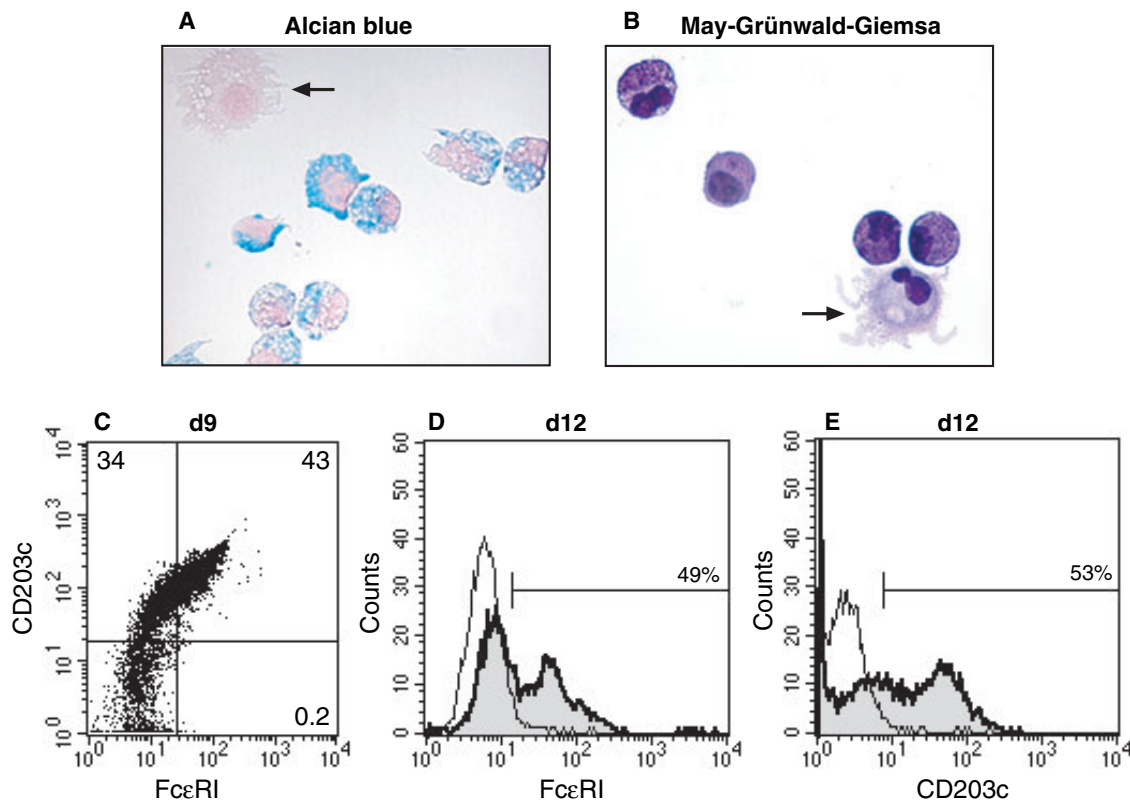


Figure 3. Morphology and surface-marker expression of enriched cord blood basophils. Cord blood cells were cultured in the presence of IL-3 and IgE for 9 days (C) or 12 days (A, B, D and E) and basophils were isolated using the MACS basophil isolation kit. The enriched cord blood basophils were stained with Alcian blue and nuclear fast red (A) or May-Grünwald-Giemsa (B). Representatives of the small fraction (<4%) of nonbasophils, i.e. large blast cells, are demonstrated for both staining techniques (arrows). The expression of FcεRI and CD203c on purified cells was analysed by flow cytometry. All viable cells were included in the analyses and quadrant gates were set to assure that, for the corresponding isotype-matched control sample (thin line in D and E), less than 1% false positives were detected. Staining is expressed as log₁₀ fluorescence intensity.

Table 1. The purity and recovery of basophils isolated from cord blood cultures

Donor	Days in culture	Method	Purity (%)	Recovery (%)
1	9	MACS kit	98	14
	12	MACS kit	97	32
2	8	MACS kit	97	25
	8	One-step cocktail	46	97
	9	One-step cocktail	57	104
	9	Two-step cocktail	94	60
3	9	Two-step cocktail	95	57

Umbilical cord blood was cultured in the presence of 10 ng/ml IL-3 and purified at the indicated days. Three different purification methods based on negative selection of magnetically labelled nonbasophils were used. MACS Basophil isolation kit (MACS kit) from Miltenyi Biotec includes antibodies against CD3, CD7, CD14, CD15, CD16, CD36, CD45RA and HLA-DR. A cocktail of magnetically labelled antibodies (one-step cocktail) directed against CD3, CD14, CD15, CD16 and CD19 were used. Alternatively, basophils were isolated by preincubation of cells with unlabelled IgG1-antibodies specific for CD3, CD16, CD36 and CD45RA followed by magnetically labelled anti-IgG1, anti-CD14 and anti-CD15 (two-step cocktail).

regulated as the culture proceeded and by day 20, less than 25% were CD203c⁺HLA-DR⁺. The expression of HLA-DR on IL-3 pulsed (3.5 h) basophils was only 5% (Fig. 4D), thus displaying a phenotype more closely resembling that of mature normal basophils. The expression of HLA-DR on basophils cultured in the constant presence of IL-3 most likely explains the low yield obtained with the Basophil isolation kit. This kit uses antibodies towards HLA-DR for negative selection. As shown in Fig. 5, the immature cultured basophils (CD203c⁺ cells) lack several other differentiation markers, including CD3, CD14, CD15, CD16, CD19 and CD21. However, small populations of CD203⁻ cells expressing CD3 (9%), CD15 (10%) or CD19 (8%) were seen among the gated leukocyte population (Fig. 5D, E and G). Note also that 90% of the large blasts present in

the IL-3 driven cultures expressed the monocyte/macrophage marker CD14 (Fig. 5C).

Purification of immature basophils

A series of experiments to optimize the purification of immature basophils from cultures was conducted. The MACS Basophil isolation kit was compared with two HLA-DR nondepleting methods: a home-made 'one-step cocktail' of magnetically labelled antibodies against CD3, CD14, CD15, CD16 and CD19, or a 'two-step cocktail' including preincubation of cells with unlabelled IgG1 against CD3, CD16, CD36 and CD45RA followed by magnetically labelled anti-IgG1, anti-CD14 and anti-CD15. In three independent experiments, purification with Basophil isolation kit resulted in high purity ($97 \pm 0.33\%$) and low recovery ($24 \pm 5.2\%$) (Table 1). In contrast, when using the one-step cocktail, a significantly higher recovery of basophils ($100 \pm 3.5\%$) was obtained, but at a very low purity ($52 \pm 5.5\%$) (Table 1). Finally, in two independent experiments a two-step cocktail resulted in high purity ($95 \pm 0.5\%$) and significantly higher recovery ($59 \pm 1.5\%$) than that obtained with the Basophil isolation kit (Table 1). Thus, the two-step cocktail proved to be the best method for isolation of immature basophils from early cord blood cultures.

Cultured basophils are transcriptionally active

Previous attempts to make cDNA libraries from mature blood basophils have not been successful, because of the low transcriptional activity of these cells. For example, a library with a total of 100 000 recombinants was constructed from RNA extracted from 40×10^6 isolated blood basophils. Almost all inserts tested from this library were found to contain fragments of chromosomal

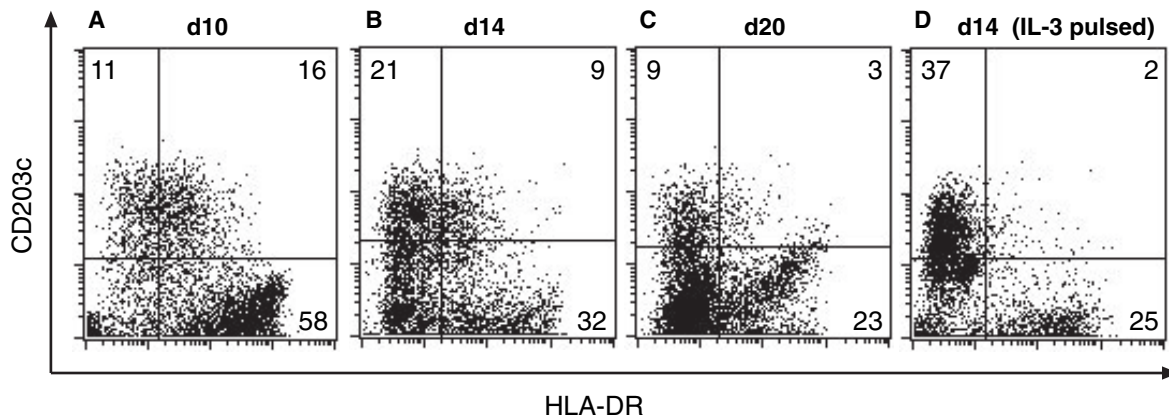


Figure 4. Expression of HLA-DR and CD203c on cultured umbilical cord blood cells. Cord blood cells, cultured in the presence of 10 ng/ml IL-3 (A–C) or pulsed with 20 ng/ml IL-3 for 3.5 h (D), were collected at the indicated time points and analysed by flow cytometry for the expression of HLA-DR and CD203c. The viable leukocyte population was gated to exclude large blasts, dead cells and erythrocytes. See Fig. 3 for details on quadrant gates and demonstration of results. Representative examples out of at least three experiments are shown in (A–C).

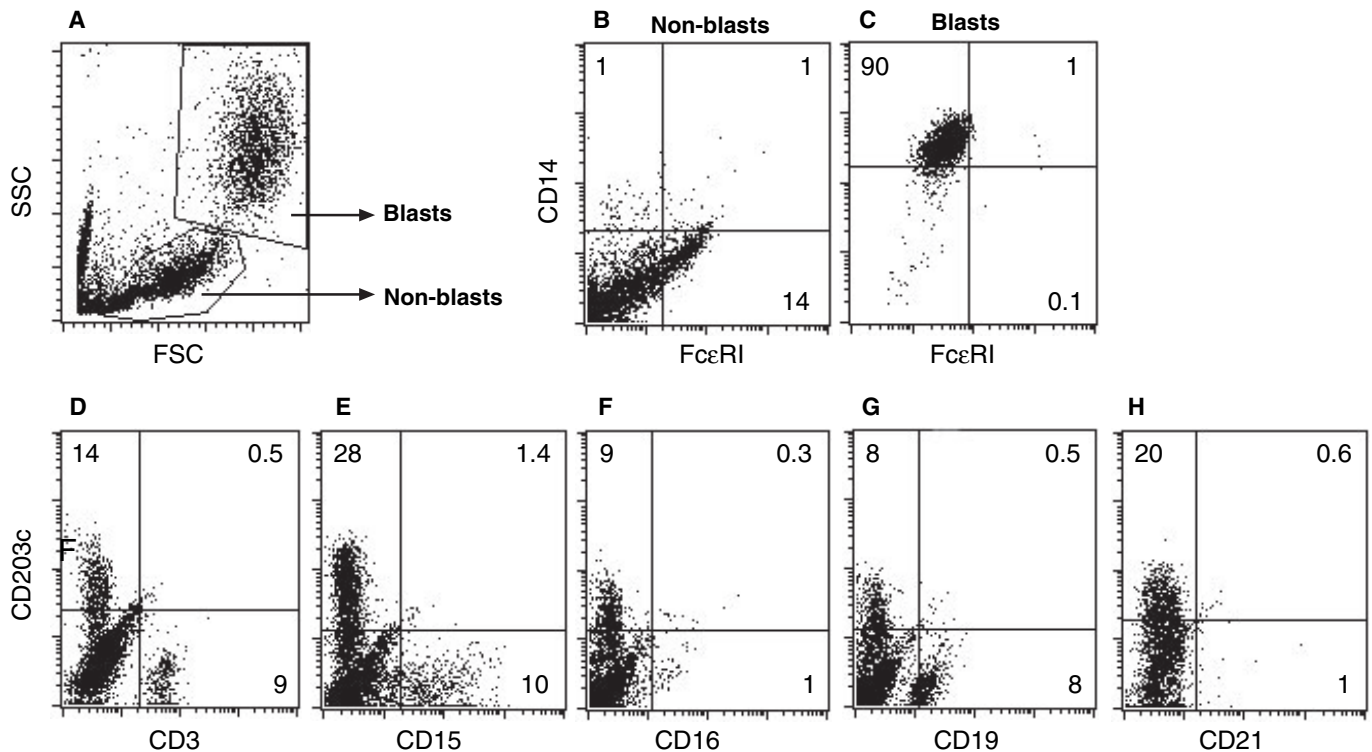


Figure 5. Surface marker expression on cultured umbilical cord blood cells. Cord blood cells, cultured in the presence of 10 ng/ml IL-3 alone (D, E and H) or together with 10% IgE supernatant (A–C, F and G), were analysed by flow cytometry for the expression of several surface markers as indicated in the figure. Cells were analysed on day 8 (A–C, F and G), day 10 (D and E) or day 14 (H). The viable leukocyte population (B, D–H) or blast population (C) was gated (A) to exclude dead cells and erythrocytes. See Fig. 3 for details on quadrant gates and demonstration of results.

DNA originating from apoptotic cells (M. Poorafshar and L. Hellman, unpubl. obs.). A very high percentage of the clones also contained repetitive sequences, a feature of chromosomal DNA. The major presence of inserts with chromosomal DNA, instead of cDNA, strongly suggests that peripheral blood basophils contain very small amounts of mRNA. In sharp contrast, a high-quality cDNA library with approximately 600 000 independent recombinants could be constructed, using the newly developed two-step protocol for the purification of 34×10^6 immature basophils from early cord-blood cultures. The majority of clones within this library contain cDNA inserts, thus reflecting the presence of sufficient amounts of mRNA in the isolated cells. This library has been screened with probes for several marker genes. We observed a level of actin mRNA in the same range as in a high-quality cDNA library from rat peritoneal mast cells (13): 480 and 397 clones in 100 000 plaques respectively. Two proteins expressed by both eosinophils and basophils, i.e. major basic protein and Charcot–Leyden crystal protein, were also highly expressed, and constituted 2% and 0.6% of the total mRNA pool respectively. This finding together with the expression of substantial levels of the FcεRI α -chain show

that we have a high-quality cDNA library with many of the key features of the proteome of mature basophils.

Discussion

The purpose of this investigation was to study the *in vitro* differentiation of basophils by flow cytometry, and to optimize the generation and purification of actively dividing basophil progenitors for future studies of the basophil transcriptome. The results from our screening of different culture conditions clearly shows that there is an advantage of culturing cells in the constant presence of IL-3 when compared with pulsing with IL-3; this despite the fact that the pulsing with IL-3 generates a more mature phenotype. The constant presence of IL-3 gives a substantially higher yield of immature cells for studies of transcriptionally active basophils.

The screening for markers to identify and purify immature basophils resulted in the identification of a major subpopulation of immature cells that co-express FcεRI and CD203c. These results confirm that CD203c is indeed a marker expressed very early during basophil differentiation and we, therefore, consider this marker a

good alternative to Fc ϵ RI, for identifying basophil precursors. The presence of a CD203c⁺Fc ϵ RI⁻ cell population already by day 9 may also indicate the presence of an initial single positive precursor population of interest for studies of the early events in basophil differentiation. Moreover, the identification of Alcian blue-positive cells without expression of either CD203c or Fc ϵ RI suggests that the formation of granules precedes the expression of both these surface markers during basophil differentiation. This is of particular interest as it suggests that high levels of mRNA for granule-stored proteins would be present in very early basophil precursors. Thus, it would be of advantage to isolate cultured basophils before they differentiate into more mature stages. However, as the fraction of basophils are low at earlier time points, effective purification protocols are needed to be able to analyse lineage-specific gene expression in these cells.

Different methods to purify blood basophils have been described by several groups (27–30). One of the currently and most commonly used purification protocol [Basophil isolation kit, Miltenyi Biotec (29)] is based on negative selection against several markers, including HLA-DR. This protocol resulted in substantially lower yields of basophils from the IL-3 driven cord blood cultures, presumably because of the expression of HLA-DR on early basophils. To solve this problem, we here describe a method based on negative selection optimal for the purification of *in vitro* cultured basophils, which omits the use of anti HLA-DR antibodies in the cocktail. This method substantially improves the yield in isolating immature human basophils.

Numerous studies have shown that basophils are potent producers of cytokines and in particular Th2-type cytokines like IL-4 and IL-13 (31–36). In the absence of potent Th1-inducing cytokines like IL-12 and interferon-

α , basophils may actually be one of the most important cell type in setting the Th2 environment (12, 37). Basophils are also known to produce and secrete the vasoactive substances leukotriene C4 and histamine, and are, thereby, important effector cells during an inflammatory response. The presence of two different antibodies binding specifically to basophil granules (3, 4) indicates the presence of lineage-specific proteins that might also be involved in the effector function of basophils. Indeed, one of these epitopes, termed basogranulin, is secreted from activated basophils in parallel with histamine (11). However, none of these potential basophil-specific proteins has yet been cloned or analysed concerning their role in basophil function.

In conclusion, the present study has generated important information as to the initial steps of basophil differentiation in IL-3 driven cord blood cultures. The recently described basophil marker CD203c as well as HLA-DR was identified as early markers on basophil precursors and the formation of granules was seen even before the expression of Fc ϵ RI and CD203c. Moreover, the improved culture conditions and purification protocol for obtaining immature basophils may facilitate the cloning of granule-stored proteins, thus adding to the understanding of the biological function of basophils.

Acknowledgments

We thank Maria Aveskogh for excellent technical assistance. This work was supported by the Swedish Society for Medical Research, the Swedish Society of Medicine, the Swedish Asthma and Allergy Association, King Gustaf V:s 80 Year Foundation, the Vardal Foundation, and the Swedish Research Council.

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