

Volume 12, issue 2, June 2001 | Recherches

Production of cytokines by immature erythroid cells derived from human embryonic liver

Abstract

It is well known that regulatory interactions between hematopoietic and lymphoid cells are mediated by different mediators. The cells of erythroid lineage are not an exception and have a regulatory effect on hemato- and immunopoiesis that can be mediated through the production of cytokines i.e. by soluble factors – a universal mechanism for cell regulation in hematopoietic and immune systems. It has been previously shown that erythroid progenitor cells from mice express mRNA of cytokines such as IL-1a and b, IL-4, IL-6, IFN-g, GM-

CSF and TGF-b. In this report we present the results of the production of the main immunoregulatory cytokines by erythroid cells derived from human embryonic liver. It was revealed that the cell population enriched with erythroid progenitors, isolated from human fetal liver, can produce IL-1b, IL-2, IL-4, IL-6. The levels of production of cytokines by immature erythroid progenitor cells is compared to the levels of corresponding cytokines produced by mitogen-stimulated peripheral blood mononuclear cells. The production of these cytokines changed quantitatively under the effect of erythropoietin, and are correlated with the expression of differentiation markers of erythroid cells such as AG-EB and Glycophorin A. The role of cytokine production by erythroid cells in hemato- and immunopoiesis and the mechanisms of self-regulation of proliferation and differentiation of erythroid progenitor cells is discussed.

Full text

INTRODUCTION

It is known that immature erythroid progenitors, and cells of other hematopoietic lineages can participate in different regulatory interactions with other hematopoietic cell populations. It is well established that erythroid cells have an immunosuppressive effect on the humoral immune response, suppressing proliferation of B lymphocytes and stimulating proliferation of T lymphocytes [1-4]. It was shown that protein synthesis in erythroid cells is necessary to bring about these effects, and they are mediated through production of soluble factors [5]. We suggest that the above mentioned properties of erythroblasts can be mediated through a general mechanism for cell interactions in hematopoietic and immune systems: the production of cytokines. Further, the authors established that mRNA of such cytokines as IL-1alpha, IL-1beta, IL-6, IL-4, GM-CSF, TGF-beta, IFN-gamma [6] is detected in nucleated, immature, erythroid cells of mice. It was found that some immunoregulatory effects of erythroid cells, mediated through soluble factors, can be partially abolished by blocking antibodies directed against IFN-gamma and TGF-beta [7, 8]. All these investigations were carried out on erythroid cells of experimental animals and it remained unclear whether human erythroid progenitors are capable of cytokine production in the same manner. Furthermore, it seemed important to elucidate whether the levels of cytokine production by erythroid and immunocompetent cells are quantitatively comparable.

The aim of the present study was to investigate the production of the main immunoregulatory cytokines by erythroid cells of human fetal liver, including those which express AG-EB and Glycophorin A surface antigens, and the qualitative and quantitative changes in cytokine production by erythroid cells during their differentiation under the effect of erythropoietin.

MATERIALS AND METHODS

Cells

Fetal liver (17-20 weeks of embryonic development) was used as a source for obtaining an enriched population of human nucleated erythroid progenitor cells. It is known, that the peak of erythropoiesis in embryonic liver occurs at this period and that the main cell population is composed of erythroid cells [9].

Retrieval of an enriched population of erythroid cells

Human fetal liver (gestational age 17-20 wk) were obtained from the 2nd Central Gynaecology Clinic (Novosibirsk) and was disaggregated in a single cell suspension as described [10].

A cell suspension was obtained by sedimentation through density gradient of Ficoll-Urografin (1,077 $g.cm^{-1}$). Ficoll was obtained from Sigma (St. Louis, USA), Urografin was from Shering AG (Germany). Adherent cells were further depleted by routine adherence procedures on plastic Petri dishes and the resultant non-adherent cells were treated with L-leucine methyl ester hydrochloride (L-LME) (Fluka Chemika AG, Buchs, Switzerland), a lysosomotropic agent capable of exerting a toxic effect on cells with well-developed lysosomal apparatus, including large granular lymphocytes, monocytes, macrophages, granulocytes, mast cells, cytotoxic T-lymphocytes [11, 12]. Briefly, cells were incubated at 37° C, at a concentration of 10^7/ml, in serum-free RPMI-1640, with 15 mM L-ME. After 30 min of incubation, cells were washed twice in cold RPMI-1640. Morphological control and staining for hemoglobin showed the purity of the cell population to be > 98%. The cells obtained were cultured for 24 hours before testing (ECL). In all experiments, cells were incubated at a concentration of 10^6/ml.

Retrieval of a population of erythroid cells carrying an erythroid surface antigen and Glycophorin A

In order to obtain cells expressing an erythroid antigen and Glycophorin A (GlA), we used the indirect panning method of cell isolation. Monoclonal mouse anti-red blood cell Glycophorin A MAS518 (Harlan-Sera Lab, England), and monoclonal HAE-9 antibodies against erythroblast antigen, kindly provided by Prof. Mechetner, Russia (AG-EB is an antigen expressed on the surface of nucleated red cells and reticulocytes) were used [13-17]. AG-EB appears first on BFU-E, 36% CFU-E carry this antigen and 90% of erythroblasts are positive for this marker. On further differentiation, the expression of this marker decreases and reaches 4% on reticulocytes [13]. Affinity-isolated polyclonal rabbit anti-mouse immunoglobulin (Biosan, Russia) was used as a second reagent to coat the Petri dishes in the panning pro- cedure. Some cells, obtained as described above, were treated with aggregated normal human immunoglobulin for FcR blocking, to avoid non-specific binding, and were incubated with anti-GlA antibodies or with HAE-9 antibodies for 50 min at 4° C. Then cells were transferred into Petri dishes, and covered with rabbit anti-mouse antibodies for panning, for 50 min at 4° C. Immobilized cells were then collected. Morphological control did not reveal cells of nonerythroid origin in the enriched cell population. All isolated cells had positive staining for hemoglobin. GlA^+ and $AG\text{-}EB^+$ cells were cultivated for 24 hours in RPMI-1640 with 400 mug/ml BSA (Sigma, St. Louis, USA). Samples were collected after culture.

Culture of an enriched population of erythroid cells with Epo

Cells at a concentration of 10^6/ml obtained after density gradient sedimentation, were placed in culture medium supplemented with BSA, and containing 2 U/ml Epo (Recormon, Boehringer Mannheim, Germany) for a 72 hours incubation, that increases viability of erythroid precursors [18]. After 72 hours incubation with Epo, the morphological control revealed no cells of nonerythroid origin. All isolated cells had positive staining for hemoglobin. Then cells were washed and cultured for an additional 24 hours, when supernatants of all cultures were collected and kept at - 26° C until cytokine determination.

Control of purity of the isolated erythroid cell population

Purity of cells was assessed by staining the smears obtained with Nocht-Maksimov [19], and staining of hemoglobin with benzidin [20].

Peripheral blood mononuclear cells

PBMC were isolated with 50 U/ml Heparin (BioChemi, Vienna, Austria) from blood of healthy donors, by density gradient sedimentation (1,082 $g.cm^{-1}$) and cultured at a concentration of 10^6/ml in the presence of ConA (10 mug/ml) for 48 hours.

Culture medium

RPMI-1640 and Iskov DMEM was from Vector (Novosibirsk, Russia) supplemented with BSA (400 mug/ml).

Electrochemiluminescence (ECL) method for the quantitative determination of cytokines

Quantitative determination of cytokines was performed using the electrochemiluminescent method [21-23] with the aid of the ORIGEN Analyzer (IGEN Inc., USA) according to the protocol of the manufacturer. Calibration curves ranged from 10 to 10,000 pg/ml. Assay sensitivities were 2.8 pg/ml, 2 pg/ml, 2 pg/ml, 6 pg/ml for IL-1beta, IL-2, IL-4 and IL-6 respectively. The ruthenylated antibody and the biotinylated antibody were diluted to working concentrations (mug/ml) 1:1, 1:1, 1:2, 2:1 for IL-1beta, IL-2, IL-4 and IL-6 respectively.

Antibodies

All polyclonal and monoclonal antibodies were purchased from R&D Systems (Abington, UK). The following antibodies were used: against recombinant IL-1beta, IL-2, IL-4, IL-6 polyclonal and monoclonal: anti-hIL-1beta #AB-201-NA, anti-hIL-1beta #MAB201, anti-hIL-2 #AB-202-NA, anti-hIL-2 #MAB202, anti-hIL-4 #AB-204-NA, anti-hIL-4 #MAB204, anti-hIL-6 #AB-206-NA, anti-hIL-6 #MAB206. None of the antibodies that were used displayed any cross-reactivity with other cytokines.

Cytokines

Recombinant cytokines were purchased from R&D Systems (Abington, UK): rhIL-1beta #201-IL, rhIL-2 #202-IL, rhIL-4 #204-IL, rhIL-6 #206-IL were used for calibration curve development. All recombinant preparations were diluted in PBS

pH-7,4, supplemented with 0.05% NaN_3 and BSA 0.1%, at a 2 mug/ml concentration (aliquots were kept at - 70° C before use).

Statistical analysis

Statistical significance was determined using the nonparametric Mann-Whitney test.

RESULTS AND DISCUSSION

Production of cytokines by erythroid cells of human fetal liver

It was found that the enriched population of erythroid cells derived from human fetal liver is capable of producing a wide range of cytokines such as IL-1beta, IL-2, IL-4 and IL-6 (Table I). Although the enriched population of immature erythroid cells of embryonic liver after isolation procedures consisted (98%) of morphologically-identified, nucleus-containing erythroid cells, we applied methods of additional positive selection using the panning with monoclonal antibodies to AG-EB and Glycophorin A technique, to isolate the highly purified population of erythroid progenitors. Cells, isolated by the presence of the surface marker of erythroid cells GI A, also produced the above mentioned cytokines. In tissue culture supernatants derived from AG-EB positive cells, we found IL-1beta, IL-2, IL-4 in significant levels and trace amount of IL-6 (Table I).

The most differentiated cells expressing surface antigen GIA produced significantly higher quantities of IL-2 compared to the total population of erythroid cells. The secretion of IL-1beta by GIA^+ cells into the culture medium was significantly lower compared to enriched populations of erythroid cells, while production of IL-4 and IL-6 differed only slightly. At the same time, in AG-EB positive cells, defined with HAE-9 antibodies, the production of IL-4 and IL-6 was significantly lower than in GIA^+ cells. The increase in the production of IL-1beta and IL-2 by AG-EB + cells involving the enriched population of erythroid cells was not significant.

The immature hematopoietic precursors are known to be able to produce IL-1beta [24, 25]. We suggest that this production is necessary for regulation of the microenvironment of precursor cells and modulation of their production of growth and differentiation factors such as IL-3, SCF. We also showed that the

population of erythroid cells carrying AG-EB$^+$ markers, such as erythroid precursor BFU-E and CFU-E [13], produced significantly more IL-1beta than differentiated GlA$^+$ cells. In our experiments, an increased production of IL-4 correlated with a decreased production of IL-1beta during the differentiation of erythroid cells. This is in agreement with the fact that IL-4 is able to suppress production of IL-1beta [26]. These data suggest the existence of a mechanism of self-regulation and self-maintenance of committed precursors in the erythroid lineage, since it is well known that IL-4 is a stimulator of erythropoiesis, being an Epo synergist [27]. An increase of IL-6 production during the differentiation of erythroid cells also confirms the existence of a mechanism of the self-maintenance of erythropoiesis, since IL-6 can be a co-stimulator of IL-4 while enhancing the proliferation of erythroid precursors [28-30]. An increase in IL-6 production by more differentiated GlA$^+$ erythroid cells of human fetal liver can also indicate the participation of these cells in the regulation of hepatogenesis since IL-6 is a growth factor for hepatocytes [31, 32]. An increase in IL-2 production in erythroid cells can indicate the possibility of the participation of erythroid cells from fetal liver in the regulation of lymphopoiesis during embryogenesis.

The differences obtained in cytokine production between AG-EB$^+$ and GlA$^+$ cells might be related initially with the stages of differentiation defined by AG-EB and GlA surface antigens expression. Some authors have previously shown that GlA expression occurring in erythroblasts is present until the complete maturation of the erythrocyte. GlA is not found in early precursors, BFU-E, CFU-E or right up to proerythroblasts. This means that GlA$^+$ cells are also at a later stage of differentiation than AG-EB$^+$ erythroid cells [16, 17]. AG-EB antigen of erythroid cells is expressed on early precursors of erythroid cells and it has been found that 36% BFU-E. CFU-E bear the AG-EB antigen. It is well established that 90% of nucleated erythroid cells are AG-EB positive. Following differentiation of erythroid cells, there is a fall in AG-EB expression to an average of 4.56% in reticulocytes of normal donors [13, 14].

Based on these data, we can assume that cytokine production at different stages of proliferation and differentiation of erythroid cells changed quantitatively and that this production can be important both in the regulation of proliferation of erythroid growth and in the regulation of proliferation and differentiation of other types of cells.

Cytokine production by an enriched population of erythroid cells in comparison with erythroid cells after treatment with erythropoietin, and by mitogen-stimulated PBMC

Epo is known as the principal specific regulatory factor that influences the proliferation, differentiation and maintenance of populations of erythroid precursor and mature erythroid cells [33, 34]. Epo promotes survival of erythroid precursors [35]. It was found that cytokine production by erythroid cells, cultivated in the presence of Epo, is greatly changed. In Epo-treated erythroid cells significantly decreased IL-1beta production was observed as compared to the more differentiated cells. This corresponds with the Table 1 results, which show that there is a decrease in IL-1beta production by GIA^+ cells compared to an enriched population of immature erythroid cells. IL-2 production was significantly increased by the incubation of erythroid cells in the presence of Epo. This can indicate the existence of a feedback regulation since the inhibitory effects of IL-2 [36] on erythropoiesis are well known. This effect can be mediated through stimulation of production of IFN-gamma that has a direct inhibitory influence on the proliferation of erythroid cells [36-38]. Besides, IL-2 is known to decrease the stimulatory effect of growth factors such as IL-3, and Epo on erythropoiesis. IL-4 production by erythroid cells was also increased by incubation of cells with Epo (Table 2).

As mentioned above, IL-4 can be a stimulator of erythropoiesis acting synergystically with Epo [27]. Furthermore, the production of this cytokine can reflect the mechanism of self-maintenance of erythroid precursors. The capacity of erythroid cells to synthesize IL-6 during cell culture is decreased. This fact may indicate the presence of specific factors in the microenvironment of embryonic erythroid cells, inducing IL-6 production and their absence in the culture medium.

In order to elucidate the significance of the level of the cytokines production in regulatory interactions, we compared cytokines production by erythroid cells with an equal number of mitogen-stimulated peripheral blood mononuclear cells. Cytokine production by mitogen-stimulated mononuclear cells was estimated after 48 hours of culture. According to our data, during this period the levels of cytokine production in cultures of mitogen-stimulated mononuclear cells reached a plateau. Cytokine production in erythroid cells was estimated for 24 hours in all experiments. Table 2 shows that the levels of cytokine production

by nucleated erythroid human cells are comparable with the levels of production of these cytokines by PBMC, and in the case of IL-1beta and IL-2, production by erythroid cells was higher.

CONCLUSION

Thus, erythroid cells, along with cells of other hematopoietic lineages, are capable to produce cytokines such as IL-1beta, IL-2, IL-4 and IL-6. Erythroid cells can affect both proliferation and differentiation of cells of erythroid cell lineage as well as other of hemato- and immunopoietic cells, by the above mentioned cytokines. The production of these cytokines in erythroid cells of human embryonic liver may be related to the different stages of proliferation and differentiation, and can be influenced by the effect of mediators. The levels of cytokine production by erythroid cells is comparable with those of mitogen-stimulated peripheral blood mononuclear cells.

The possibility of participation of erythroid cells in regulation by the production of cytokines allows to consider the importance of erythroid cells in the regulation of hematopoiesis and in particular in the regulation of hematopoiesis in embryonic liver. It is known that erythropoesis is the first to be developed in ontogenesis. Therefore, the synthesis of regulatory cytokines in erythroid cells can influence the self-maintenance of the erythroid cell compartment, as well as the lymphoid, monocyte-macrophage and other lineages of hematopoiesis precursors which appear later than erythroid cells. Our results confirm that the capacity of hematopoietic cells to produce regulatory cytokines may be one of the general mechanisms of mediation of regulatory interactions of cells, and that erythroid cells play a very important role in these interactions, which merits further study.

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Figures

Table 1
Production of cytokines by an enriched population of erythroid cells from fetal liver compared to the production of these cytokines in GIA+ and AG-EB+ populations of erythroid cells

Cells	IL-1 beta (rhôg/ml)	IL-2 (rhôg/ml)	IL-4 (rhôg/ml)	IL-6 (rhôg/ml)
Fetal liver erythroid cells (> 98%)	2,536.0 ± 575.3	107.0 ± 57.43	651.0 ± 147.91	232.2 ± 72.04
Glycophorin A (+) cells	187.6 ± 124.21***	2,065 ± 407.8**	477.7 ± 235.87	322.0 ± 68.02
AG-EB (+) cells	6,464.5 ± 2,820.67	840.64 ± 376.36	94.5 ± 24.59**	6 ± 6***

n = 10, *** p < 0.001, ** p < 0.01. Fetal liver erythroid cells compared to GIA+ or AG-EB+ cell populations.

Table 2
Cytokine production by eythroid cells after Epo treatment

Conditions	IL-1 beta (rhôg/ml)	IL-2 (rhôg/ml)	IL-4 (rhôg/ml)	IL-6 (rhôg/ml)
Fetal liver erythroid cells (> 98%)	2,536.0 ± 575.3	107.0 ± 57.4	651.0 ± 147.9	232.2 ± 72.0
Fetal liver erythroid cells after Epo treatment	931 ± 340**	715.0 ± 409.0*	1,177.2 ± 141.7*	96.6 ± 36.0*
PBMC, ConA.***	1,089.3 ± 555.1	24.2 ± 11	2,045.5 ± 778	1,720 ± 621

n = 10, ** p < 0.01, * p < 0.05. Fetal liver erythroid cells compared to the same cells afer Epo treatment.
*** production of cytokines by ConA. treated PBMC after 48 hours of incubation.

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macrophages, NOS2, Herpes simplex virus, NF-kB, IRF-1.

Negative regulation of onconstatin M signaling by suppressor of cytokine signaling (SOCS-3)

F. Magrangeas, O. Boisteau, S. Denis, Y. Jacques, S. Minvielle
cytokine signaling, inhibition, Jak-STAT pathway.

Increased expression of interleukin-8 and aminopeptidase N by cell-cell contact: interleukin-8 is resistant to degradation by aminopeptidase N/CD13

A. Kehlen, I. Egbert, K. Thiele, K. Fischer, D. Riemann, J. Langner
cell co-culturing, leucine aminopeptidase, plasma membrane preparations, CD13, proteolysis of IL-8, interleukin-8.

Functional characterization of the mouse lymphotoxin-beta receptor promoter

P. Muller, D.N. Männel, T. Hehlgans
LTbR-promoter, transcriptional activation, dexamethasone.

Glucose potentiates interleukin-1b (IL-1b)-induced p38 mitogen-activated protein kinase activity in rat pancreatic islets of Langerhans

A.M.E. Sprinkel, N.A. Andersen, T. Mandrup-Poulsen
interleukin-1b, p38, ERK1/2, JNK, nitric oxide, diabetes mellitus.

Sustained parasite burden in the spleen of Leishmania infantum-infected BALB/c mice is accompanied by expression of MCP-1 transcripts and lack of protection against challenge

D. Rousseau, S. Demartino, F. Anjuère, B. Ferrua, K. Fragaki, Y. Le Fichoux, J. Kubar
murine leishmaniasis, acquired immunity, inflammation, chemokines, spleen cell subpopulations.

Effects of cytokines and prolactin on the replication of Toxoplasma gondii in murine microglia

N. Benedetto, A. Folgore, C. Romano-Carratelli, F. Galdiero
T. gondii, cytokine, PRL, MGC.

Strip test for bedside detection of interleukin-6 in cervical secretions is predictive for impending preterm delivery

H. Trébéden, F. Goffinet, G. Kayem, F. Maillard, E. Lemoine, D. Cabrol, B. Weill, F. Batteux
cytokine, IL-6, prematurity, cervicovaginal secretions, strip test.

Serum levels of interleukin-18 in patients with uncomplicated Plasmodium falciparum malaria

D. Torre, M. Giola, F. Speranza, A. Matteelli, C. Basilico, G. Biondi
interleukin-18, malaria, Plasmodium falciparum, gamma interferon.

The ecdysone inducible gene expression system: unexpected effects of muristerone A and ponasterone A on cytokine signaling in mammalian cells

S. Constantino, R. Santos, S. Gisselbrecht, F. Gouilleux
ecdysone, PI 3-kinase, AKT, survival.

Presence of estrogen receptors in human myeloid monocytic cells (THP-1 cell line)

M. Cutolo, B. Villaggio, A. Bisso, A. Sulli, D. Coviello, J.M. Dayer
THP-1 cells, macrophages, estrogen receptors, estrogens, monocytes.

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