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GM-CSF and IFN-γ-induced expression of human leucocyte antigen class Ⅱ molecules on basophils of umbilical cord blood[1]

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KEY WORDS basophils; HLA antigen; granulocyte-macrophage colony-stimulating factor; interferon-gamma

ABSTRACT

AIM: To determine whether basophils expressed human leucocyte antigen (HLA) class Ⅱ molecules. **METHODS**: Basophils in umbilical cord blood were separated and purified with methods of density gradient centrifugation and immunomagnetic microbeads. The isolated basophils were cultured in RPMI-1640 plus 10 % fetal calf serum at 37 ℃ in a humidified atmosphere with 5 % CO_2 and stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) or interferon (IFN)-γ for 20 – 60 h. The expression of HLA class Ⅱ molecules on basophils was measured with fluorescence activated cell sorter (FACS). **RESULTS**: The purity of isolated basophils was ≥83.5 %. After treated with GM-CSF 10 μg/L or IFN-γ 100 kU/L, the expression of HLA class Ⅱ molecules became detectable on membranous surface and at a higher level at 20 h, the percentage of expression was 10.2 % ± 2.1 % and 11.3 % ± 1.0 %, respectively. **CONCLUSION**: Basophils possessed the potency of HLA class Ⅱ molecular expression.

INTRODUCTION

The human leucocyte antigen (HLA) class Ⅱ molecules are encoded by genes located within the major histocompatibility complex that is mapped on the short arm of chromosome 6, the expression of HLA class Ⅱ molecules is a prerequisite condition for an antigen-presenting cell to generate a T-helper cell-mediated immune response. Recent studies have shown that HLA class Ⅱ genes are involved in the pathogenesis of asthma induced by some low molecular weight compounds as antigens[1-3], and that the basophils are known as the main cells for development of pathological process[4]. These findings prompted us to investigate the biological functions of basophils participated in the immune response. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon (IFN)-γ are two important cytokines, and they can induce the expression of HLA class Ⅱ molecules on a variety of cell types[5,6]. The purpose of the present studies was to evaluate whether basophils had the potency of HLA class Ⅱ molecular expression after they were exposured to stimulation of GM-CSF or IFN-γ.

MATERIALS AND METHODS

Materials Umbilical cord blood was supplied by Shanghai International Peace Maternity and Infant Health Institute. Recombinant human GM-CSF was purchased from Schering-Plough (Brinny) Co, Ireland. Recombinant human IFN-γ was a product of Shanghai Clonbiotech Co Ltd, China. Basophil isolation kit, type BS separation column and vario-magnetic activated cell sorting (MACS) were the products of Miltenyi Biotec Co, Germany. The cell isolation kit contained a cocktail of hapten-conjugated CD3 (mouse IgG_{2a}), CD7 (mouse IgG_{2a}), CD14 (mouse IgG_{2a}), CD15 (mouse IgM), CD16 (mouse IgM), CD36 (mouse IgG_{2a}), CD45RA (mouse IgG_1) as well as anti-HLA-DR antibodies and MACS microbeads coupled to an anti-hapten monoclonal antibodies. Antibodies of FITC-conjugated mouse anti-human HLA-DR, DP, DQ, and FITC-conjugated mouse $IgG_{2a,\kappa}$ immunoglobulin isotype control were obtained from Pharmingen Co. Monoclonal antibodies of CY-conjugated anti-human CD45 and CY-conjugated mouse $IgG_{1,\kappa}$ immunoglobulin isotype control were kindly provided by Flow Cytometric Unit of Shanghai Second Medical University, China.

Isolation and purification of basophils Every

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batch of the umbilical cord blood (40 mL) was treated with the anticoagulant of heparin at the final concentration of 50 kU/L. It was mixed with 6 % dextran (5 : 1, v:v) and incubated at 37 ℃ for 40 min, the obtained supernatant was then spun at 140 × g, at 4 ℃ for 10 min. The pellet was resuspended in 4 – 6 mL of plasma originally derived from the heparin treated umbilical cord blood to make the cell suspension, which was then layered onto a two-step Ficoll-Hypaque gradient solutions (1.065/1.076, 3 mL each). The prepared sample was centrifuged at 2100 × g, at 4 ℃ for 20 min and the basophil-containing interface between the cell suspension and the buffer with the density of 1.065 was harvested. Basophil-enriched preparation was washed and centrifuged once at 140 × g, at 4 ℃ for 10 min with buffer A, containing NaCl 130 mmol/L, Na_2HPO_4 10 mmol/L, NaH_2PO_4 10 mmol/L, edetic acid 2 mmol/L and 0.5 % bovine serum albumin (pH 7.2), and finally resuspended in 100 μL of the same buffer A. In order to obtain high purity of basophils, the basophil-enriched fraction was further purified by negative selection using immunomagnetic microbead method[7], with which the fraction was exposed to a MACS and the labeled cells would be depleted.

Evaluation of basophil purity The basophil number and purity were assessed by count and fluorescence activated cell sorter (FACS, Becton Dickinson, USA). For counting, basophils were stained with alcian blue[8]. Using forward and side scatter characteristics of flow cytometer, the percentage of basophil populations was determined by display of CD45 (X axis) *vs* side light scatter (Y axis)[9].

Culture studies Basophils were cultured in RPMI-1640 plus 10 % fetal calf serum at 37 ℃ in a humidified atmosphere with 5 % CO_2, and incubated in 96-well culture plates and the number of basophils was adjusted to 1 × 10^5 cells per well. To determine whether basophils expressed the potency of HLA class Ⅱ molecules, basophils were stimulated with either GM-CSF 10 μg/L or IFN-γ 100 kU/L for 20 – 60 h.

FACS analysis The expression of HLA class Ⅱ molecules was measured with FACS according to the procedure provided. Briefly, 10 μL of the FITC-conjugated anti-HLA class Ⅱ molecule antibody was added to harvested cells in the dark and kept for 30 min. The reaction was terminated by washing with buffer A, the cells were then fixed with 4 % paraformaldehyde and analysed on a FACS. The FITC-conjugated mouse $IgG_{2a,\kappa}$ immuno-globulin isotype was saved as negative control.

Statistical analysis The data were expressed as $\bar{x} \pm s$. Intergroup statistical analysis was performed using the unpaired Student's t test.

RESULTS

Purity of basophils After density gradient centrifugation and MACS isolation, purity of basophils was ≥83.5 % as determined by count method (Tab 1) and FACS analysis (Fig 1), respectively. Contaminating cells were mainly juvenile cells. The phenotype of purified basophils had been well defined by microscopic examination (Fig 2), and changes in morphology of basophils were not found in this isolation procedure.

Tab 1. Purity and number of basophils after density gradient centrifugation and MACS isolation.

Sample	Purity /%	10^{-5} × Total basophils
1	85.7	6.0
2	93.9	7.7
3	85.1	5.0
4	85.7	6.0
5	88.6	6.5
6	90.1	6.8
7	93.0	5.8
8	87.1	8.0
9	88.0	10.0
10	98.0	5.5

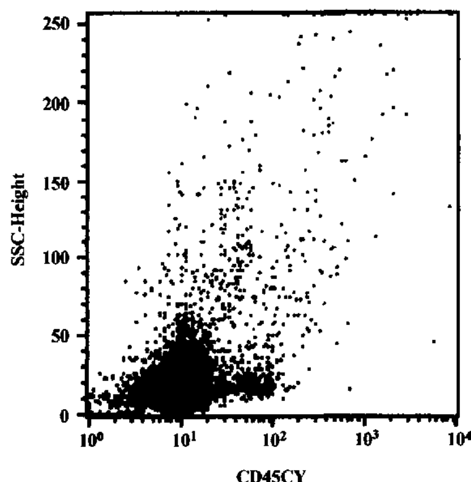

Fig 1. FACS profile for the isolation of basophils from umbilical cord blood. More than 83.5 % of the cells was clustered on left lower position and identified as basophils.

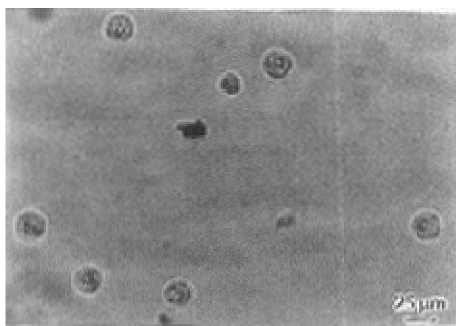

Fig 2. Microscopic photograph of isolated basophils. ×400.

Effect of GM-CSF or IFN-γ on expression of HLA class Ⅱ molecules After basophils were incubated in medium with GM-CSF or IFN-γ for 20 – 60 h, the expression of HLA class Ⅱ molecules on basophils was evaluated by FACS analysis. The results showed that expression of HLA class Ⅱ molecules was detectable on membranous surface and at a higher level at 20 h post-stimulation with GM-CSF 10 μg/L or IFN-γ 100 kU/L (Tab 2, 3), but the percentage of expression was decreased with stimulative time prolonging. No significant difference on the expression of HLA class Ⅱ molecules was found between the basophils stimulated by GM-CSF and IFN-γ, respectively.

Tab 2. Time responses of GM-CSF-stimulated expression of HLA class Ⅱ molecules by basophils. $n=4$. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* negative control.

Stimulative time/h	Fluorescent intensity/%	
	Negative control	GM-CSF 10 μg/L
20	0.96±0.25	10.2±2.1[c]
40	1.5±0.4	3.8±1.2[b]
60	0.68±0.21	1.2±0.5[a]

Tab 3. Time responses of IFN-γ-stimulated expression of HLA class Ⅱ molecules by basophils. $n=4$. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* negative control.

Stimulative time/h	Fluorescent intensity/%	
	Negative control	IFN-γ 100 kU/L
20	0.80±0.06	11.3±1.0[c]
40	1.26±0.08	3.6±1.4[b]

DISCUSSION

Basophils are the least common circulating leucocytes in blood and account for only 0.5 % – 1.0 % of total leukocytes, and express IgE, CD11, CD13, CD18, CD26, CD31, CD32, CD33, CD40, CD43, CD44, CD45, and CD54 on their surface[10,12], but none of these antigens is specific for them. Therefore, it is difficult to isolate and purify basophils in the early, the attempts to purify basophils have only been partially successful such as affinity chromatograph[13], density gradient centrifugation *et al*[14,15], and the purity of basophils was 8 % – 80 %. In present studies, density gradient centrifugation and immunomagnetic microbead methods were used to separate and purify the basophils from umbilical cord blood. For the depletion of B cells, monocytes, NK cells, dendritic cells, early erythroid cells, platelets, neutrophils, eosinophils, and T cells, a cocktail of CD3, CD7, CD14, CD15, CD16, CD36, CD45RA, anti-HLA-DR antibodies, and MACS microbeads coupled to these monoclonal antibodies is used, the labeled cells are then depleted by retaining them on a MACS column in the magnetic field. The results showed that the purity of basophils was ≥83.5 % and higher than that in previous reports. The results also indicated that the useful means for purification of basophils could be provided for us to study the biological aspects of basophils.

Recently, the function of basophils has been more extended toward immunologic processes. Detection of HLA class Ⅱ molecules was first reported by Stain *et al*[6] on basophils under some cultured condition. The expression of HLA class Ⅱ molecules on basophils isolated from chromic granulocytic leukemia patients became detectable after IFN-γ stimulation for 2 d and 5 d, the positive rates were 18 % and 45 %, respectively. However, the expression was not detectable on freshly obtained basophils. The detection of HLA class Ⅱ molecules on basophil cell line also demonstrated by Mizobuchi *et al*[16], but the percentage of expression was very low and only 0.3 %. The present results showed that HLA class Ⅱ molecules expressed on basophils derived from umbilical cord blood after stimulated with GM-CSF or IFN-γ, which suggested that basophils possessed the potency of HLA class Ⅱ molecular expression, but the pathophysiologic significance of HLA class Ⅱ molecules expressed on the basophils is not clear and requires to be investigated further. On the other hand, the result that the most high level of HLA class Ⅱ

molecular expression has been found at the point after 20 h incubation of basophils with the stimuli obviously differs from previous observation[6]. So we speculated that the different results might reflect the different source of the tested cells and the different cell cultured conditions.

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粒细胞-巨噬细胞集落刺激因子和干扰素-γ诱导脐血嗜碱粒细胞表达人类白细胞抗原Ⅱ类分子[1]

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关键词 嗜碱粒细胞; HLA 抗原; 粒细胞-巨噬细胞集落刺激因子; 干扰素-γ

目的: 探讨嗜碱粒细胞是否可表达人类白细胞抗原(HLA) Ⅱ类分子. **方法**: 采用密度梯度离心和免疫磁珠方法分离和纯化脐血嗜碱粒细胞, 通过体外培养及粒细胞-巨噬细胞集落刺激因子(GM-CSF)和干扰素(IFN)-γ 刺激 20-60 小时后, 用流式细胞仪分析 HLA Ⅱ类分子表达的情况. **结果**: 嗜碱粒细胞的纯度≥83.5 %, 在 GM-CSF 10 μg/L 或 IFN-γ 100 kU/L 刺激后, 其膜表面可表达 HLA Ⅱ类分子, 表达百分率在刺激后 20 小时较高, 分别为 10.2 % ± 2.1 %和 11.3 % ± 1.0 %. **结论**: 嗜碱粒细胞具有表达 HLA Ⅱ类分子的潜在能力.

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