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Immunoregulatory function of neonatal nucleated red blood cells in humans

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ABSTRACT

We found that human cord blood nucleated red blood cells (NRBCs) have a regulatory function in the innate immune reaction. These cells suppressed the production of inflammatory cytokines including TNF-α and IL-1β from monocytes in response to lipopolysaccharide (LPS). The NRBCs exerted their regulatory function even without cell-to-cell contact with the monocytes. However, IL-10 production from the monocytes by LPS stimulation in the presence of NRBCs was higher than that from LPS-stimulated monocytes cultured in the absence of NRBCs. Addition of an anti-IL-10 receptor blocking antibody restored the inflammatory cytokine production from the monocytes, suggesting that the functional change of the monocytes caused by the interaction with NRBCs was mediated by the increased IL-10 production. A whole-genome microarray analysis revealed that the monocytes expressed increased amounts of IL-10 superfamily genes after interacting with NRBCs. IL-19, which is a member of the IL-10 superfamily, enhanced IL-10 production from the monocytes, which suggested a cooperative role of the IL-10 superfamily in the suppression of inflammatory cytokine production from monocytes. Arginase, which was reported to play an important role in the suppressive function of NRBCs in mice monocytes, was found to have no significant role in human monocytes. The NRBCs seem to have a regulatory role through the induction of IL-10/IL-19 production by monocytes to suppress a vigorous innate immune reaction, which can be harmful to fetuses.

1. Introduction

Neonates are particularly vulnerable to infectious agents (PrabhuDas et al., 2011). They are exposed to a variety of microbes after birth despite the limited exposure to antigens *in utero* to induce adaptive immunity. Additionally, it has been reported that the responsiveness of cord blood (CB) T cells against antigens differs from that of adult peripheral blood (PB) T cells (Basha et al., 2014). The CB $CD4^+$ T cells are polarized toward a Th2 response with a reduced production of Th1 cytokines (Webster et al., 2007 and Zaghouani et al., 2009). The $CD8^+$ T cell function of neonates has been reported to be insufficient compared with that of adults (McCarron and Reen, 2010). Unlike adaptive immune responses, the innate immune system is an evolutionarily ancient, antigen-non-specific host defense system that provides immediate protection against invasive microorganisms (Janeway and Medzhitov, 2002 and Strunk et al., 2011). It has been reported that neonates are more dependent on innate immunity than acquired immunity, because the production of various inflammatory cytokines by neonatal monocytes and antigen presenting cells after Toll-like receptor (TLR) stimulation is not lower than that of adults (Vanden Eijnden et al., 2006 and Angelone et al., 2006). However, Chelvarajan et al. (2004) reported that the macrophage production of inflammatory cytokines, such as IL-1β and IL-6 in neonates was defective as a result of an excessive production of IL-10. Therefore, it is possible that the neonatal innate immune response is regulated by immune suppressive cytokines or other factors that are unique to neonates.

Nucleated red blood cells (NRBCs) are normally present in fetal circulation. After birth, NRBCs gradually disappear within one month (Hermansen, 2001). Recently, Elahi et al. (2013) identified an immunosuppressive erythroid cell population in humans and mice. Moreover, they reported that $CD71^+$ erythroid cells had an immunosuppressive function via arginase-2 in mice. In this study,

Abbreviation: NRBCs, nucleated red blood cells.

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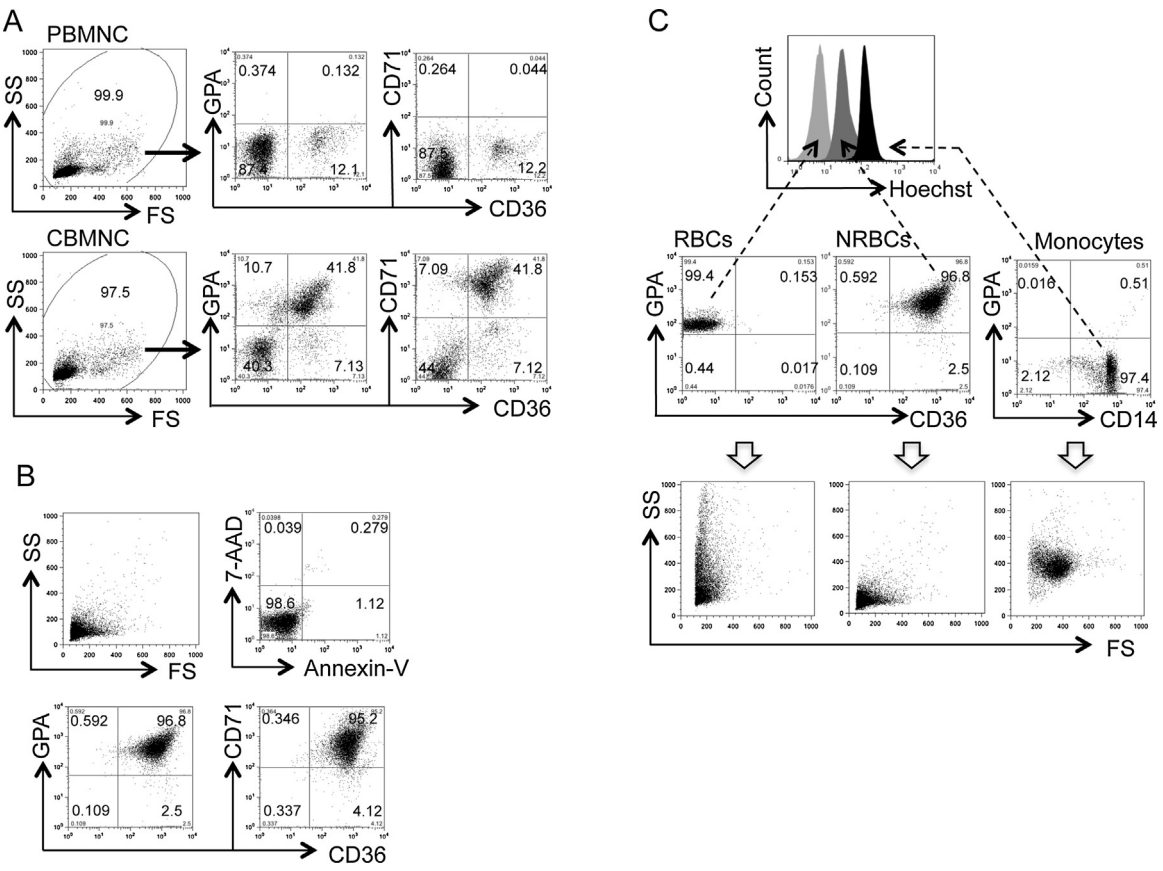

Fig. 1. Phenotypic characteristics of CD36$^+$CD71$^+$GPA$^+$ NRBCs in CB MNCs.
(A) Phenotypic characteristics of CB NRBCs. Mononuclear cells obtained from adult PB (upper panel) and CB (lower panel) were stained with erythroid lineage-specific markers by fluorescein isothiocyanate (FITC) conjugated anti-CD36, PE-conjugated anti-CD71 and APC-conjugated anti-GPA antibodies.
(B) The NRBCs after purification from the CB mononuclear cells using microbeads. After the separation of the CD45$^+$ and CD61$^+$ cells from the MNCs using anti-CD45 mAb and anti-CD61 mAb, CD36$^+$ cells were purified by positive selection using FITC-conjugated anti-CD36 mAbs and anti-FITC microbeads as described in the Supplementary Materials and Methods. The cells were stained with 7-AAD and annexin-V to identify dead cells. The purity of the NRBCs was analyzed by a flow cytometer after staining with FITC-conjugated anti-CD36, PE-conjugated anti-CD71 and APC-conjugated anti-GPA.
(C) Hoechst 33342 staining of CD36$^+$ cells from CB mononuclear cells. The mature erythrocytes and CB monocytes were obtained by density gradient centrifugation and positive selection using anti-CD14 microbeads (middle panel), respectively, and were used as controls. These cells and purified NRBCs were stained with Hoechst 33342 (upper panel). The staining results of the mature erythrocytes (RBCs), NRBCs and CB monocytes are shown in light gray, dark gray and black, respectively. The forward and side scatter distributions of these three cell populations are shown in the lower panel.

we investigated a possible role for human NRBCs in regulating the innate immune reaction.

2. Materials and methods

2.1. Isolation of NRBCs, monocytes and naïve T cells

Fresh human umbilical CB samples were obtained from Kyushu University Hospital after informed consent was obtained. All mothers had no complications including infectious diseases during the perinatal period. Adult PB was obtained from healthy adult volunteers aged 30–40 years. Mononuclear cells (MNCs) were separated by density gradient centrifugation using a lymphocyte separation medium (LSM: density 1.077 g/ml: MP Biomedicals, Santa Ana, CA). The CD45$^+$ and CD61$^+$ cells were separated from the MNCs using anti-CD45 monoclonal antibody (mAb)-conjugated and anti-CD61 mAb-conjugated microbeads, respectively. The NRBCs were positively selected from the CD45$^-$CD61$^-$ fraction using fluorescein isothiocyanate (FITC)-conjugated anti-CD36 mAb and anti-FITC mAb-conjugated microbeads. To obtain CD14$^+$ monocytes and CD4$^+$CD45RA$^+$ naïve T cells, MNCs were positively selected using anti-CD14 mAb-conjugated microbeads and a naïve T cell isolation kit, respectively. The purity of the NRBCs was more than 94% as determined by the flow cytometric analysis after staining with FITC-conjugated anti-CD36, phycoerythrin (PE)-conjugated anti-CD71 and allophycocyanin (APC)-conjugated anti-CD235a (glycophorin A: GPA). The purity of the monocytes and naïve T cells was more than 96% as determined by the flow cytometric analysis after staining with FITC-conjugated anti-CD14, FITC-conjugated anti-CD45RA and PE-conjugated anti-CD4. Dead cells were identified by 7-amino-actinomycin D (7-AAD) and annexin V-PE staining (BD Biosciences). All microbeads and the naïve T cell isolation kit were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). The anti-CD4, anti-CD14, anti-CD36, anti-CD45RA and anti-CD235a mAbs were purchased from Beckman Coulter (Marseille, France). The anti-CD71 mAb was purchased from eBiosciences (San Diego, CA). Flow cytometric analysis was performed using the EC-800 (Sony Biotechnology, Champaign, IL, USA).

2.2. Cell conditions and stimulation

Purified CD14$^+$ cells were cultured in RPMI-1640 culture medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum and 1% gentamicin in a 96-well round bottom plate at a concentration of 5×10^5 cells/ml with or without LPS (Sigma, L2630). The NRBCs were added at specific concentrations. After 36 h, the culture supernatant was collected