Bacterial infection of human hematopoietic stem cells induces monocytic differentiation

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Abstract

Differentiation of hematopoietic stem cells (HSCs) can be influenced by different stimuli, including cytotoxic agents, certain cytokines, and contact with pathogens. Infection may result in dysregulation of these important progenitor cells and therefore interfere with the availability of blood cells. In this study we analyzed the effect of bacterial infection on HSCs concerning surface marker expression and cytokine release. Listeria monocytogenes and Yersinia enterocolitica accelerated maturation of hematopoietic progenitor cells along the myeloid lineage, as demonstrated by the upregulation of CD13, CD14, and costimulatory signals. By screening cytokine secretion, granulocyte-macrophage colony-stimulating factor, interleukin (IL)-6, IL-8, IL-10, IL-12, and tumor necrosis factor-α were found to be induced by bacterial infection. These data indicate that infection of HSCs with L. monocytogenes and Y. enterocolitica affects the differentiation of CD34+ hematopoietic progenitors in vitro and may lead to secretion of cytokines that can influence the HSC differentiation capacity and immune response.

Keywords: Hematopoietic stem cell; Bacterial infection; Cytokine; Cell differentiation

1. Introduction

The availability of blood cells is sustained by pluripotent hematopoietic progenitors. These hematopoietic stem cells (HSCs), which have in common the expression of CD34 surface molecules, are rare pluripotent cells in the bone marrow with the capacity to differentiate into erythrocytes, megakaryocytes, granulocytes, monocytes, and lymphocytes [1,2].

Hematopoiesis is a complex process in which multiple factors of the microenvironment are involved. The production of peripheral blood cells is regulated by hematopoietic growth factors and related cytokines which promote the survival of stem cells and stimulate the proliferation and differentiation of progenitors [3,4]. Disturbance of this complex network by infectious agents is critical, since infection can lead to dysregulation and destruction of these important progenitor cells [5]. Infection may augment the production of cytokines, and change proliferation and differentiation of stem cells. Concerning the interaction between infectious agents and HSCs the repertoire of cellular surface receptors on HSCs plays an important role in differential targeting of infectious agents. Therefore identification of surface phenotypes of HSCs is important for the understanding of basic steps in hematopoiesis. Recently, we have shown that human HSCs are fully resistant to infection with Listeria monocytogenes and Yersinia enterocolitica. However, when CD34+ HSCs were cultured in a cytokine mixture with stem cell factor, thrombopoietin, and flt-3 ligand to promote myeloid development [6,7],

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these partially differentiated HSCs were able to take up pathogens [8].

In this study, we therefore characterize the developmental changes of HSC surface molecule expression and cytokine release after infection with Gram-negative Y. enterocolitica and Gram-positive L. monocytogenes.

2. Materials and methods

2.1. Isolation of human CD34+ progenitor cells derived from peripheral blood cells

After informed consent, cells were collected by apheresis during hematopoietic recovery after chemotherapy and treatment with recombinant s.c. granulocyte colony-stimulating factor (G-CSF). 4 ml cell suspension from the pilot aliquot was used for positive selection of CD34+ cells.

2.2. Positive selection of CD34+ cells with the CD34+ selection kit (Miltenyi Biotec, Bergisch Gladbach, Germany)

After washing, the cell pellet was resuspended in 300 µl buffer (phosphate-buffered saline supplemented with 0.5% bovine serum albumin, and 2 mM EDTA) per 5×10⁸ cells. The cell suspension was incubated with Fc receptor-blocking reagent (human immunoglobulins) and subsequently with monoclonal hapten-conjugated anti-CD34 antibody (clone QBEND/10, mouse IgG1) for 15 min at 6°C. Following an additional washing step, the stained cell suspension was incubated with colloidal super-paramagnetic MACS MicroBeads conjugated to an anti-hapten antibody in a final volume of 500 µl per 5×10⁸ cells for 15 min at 6°C. For positive selection cells were again washed and resuspended in buffer (2×10⁶ cells ml⁻¹). After passing through a filter to remove clumps, cells were applied to the selection column (type VS 0⁺/10, mouse IgG1) for 15 min at 6°C. For selective removal of extracellular bacteria, 100 µg ml⁻¹ gentamicin (Gibco, Karlsruhe, Germany) was added. Twenty-four hours post infection (p.i.) cells were analyzed by flow cytometry and the supernatant was collected and stored at −80°C.

2.3. Differentiation of HSCs towards monocytes

HSCs were differentiated towards monocytes by incubation in long-term cell culture medium (Cell Systems, Vancouver, BC, Canada) supplemented with stem cell factor (100 ng ml⁻¹), thrombopoietin (75 ng ml⁻¹) and flt-3 ligand (15 ng ml⁻¹) (Pepro Tech, Rockyhill, SC, USA) [9].

2.4. Bacteria

Bacterial strains used in this study are: L. monocytogenes Sv 1/2a EGD and Y. enterocolitica. The bacteria were grown in brain heart infusion medium at 30°C (Yersinia) or 37°C (Listeria) until they reached the mid-exponential phase of growth (circa 1×10⁸ bacteria ml⁻¹).

2.5. In vitro bacterial infection of HSCs

5×10⁵ cells ml⁻¹ were infected with bacteria. Bacteria were added at a multiplicity of infection (MOI) of 10. The cultures were incubated in RPMI 1640 medium supplemented with 10% pooled human AB serum at 37°C for 1 h. For selective removal of extracellular bacteria, 100 µg ml⁻¹ gentamicin (Gibco, Karlsruhe, Germany) was added. Cytokine concentrations in the supernatants generated

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in the infection experiments 24 h p.i. were measured with the Luminex technology for multiplex cytokine immunoassay (obtained from BioSource, Nivelles, Belgium) using beads for G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-4, IL-5, IL-6, IL-8, IL-1β, IL-12p40, IL-10, IL-13 and interferon-γ (IFN-γ). Supernatants were diluted 1:10 in sterile water; 50 μl of the dilution was used in the assay according to the detailed description supplied by the manufacturer. At least 100 beads per cytokine and sample were measured. Data were evaluated with the MasterPlex QT software (MiraBio).

Alternatively, standard enzyme-linked immunosorbent assay kits were used to determine TNF-α, IL-6, IL-8, IL-1β, IFN-γ (ImmunoKontact, AMS Biotech, Wiesbaden, Germany) and IL-10, IL-12p70 (Biocarta, Hamburg, Germany).

3. Results and discussion

3.1. CD34+ HSCs change surface marker expression after infection with L. monocytogenes and Y. enterocolitica

The purified fraction of CD34+ HSCs from human blood consisted of >95% CD34+ cells. Specific markers for T-cells, B-cells, monocytes, and mature dendritic cells (CD3, CD19, CD14, or CD83) were found on <1% of the cells. CD34+ HSCs showed a slight expression of CD54 (ICAM-1), while other costimulatory signals such as B7.1 (CD80), B7.2 (CD86), and B7-H1 were almost not detectable (Fig. 1a).

HSCs were incubated with the Gram-positive bacterium L. monocytogenes and the Gram-negative bacterium Y. enterocolitica. Four hours and 24 h p.i. the surface marker expression of CD13, CD14, CD16, CD33, CD34, CD54, CD80, CD83, CD86, and B7-H1 was evaluated. CD13 and CD33 are expressed on myeloid progenitors, monocytes, and mast cells. Four hours p.i. infected HSCs showed no altered surface molecule expression compared to uninfected HSCs. Twenty-four hours p.i. we observed a prominent upregulation of CD54 (ICAM-1). In addition to ICAM-1, the costimulatory molecules CD86 (B7.2) and B7-H1 [10] were upregulated, in particular after infection with Y. enterocolitica (Fig. 1b).

The change in surface marker expression was unexpected, since recent studies have shown that undifferentiated HSCs are not able to perform macrophagocytosis or receptor-mediated phagocytosis because essential receptors and internalization mechanisms for bacterial uptake have not yet been developed [8]. Therefore we examined whether these cells already express an important pathogen recognition receptor, namely TLR-4. Pathogen-recognition receptors include the family of TLRs, which recognize conserved motifs of pathogens [11]. We investigated TLR-4, which is known to bind bacterial lipopolysaccharide (LPS) of Gram-negative bacteria and might therefore account for the upregulation of surface markers induced by Y. enterocolitica. While we could clearly show TLR-4 expression on monocytes, HSCs did not express TLR-4 (Fig. 1c). Therefore the altered surface marker expression profile after infection is not mediated via TLR-4.

HSCs were then differentiated towards monocytes using a cytokine mixture consisting of stem cell factor, thrombopoietin, and flt-3 ligand (see Section 2). Incubation of HSCs in this differentiation medium leads to the upregulation of the myeloid markers CD13 and CD33, and monocyte-specific antigens such as CD14, while the stem cell marker CD34 was downregulated (Fig. 2a). CD54 (ICAM-1) was still detectable at low levels, but other costimulatory molecules were not expressed after incubation with the cytokine combination. Surface markers characteristic for T- and B-cells (CD3 and CD19), as well as for mature dendritic cells (CD83) were also undetectable.

Infection studies were carried out after incubation of HSCs for 3 (d3 HSCs) or 6 days (d6 HSCs) in the cell culture medium. These time points were selected since monocytic differentiation occurs within this period [8]. In comparison to the uninfected situation infection of d6 HSCs with Y. enterocolitica and L. monocytogenes led to an enhanced upregulation of CD13 and CD14 24 h p.i., which is expressed at high levels on monocytes (Fig. 2b). This effect of altered cell surface expression was less pronounced after infection of d3 HSCs (data not shown).

Our data indicate that Y. enterocolitica and to a lesser degree L. monocytogenes induced a more pronounced differentiation of HSCs towards monocytes. CD14 has been identified as a high-affinity cell surface receptor for complexes of LPS and serum LPS-binding protein. Therefore, the more pronounced CD14 surface expression on HSCs could be a reaction to cope with further bacterial encounters. Although we noticed differentiation towards monocytes, infected cells showed an upregulation of CD34. In addition HSCs express CD54 (ICAM-1) and B7-H1, and to a much lesser degree CD80 (B7.1), CD86 (B7.2), and CD83, a marker of mature dendritic cells after bacterial stimulation. In general the effects were more pronounced after infection with Y. enterocolitica than with L. monocytogenes (Fig. 2b).

To investigate if these changes in surface marker expression are related to bacterial uptake by partially differentiated HSCs, we evaluated the surface marker expression after the addition of cytochalasin D, an inhibitor of bacterial uptake, to the culture medium. We observed no difference between molecule expression in the presence or absence of the uptake inhibitor. These results underline that the uptake of bacteria was not essential for the surface molecule changes. In addition, these data indicate that reorganization of the cell membrane protein composition can occur independent of the actin cytoskeleton. Furthermore, we could show that changes in surface
marker expression occur with both viable and heat-killed bacteria.

3.2. Cytokine profile of HSCs after bacterial infection

To investigate whether secreted factors are released from human HSCs after incubation with *L. monocytogenes* or *Y. enterocolitica*, we evaluated a panel of major cytokines and growth factors. We measured G-CSF, GM-CSF, IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p40), IFN-γ, and TNF-α 24h p.i. in cell culture supernatant.

Uninfected HSCs, either undifferentiated or grown for 6 days in the monocytic differentiation cytokine combination, did not produce detectable levels of the majority of the tested cytokines. Only a slight release of G-CSF (1 ng ml⁻¹), GM-CSF (500 pg ml⁻¹), and IL-1β (170 pg ml⁻¹) was observed in these cells.

Subsequently, undifferentiated HSCs, d3 HSCs and d6 HSCs were incubated for 24 h with either *Y. enterocolitica* or *L. monocytogenes* and the production of cytokines was determined.

In general, bacterial infection did not lead to an increased production of G-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, or IL-13. Undifferentiated HSCs produced a moderate level of GM-CSF (up to 3 ng ml⁻¹), while all other cytokines were hardly detectable. Infection of d3 and more pronouncedly of d6 HSCs resulted in IL-6 (Fig. 3a), IL-8, IL-10 (Fig. 3b), IL-12 (Fig. 3c), TNF-α (Fig. 3d), and
GM-CSF upregulation. In comparison to L. monocytogenes infection, infection with Y. enterocolitica resulted in particular in a stronger upregulation of IL-10, TNF-α, and IL-12 p40 (Fig. 3). These differences may be due to an alternative pathway of signal transduction induced by Gram-negative and Gram-positive bacteria and LPS and lipoteichoic acid (LTA). It was shown recently that human dendritic cells require a higher concentration of listerial LTA in comparison to LPS to become stimulated and to release cytokines [12].

Investigations with viable and heat-killed bacteria showed only one major difference concerning the production of cytokines. Infection of d3 and d6 HSCs with heat-killed bacteria led to an approximately 10-fold increased release of IL-8 in comparison to viable bacteria. This could be explained by the fact that heat killing of the bacteria will lead to a release of bacterial components, namely bacterial Cpg DNA which could be responsible for the increased IL-8 production. This is underlined by observations of Akhtar et al., who showed that bacterial DNA evokes epithelial IL-8 production in a time- and dose-dependent manner by a mitogen-activated protein kinase-dependent pathway, while other cytokines are dependent on the activation of nuclear factor κB [13].

This study clearly demonstrates that expression of surface antigens and cytokine release of HSCs can be influenced by pathogens. Infection with bacteria promotes differentiation towards a monocytic phenotype. This effect is more pronounced when HSCs have already started to differentiate as shown by infection experiments with undifferentiated HSCs cultured in a differentiation medium. These cells express characteristic monocyte surface markers and adopt a monocytic cytokine secretion profile. Interestingly, infection also results in secretion of GM-CSF, even when undifferentiated HSCs encounter bacteria. This upregulation of GM-CSF might result in an increased amount of progenitor cells. Indeed, when we counted the number of viable cells 24 h p.i. and compared the results with the number of viable cells in uninfected controls we found no significant difference, although L. monocytogenes and Y. enterocolitica are known to kill host cells upon encounter. In line with this observation, we could show that bacterial infection results in an elevation of the CD34+ cell fraction. This enhanced reconstitution of the myeloid progenitor pool might offer protection against challenge with bacterial pathogens [14] and is one possible explanation for leukocytosis during bacterial infections. Proliferative kinetics of the progenitor cells, in particular survival and expansion, are controlled by interacting
cytokines. An effect was described for IL-3, IL-4, IL-6, GM-CSF, G-CSF, IL-11, IL-12, leukemia inhibitory factor, and stem cell factor [15,16]. In addition the expression of receptors for IL-3, IL-6, and GM-CSF appears to be very low on the most immature subsets of CD34+ cells, but increases progressively during successive stages, in particular during myelo-monocytic differentiation [17]. Therefore, the cytokine release of hematopoietic progenitor cells after bacterial infection is likely to influence proliferation and differentiation of HSCs. However, it is so far not clear if the changes in the surface molecule receptors result from cytokine release or are mediated by direct signaling via pathogen recognition receptors.

Since infection of bone marrow progenitors may contribute to hematologic manifestation of diseases, further studies are needed to elucidate this important interaction between pathogens and stem cells.

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