Impaired clearance of apoptotic cells leads to HMGB1 protein release in the bone marrow of patients with Myelodysplastic Syndromes and induces TLR4-mediated proinflammatory cytokine production

Maria Velegraki,1 Evaggelia Papakonstanti,2 Irene Mavroudi,1 Maria Psyllaki,1 Christina Kalpadaki,1 Christos Tsatsanis,2 and Helen A. Papadaki1

1Department of Hematology; 2Department of Biochemistry; and 3Department of Clinical Chemistry, University of Crete School of Medicine, Heraklion, Greece

BACKGROUND

Excessive pro-inflammatory cytokine production in the bone marrow (BM) has been recognized as a prominent pathogenetic mechanism for the disturbed hematopoiesis in patients with Myelodysplastic Syndromes (MDS). However, the upstream pathways, the exact cellular source and the triggering events related to cytokine excess in MDS BM remain unknown. The aim of this study was to investigate the possible involvement of Toll-like receptors (TLRs) and their endogenous ligands in the induction/maintenance of the inflammatory process in MDS BM.

METHODS

TLR expression was evaluated in the BM of MDS patients (n=27) and healthy controls (n=25) using flow-cytometry. Quantitative PCR analysis of 84 genes related to TLR-mediated signal transduction was performed using a commercially available PCR array in immunomagnetically sorted CD14+ BM cells of patients and controls. Results were confirmed by testing separately three significantly over-expressed genes.

The pro-inflammatory cytokine production by patient monocytes treated with autologous plasma in the presence/existence of a TLR4 inhibitor were measured with chemoluminescence and the levels of the high mobility group box 1 (HMGB1) protein, a TLR4 endogenous ligand, were assayed by ELISA in long-term BM culture (LTBMC) supernatants. To examine whether the excess of HMGB1 in patients’ BM is due to macrophage dysfunctions we used a fluorescent microscopy-based assay to estimate the macrophage capacity to phagocytose apoptotic cells. To examine the biological consequences of the impaired clearance of apoptotic cells we assessed the HMGB1 levels in co-cultures of patient macrophages with different numbers of apoptotic cells.

RESULTS

A statistically significant increase in the proportion of TLR4+ cells within the CD14+ BM cell fraction was observed in patients (6.10±2.27%) compared to controls (2.01±1.38%) along with an up-regulation of TLR4 expression as was indicated by the TLR4 MRP1 in MDS patients. (Fig. 1)

To determine whether TLR4 over-expression in BM monocytes of MDS patients is associated with an activated TLR-mediated signaling, we screened 84 TLR-associated genes. Fifty-three out of 84 TLR-related genes displayed at least a four-fold increase mRNA expression in MDS patients compared to controls. (Fig. 2a)

To validate the data obtained from the PCR array analysis, we measured the mRNA expression of three representative genes, namely the MyD88, TRIF/TICAM1 and TRAM/TICAM2 by means of individual quantitative RT-PCR reactions. The relative mRNA expression of MyD88, TRIF/TICAM1 and TRAM/TICAM2 was significantly increased in MDS patients. (Fig. 2b)

Incubation of patient monocytes with autologous BM plasma resulted in a TLR4-dependent production of proinflammatory cytokines as was indicated by the significant decrease of IL-1β, IL-6 and TNFα levels in the presence of a TLR4 inhibitor compared to cultures treated with the BM plasma alone (Fig. 3). These data suggest that soluble factor(s) present in the BM of MDS patients apparently induce the production of proinflammatory cytokines by MDS BM monocytes via a TLR4-mediated pathway.

MDS patients displayed increased expression of IRAK4 and SHIP1 (Fig. 4), two molecules which negatively regulate TLR-signaling contributing therefore to the resolution of the TLR-induced inflammatory reactions. These data indicate that the activated TLR signaling in patient BM monocytes is unlikely to be due to inadequate suppressor mechanisms but is apparently due to constant stimulatory effects.

HMGB1 levels were significantly increased in LTBMC of patients compared to controls suggesting that HMGB1 might constitute an endogenous TLR4-activating ligand in MDS BM (Fig. 5).

To probe the hypothesis that increased HMGB1 levels in MDS BM microenvironment might be the result of ineffective clearance of apoptotic cells by BM macrophages, we examined their capacity to engulf apoptotic cells. Indeed, BM macrophages from MDS displayed decreased apoptotic cell phagocytosis capacity in comparison to healthy individuals (Fig. 6).

HMGB1 release by MDS BM macrophages loaded with increasing numbers of apoptotic cells for different time periods was dependent on the apoptotic cell load (P<0.001) and incubation time (P<0.0417) (Fig. 7).

In conclusion, these data indicate novel pathophysiological mechanisms for the immunologically disturbed BM microenvironment in MDS. A primary apoptotic cell clearance defect of BM macrophages might contribute to the inflammatory process in MDS BM through aberrant release of TLR4-inducing molecules such as HMGB1, from the apoptotic/necrotic cells present in patients’ marrow microenvironment.

DOI: 10.3252/pso.eu.17eha.2012