European Journal of Cell Biology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Short communication

Cytokine-mediated induction of MHC class II in human neutrophils is dependent on NADPH oxidase activity

Boris K. Pliyev*, Tatyana V. Dimitrieva, Valery G. Savchenko

Hematology Research Center, Novy Zykovsky Pr. 4, Moscow 125167, Russia

ARTICLE INFO

Article history:
Received 11 September 2014
Received in revised form 31 October 2014
Accepted 3 November 2014

Keywords:
Neutrophils
MHC class II
NADPH oxidase
ROS

ABSTRACT

In response to selected cytokines, neutrophils are induced to express MHC class II and acquire properties of antigen-presenting cells. Here we show that (a) GM-CSF- and IFN-γ-mediated induction of MHC class II in human neutrophils is associated with intracellular ROS up-regulation, (b) cell-permeable ROS scavengers MnTMPyP and polyethylene glycol-conjugated superoxide dismutase and NADPH oxidase inhibitors diphenylene iodonium and apocynin abrogate both the cytokine-mediated ROS elevation and the induction of MHC class II and (c) neutrophils from chronic granulomatous disease patients which lack NADPH oxidase activity fail to express MHC class II in response to the cytokines. Thus, NADPH oxidase activity is required for the cytokine-mediated induction of MHC class II expression in neutrophils.

Introduction

Both neutrophil precursors and mature neutrophils can be driven to differentiate into cells with characteristics of dendritic cells and the capacity to present antigens (Iking-Konert et al., 2001; Matsushima et al., 2013; and references therein). This lineage switch is sometimes referred to as neutrophil transdifferentiation (Iking-Konert et al., 2001). The neutrophil-generated dendritic-like cells express MHC class II, co-receptor molecules CD80 and CD86, and markers of dendritic cells, such as CD83. Although neutrophil transdifferentiation has repeatedly been shown to be induced in response to selected cytokines in vitro and in diverse inflammatory settings in vivo (Wagner et al., 2006; Geng et al., 2013), molecular mechanisms of this phenomenon are obscure.

Reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide, are unstable and highly reactive compounds that are formed upon incomplete reduction of oxygen (Finkel, 2011). There are many potential sources of ROS in the cell but the major ones are mitochondria and NADPH-dependent oxidases (NOX), a family of membrane-bound enzymes that rely on NADPH for their activity. Excessive ROS production can cause deleterious effects and is related to many pathological conditions and aging. However, emerging evidence suggests that moderate levels of ROS are important for normal physiology. ROS are now considered to function as signaling molecules that regulate cellular functions (Finkel, 2011).

One of the hallmarks of neutrophil physiology is the relatively high concentration of ROS that are generated by NADPH oxidase (NOX2) (Fialkow et al., 2007). NADPH oxidase is inactive in resting cells, but can be activated by a variety of endogenous and exogenous inflammatory mediators. Its activation is associated with an increase in oxygen consumption (neutrophil respiratory burst) and concomitant generation of ROS. As neutrophils generate large amounts of ROS via the activation of NADPH oxidase, redox regulation of cellular signaling has particularly important physiological consequences in these cells. Extensive evidence indicates that endogenous ROS regulate neutrophil effector functions and neutrophil viability (reviewed in Fialkow et al., 2007).

Results from previous studies indicate that intracellular ROS can regulate expression and function of MHC class II. For example, IFN-γ-mediated elevation of intracellular ROS has been shown to be necessary for the induction of MHC class II expression in vascular endothelial cells (Grimm et al., 2002). Up-regulation of MHC class II in Kupffer cells and glial cells and MHC class II-restricted antigen presentation by the cells are also dependent on intracellular ROS generation (Maemura et al., 2005; Tezel et al., 2007). Functional NADPH oxidase appears to be required for efficient MHC class II antigen presentation by B cells and monocytes (Heijnen et al., 1986; Crotzer et al., 2012). In this study, we addressed whether ROS regulate cytokine-mediated induction of MHC class II in human neutrophils.

* Corresponding author. Tel.: +7 495 612 21 23; fax: +7 495 612 21 23.
E-mail address: bpliyev@cardio.ru (B.K. Pliyev).

http://dx.doi.org/10.1016/j.ejcb.2014.11.001
0171-9335/

Please cite this article in press as: Pliyev, B.K., et al., Cytokine-mediated induction of MHC class II in human neutrophils is dependent on NADPH oxidase activity. Eur. J. Cell Biol. (2014), http://dx.doi.org/10.1016/j.ejcb.2014.11.001

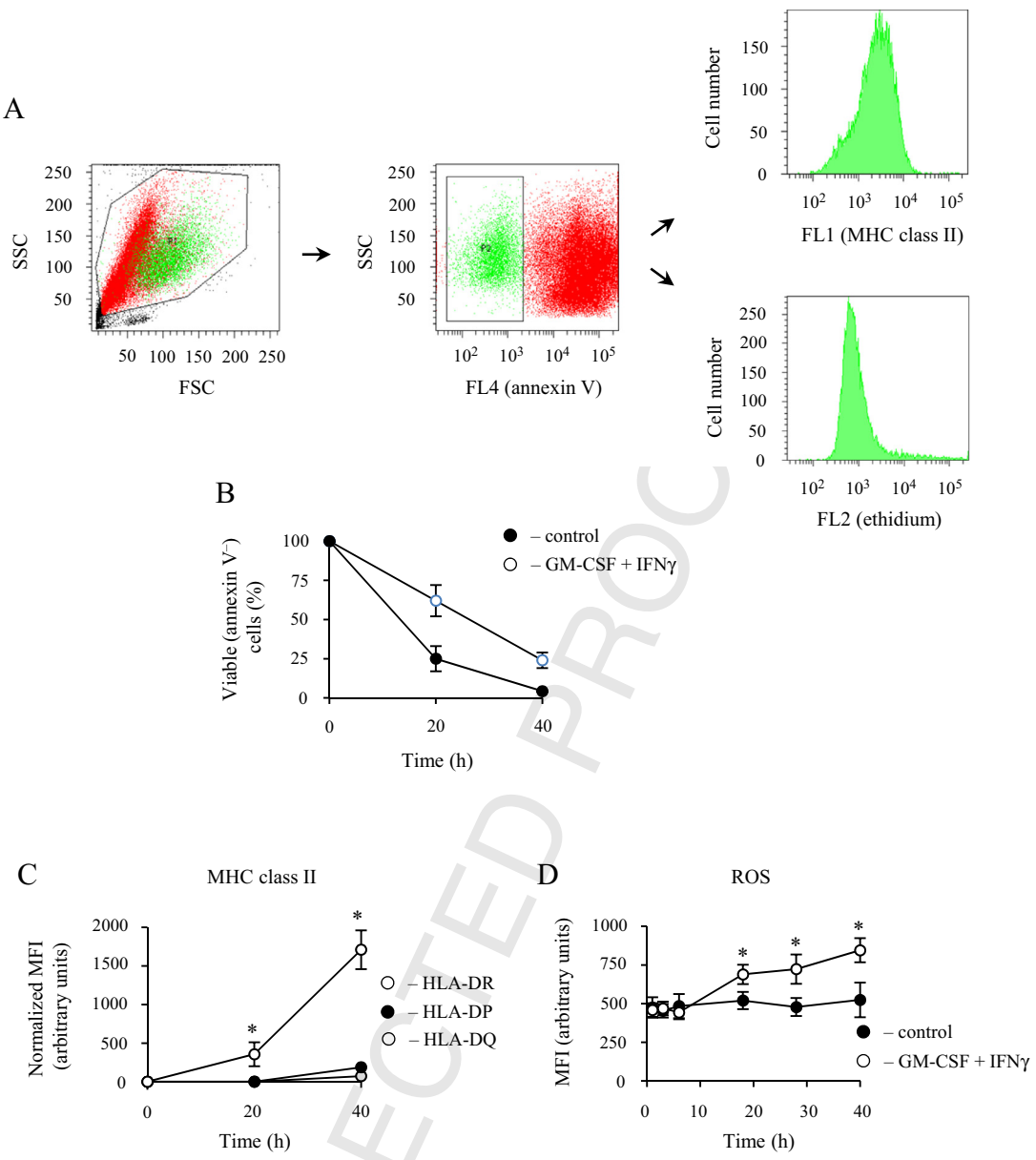

Fig. 1. GM-CSF/IFN-γ-mediated induction of MHC class II in neutrophils is associated with elevation of intracellular ROS. (A) Analysis of MHC class II surface expression and intracellular ROS by flow cytometry. The samples were co-stained with annexin V and MHC class II surface expression and intracellular ROS were measured in viable (annexin V-negative) cells. (B–D) Neutrophils were cultured with or without GM-CSF (25 ng/ml) and INF-γ (50 ng/ml) for up to 40 h and percentages of viable (annexin V-negative) cells (B), surface expression of MHC class II isotypes (C) and intracellular ROS (D) were measured at the indicated time points as described in Materials and Methods. Shown are mean values (±SEM) of five independent experiments. *$p < 0.05$ compared with the corresponding controls (no GM-CSF and IFN-γ added).

Materials and methods

Materials

Diphenylene iodonium (DPI), apocynin and polyethylene glycol-conjugated superoxide dismutase (PEG-SOD) were from Sigma (St Louis, MO). MnTMPyP was from Calbiochem (San Diego, CA). FITC-conjugated mAb against HLA-DR was from BD Pharmingen (San Diego, CA). Dihydroethidium and APC-conjugated annexin V were from Molecular Probes (Eugene, OR).

Neutrophil isolation and culture

Human neutrophils were isolated from peripheral blood of healthy donors as described (Pliyev et al., 2011) by dextran sedimentation followed by Histopaque-1077 density gradient centrifugation. The cell pellet containing neutrophils was then recovered and contaminating erythrocytes were removed by hypotonic lysis with H_2O. Neutrophil purity, as assessed flow cytometrically by staining for CD66b (positive) and CD49d (negative), was greater than 95%. The cell viability, as determined by trypan blue exclusion, was greater than 99%.

Neutrophils were cultured at a density of 4×10^6 cells/ml in 48-well cell culture plates (Costar, Corning, NY) in a total volume of 0.25 ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated FBS at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were treated as described in figure legends.

Please cite this article in press as: Pliyev, B.K., et al., Cytokine-mediated induction of MHC class II in human neutrophils is dependent on NADPH oxidase activity. Eur. J. Cell Biol. (2014), http://dx.doi.org/10.1016/j.ejcb.2014.11.001