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ORIGINAL ARTICLE

Comparing effects of interleukin-2 and interleukin-4 on the expression of MHC class II, CD80 and CD86 on polymorphonuclear neutrophils

Bahaa Kenawy Abuel-Hussien Abdel-Salam *

Department of Zoology, Faculty of Science, Minia University, 61519 El-Minia, Egypt

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KEYWORDS

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Abstract Polymorphonuclear neutrophils (PMN) have contributed only to the innate immune response, due to their phagocytosis activity. They have a short life time. Some studies of PMN cytokine production and expression of numerous cell surface proteins have suggested that PMN are likely to influence adaptive responses and may satisfy the criteria of antigen presenting cells. Flow cytometry was used in the present study for the detection of cell surface major histocompatibility complex (MHC) class II, CD80 and CD86 required for antigen presentation and subsequent T-cell activation. Human peripheral blood neutrophils were used for this purpose. *In vitro* stimulation of PMN with IL-2 or IL-4 for 24 h showed expression of MHC class II, CD80 and CD86. These observations, therefore, provide support to the hypothesis that human PMN have the potential to express molecules required for antigen presentation.

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1. Introduction

Polymorphonuclear neutrophils (PMN) possess a very short half-life in the circulation because they constitutively undergo apoptosis [1,2]. Under certain conditions PMN play an important role in the effectors arm of host immune defense through the clearance of immune complexes, phagocytosis of opsonized particles, and release of inflammatory mediators [3–5]. During the last few years the image of PMN has changed considerably. Traditionally considered to be the first line defense against bacterial infection it became increasingly clear that PMN also participate in chronic inflammation disease and regulation of the immune response when appropriately activated [6]. For surface molecules, there are several reports that PMN from a variety of species can express major histocompatibility complex (MHC) class II and costimulatory molecules (CD80 and

Abbreviations: PMN, polymorphonuclear neutrophil; MHC, major histocompatibility complex; IL, interleukin

* Tel.: +2 086 2357837; mobile: +2 0160904110; fax: +2 086 2342601.
E-mail address: Manar_Muhamad@yahoo.com.

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CD86) [6–10]. Under certain stimulation murine neutrophils could present Class II restricted antigen [11].

PMN function and recruitment to the site of inflammation have been shown to be upregulated by various cytokines, including interleukin (IL)-1, IL-8, tumor necrosis factor (TNF), interferon-γ (IFN-γ), and granulocyte macrophage-colony stimulating factor (GM-CSF) [5,12]. In addition IL-15 stimulated PMN acquire HLA-DR [13].

Classically, IL-2 has been considered to be a lymphocyte-activating and growth-promoting factor, and has been widely studied on T cells and NK cells [14]. Monocytes have been reported to express IL-2Rβ and to be activated by IL-2 for tumoricidal activity [15]. Thus far, PMN have not been studied for their interaction with IL-2, and their possession of IL-2R is unknown. The direct effect of IL-2 on PMN, especially the mechanisms involved in the activation of PMN, is unknown, although the ability of other immune cells to respond to IL-2 is well studied. Preliminary studies have shown that PMN have the capacity to respond to IL-2 with increased antifungal activity [16]. More importantly, it has been identified that PMN express surface receptors for IL-2, but only IL-2Rβ and not IL-2Rα is present [16].

IL-4 production has been found to occur in thymocytes, mature T-cells, certain malignant T-cells, mast-cells and basophiles and occasionally, in transformed B-cells [17]. In addition, it has an effect on B-cells, T-cells, monocytes, mast-cells, endothelial cells, and fibroblasts [18]. Directly and/or indirectly, IL-2 has a prominent role in the regulation of IL-4 producing cells [17]. IL-4 binds to a high-affinity cell-surface receptor (IL-4R) to exert its effects [19]. It promotes the growth and differentiation of activated human B-lymphocytes and shares many biological functions with IL-13 [20].

Following this approach, the present study was undertaken to estimate the expression of the antigen presenting molecule MHC class II and the co-stimulatory molecules CD80 and CD86 on PMN stimulated with IL-2 or IL-4.

2. Materials and methods

2.1. Donors, media, reagents and antibodies

Blood was taken by venous puncture using 7·5 ml heparin-coated tubes (Sarstedt, Nümbrecht, Germany) and analyzed within 2 h. Recombinant human (rh) IL-2 and IL-4 were purchased from Sigma (St Louis, MO, USA). For fluorescence-activated cell sorter (FACS) analysis of whole blood, erythrocyte FACS lasing solution was obtained from Becton Dichinson (Heidelberg, Germany) and diluted 1:10 by bidistilled water. For cytofluorometry fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-labeled murine MoAbs were used.

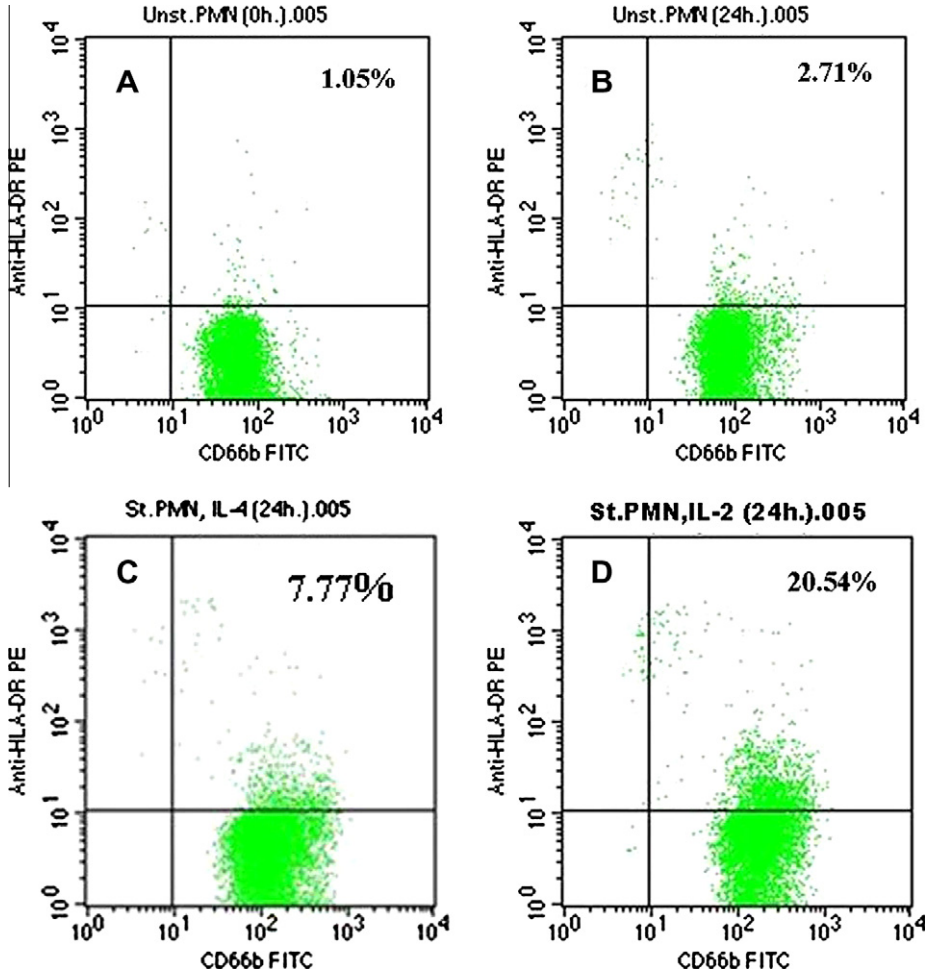

Figure 1 Cytoflourometry of the MHC class II induction in the whole blood PMN. (A) Unstimulated PMN (0 h). (B) Unstimulated PMN (24 h). (C) Stimulated PMN with IL-4 (24 h). (D) Stimulated PMN with IL-2 (24 h).

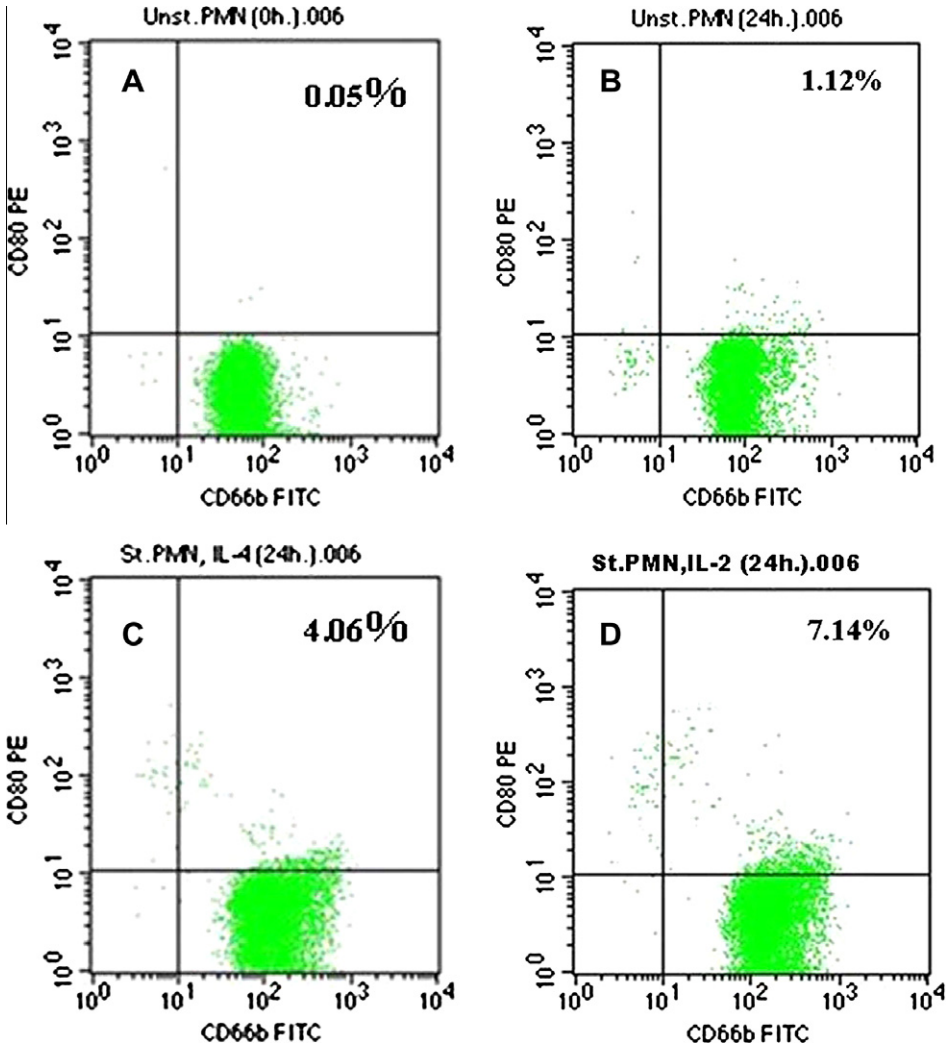

Figure 2 Flow cytometry of the CD80 induction in the whole blood PMN. (A) Unstimulated PMN (0 h). (B) Unstimulated PMN (24 h). (C) Stimulated PMN with IL-4 (24 h). (D) Stimulated PMN with IL-2 (24 h).

Mouse IgG1 FITC, IgG2a PE, CD66b-FITC, HLA-DP + DQ + DR:PE, CD80:PE and CD86:PE were obtained from Coulter Immunotech (Marseilles, France).

2.2. Direct flow cytometry analysis

To avoid alteration of antigen expression, PMN from healthy donors were analyzed in whole blood. For double labeling, anti-CD66b-FITC as a PMN marker and the respective PE-labeled antibody were used in equal protein concentrations. Cells were analyzed by FACSCalibur and CellQuest software (Becton Dickinson, SanDiego, CA). Results were expressed as the percentage of positive cells in the respective gate or quadrant.

2.3. Cultivation and stimulation of PMN

In all sets of experiments PMN in whole blood were cultured in the presence and absence of IL-2 or IL-4 for 24 h at 37 °C with 5% CO_2 in air. Heparinized blood was placed into 24-well plate, 2 ml/well and incubated in the presence or absence of the activator IL-2 (10 ng/ml) or IL-4 (6 ng/ml) and incubated for about 24 h at 37 °C with 5% CO_2.

2.4. Statistical analysis

Statistical analysis of the obtained data was performed using one way analysis of variance (ANOVA) test followed by least square differences (LSD) analysis (for comparison between means). Results were expressed as mean ± standard error (S.E.), and differences were considered to be significant at ($P < 0.05$).

3. Results

Results were expressed as percentages of positive cells in the respective gate or quadrant. Each set of experiments was repeated *in vitro* several times.

3.1. In vitro expression of MHC class II on PMN

The majority of healthy donors PMN expressed CD66b (Fig. 1A–D). In the first set of experiments the effect of IL-2 on the expression of MHC class II was tested, where the study found that PMN on whole blood showed expression of MHC

class II recording 20.54% (Fig. 1D) and 7.77% (Fig. 1C) by using IL-2 and IL-4, respectively, as stimulators. In contrast, fresh and unstimulated cells cultured with medium only counting 1.05% (Fig. 1A) and 2.71% (Fig. 1B), respectively. Statistical analysis showed that, there is a significant difference between IL-4 or IL-2 stimulated cells and unstimulated cells, also IL-4 stimulated PMN had a significant difference from IL-2 stimulated cells, $P < 0.05$ (Fig. 4A).

3.2. In vitro induction of CD80 on PMN

As shown for MHC class II, surface expression of CD80 was most impressive following cultivation of whole blood with the stimuli for 24 h. The proportion of double-positive cells (right upper quadrant) was estimated, where CD80 positive cells was slightly higher in stimulated cells recording 4.06% by using IL-4 (Fig. 2C) and 7.14% by stimulation with IL-2 (Fig. 2D) than unstimulated cultured with medium for 24 h (1.12%) and fresh PMN, 0.05% (Fig. 2B and A, respectively). Fig. 4B showed that, there is a significant difference between IL-4 or IL-2 stimulated cells and unstimulated cells, also IL-4 stimulated PMN had a significant difference from IL-2 stimulated cells ($P < 0.05$).

3.3. In vitro induction of CD86 on PMN

For CD86, high expression was recorded on the surface of both IL-2 stimulated PMN counting 24.20% (Fig. 3D) and IL-4 stimulated cells recorded 20.48% (Fig. 3C), while percentage of CD86 molecules on the surface of unstimulated PMN cultured with medium alone measured 6.97 (Fig. 3B). Fresh PMN had 1.55% (Fig. 3A). Statistical analysis also showed that, there is a significant difference between IL-4 or IL-2 stimulated cells and unstimulated cells ($P < 0.05$). In contrast to MCH Class II and CD80, there is no significant difference between IL-4 stimulated PMN and IL-2 stimulated cells, $P > 0.05$ (Fig. 4C).

4. Discussion

All sets of experiments were carried out in whole blood to avoid any receptors changes. More than 99% of PMN were

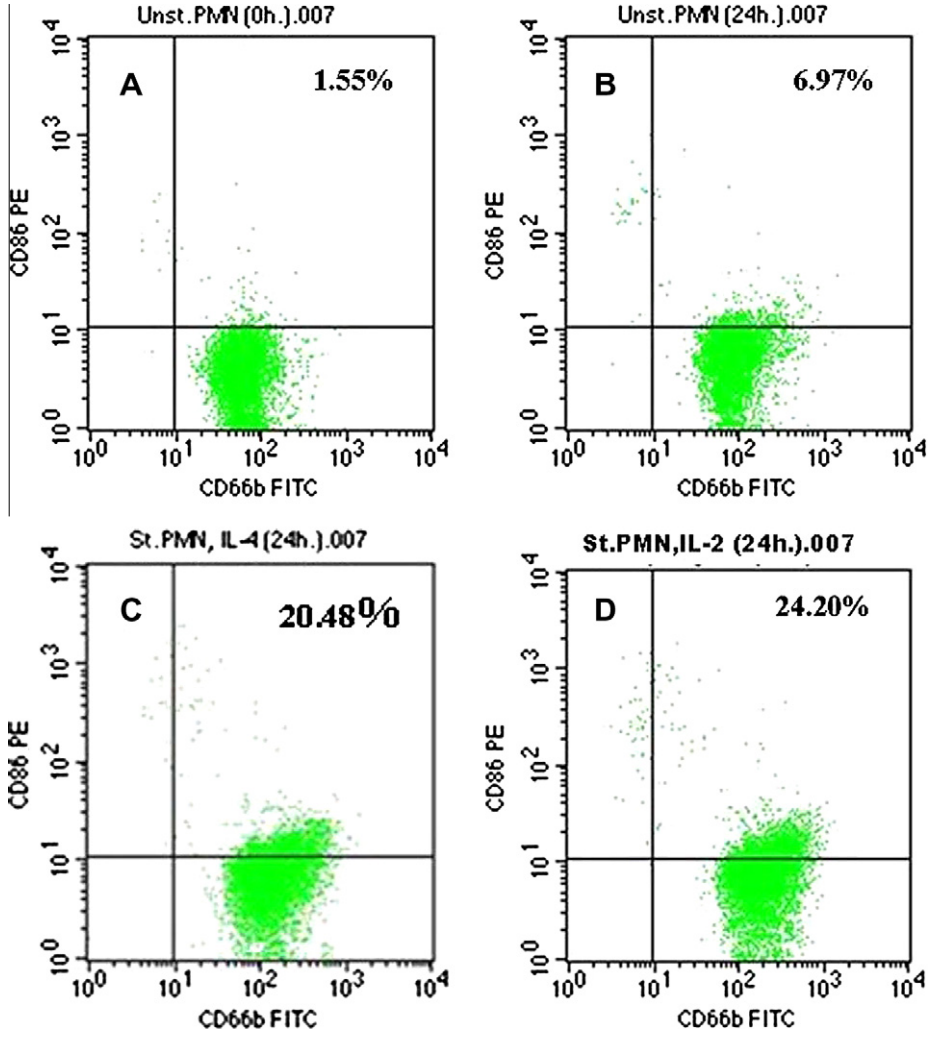

Figure 3 FACS for CD86 surface expression in the whole blood PMN. (A) Unstimulated PMN (0 h). (B) Unstimulated PMN (24 h). (C) Stimulated PMN with IL-4 (24 h). (D) Stimulated PMN with IL-2 (24 h).

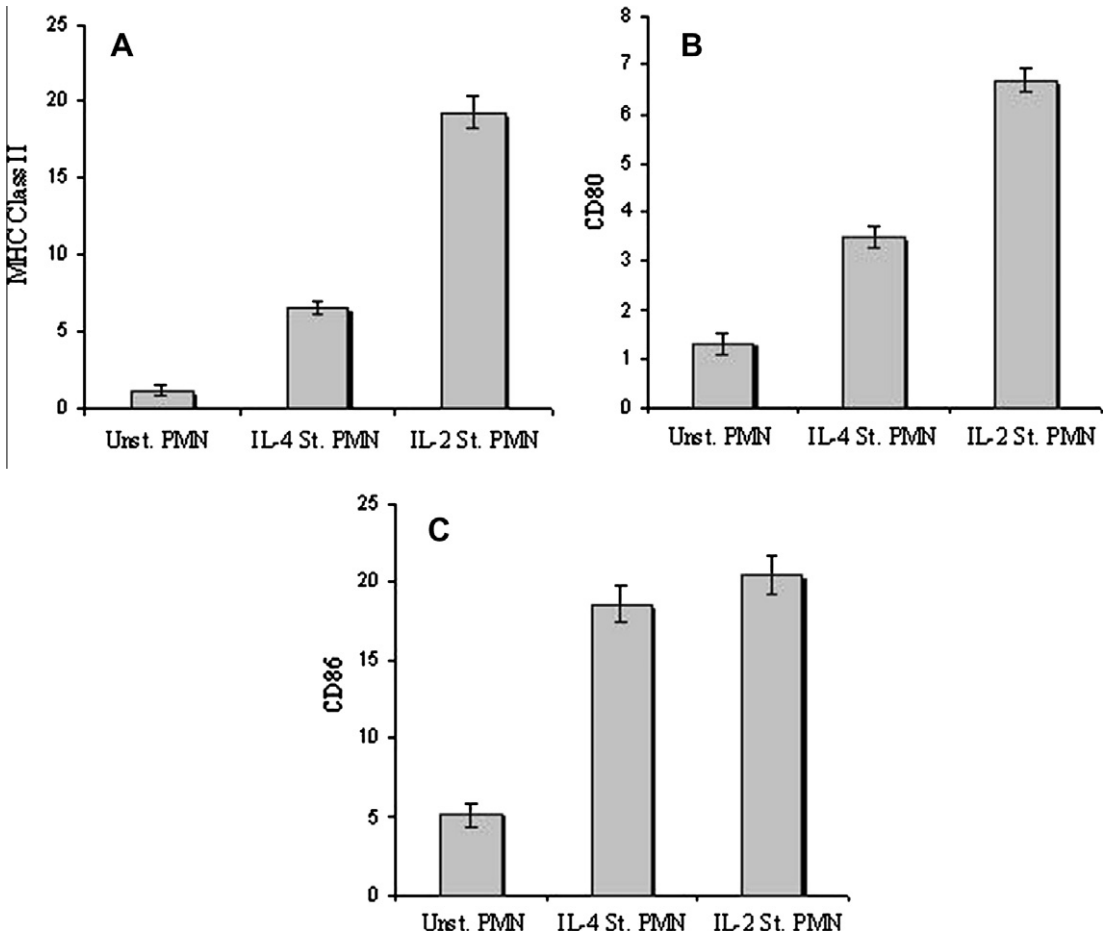

Figure 4 Expression of some surface molecules on PMN stimulated with either IL-2 or IL-4. (A) MHC class II expression. (B) CD80 expression. (C) CD86 expression.

selected by making a gate around a group of cells using the positive marker of PMN (anti-CD66b-FITC).

PMN are considered short-lived cells undergoing spontaneous apoptosis *in vivo* as well as in culture [21]. Previous studies have demonstrated that PMN can be induced *in vitro* to synthesize and release various cytokines, suggesting that these cells can contribute significantly to the initiation and amplification of cellular and humoral immune responses [5]. The detection of these molecules, therefore, provides strong support for the hypothesis that human PMN can actively synthesize immunoregulatory molecules [22] and have the potential to act as antigen presenting cells [8].

During the last period, it has become increasingly evident that culturing PMN in the presence of cytokines extends their life span [23–26]. Cultured PMN synthesize and release immunomodulatory cytokines by which they may participate in the afferent limb of the immune response [5].

This study has clearly shown that PMN could be induced to express MHC class II, CD80 and CD86 after activation with either IL-2 or IL-4. These data are in accordance with other results where MHC class II, CD80 and CD86 were expressed on the PMN surface after exposure to GM-CSF and/or INF-γ [6].

The antigen presenting molecule MHC class II and the co-stimulatory molecules CD80 and CD86 play an important role in T-cells proliferation, where MHC class II presents the engulfed antigen to T-cells [6]. Additionally, CD80 and CD86 act as second signal molecules involving in the stimulation of T-cells to produce the autocrine growth factor IL-2 without which T-cells are unable to proliferate [27].

The activation and recruitment of PMN were also regulated by IL-15 [13], IFN-γ [28], CSF-CSF [29–31] and IL-8 [32]. In addition, PMN possess IL-2Rβ chain [16,33] and IL-2Rγ chain [34] that have the ability to bind with IL-4 [35]. These published data prompted us to study the effect of IL-2 and IL-4 on PMN functions.

In conclusion, stimulated PMN with either IL-2 or IL-4 lead to expression of the antigen presenting molecules (MHC class II) and the co-stimulatory molecules (CD80 and CD86). These molecules play an important role in antigen presentation and consequently T-cell proliferation [36]. This means that IL-2 and also IL-4 stimulated PMN might be involved indirectly in acquired immune response in addition to their role in innate immunity.

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