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Original Article

Upregulation of major histocompatibility complex class II, CD83, CD64, and CD14 on polymorphonuclear neutrophils stimulated with interleukin-15

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Background and Purpose: Polymorphonuclear neutrophils (PMNs) act as a first line of defense against bacterial infections. Recent studies indicated that PMNs could act as antigen-presenting cells. The aim of this study was to detect the expression of major histocompatibility complex (MHC) class II and certain molecules and receptors (CD83, CD64, and CD14) on the surface of PMNs stimulated with interleukin (IL)-15.

Methods: PMNs were isolated from heparinized whole blood of healthy volunteers. Cells were incubated for 48 h at 37°C in a humidified incubator with 5% carbon dioxide. After incubation for 48 h with and without IL-15, surface molecules were assayed by cytofluorometry.

Results: Stimulation with IL-15 produced an increase in the expression of MHC class II and upregulation of the costimulatory molecules CD83, the immunoglobulin G receptor CD64, and the lipopolysaccharide receptor CD14 on the surface of PMNs.

Conclusions: The expression of MHC class II, CD83, CD64, and CD14 indicates that PMNs may be involved in both innate and acquired immunity.

Key words: Antigens, CD; Interleukin-15; Major histocompatibility complex; Neutrophils; Receptors, IgG

Introduction

Cytokines are important protein mediators of the immune response [1], and play a central role in the regulation of cell differentiation and proliferation, and cell-cell communication [2]. In addition, some cytokines have important effector functions via activation of directly cytotoxic compounds (e.g., perforin, oxygen, and nitric oxide radicals). Several studies have indicated that variations in cytokine expression are associated with disease activity in immune-mediated or inflammatory disorders [3].

Interleukin (IL) acts on a specific group of cells that express the correct receptor for the IL. The same IL can be produced or acted on by different cell types eliciting variable biological responses depending on the particular cell and its environment [4].

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IL-15 is a pleiotropic cytokine, which shares biological activities with IL-2. IL-15 uses both beta- and gamma (γ)-chains of the IL-2 receptor for binding and signaling. The IL-15 receptor (IL-15R) complex also includes a specific alpha (α) subunit (IL-15Rα), distinct from the IL-2Rα chain. IL-15R is expressed on various cells involved in the immune response, including T and B cells, natural killer (NK) cells and, more recently, peripheral blood neutrophils [5,6].

IL-15 plays an important role in both innate and adaptive immunity. IL-15 induces T-cell proliferation and cytokine production, and stimulates the locomotion and chemotaxis of T-cells and delays their apoptosis. IL-15 has been shown to stimulate the growth and cytotoxicity of NK cells and to induce antibody-dependent cell-mediated cytotoxicity. It has also been demonstrated that this cytokine induces macrophage functions and B-cell proliferation [5-7]. Investigations have shown that IL-15 potentiated several antimicrobial functions of normal polymorphonuclear neutrophils (PMNs) involved in the innate immune

response against invading pathogens [8]. IL-15 was observed to enhance phagocytosis, nuclear factor-kappaB activation and IL-8 production and to delay apoptosis of these cells. In addition, IL-15 has been shown to prime the metabolic burst of PMNs in response to N-formyl-methionyl-leucyl-phenylalanine [8-12]. Sources of IL-15 include macrophages/monocytes, and lymphocytes and neutrophils [5,8,13,14].

The understanding of PMNs has gradually improved over the years; it is now increasingly appreciated that PMNs are not only the short-lived 'first-line defense' against bacterial infection, but also involved in defense against parasites, fungi, and tumors, as well as playing a role in wound healing and angiogenesis [15]. Moreover, PMNs provide a link between the innate and the adaptive immune response. A regulatory role was presumed because PMNs synthesize and release numerous cytokines [15,16].

The aim of this study was to estimate the upregulation of the antigen-presenting molecule major histocompatibility complex (MHC) class II, important costimulatory molecules CD83, lipopolysaccharide (LPS) receptor CD14, and immunoglobulin G (IgG) Fc receptor CD64 on PMNs stimulated with IL-15.

Methods

Reagents and antibodies

Phycoerythrin (PE)-labeled monoclonal antibodies to the following antigens were used: CD14, CD64 ($Fc_{\gamma}R1$), CD83, human leukocyte antigen-DPDQDR, a fluorescein isothiocyanate (FITC)-labeled antibody to CD66b, and PE- and FITC-labeled mouse IgG as isotype controls (Coulter Immunotech, Marseille, France). Blood was taken from healthy volunteers by venous puncture using 7.5-mL heparin-coated syringe tubes (Sarstedt, Nümbrecht, Germany). The age range of the donor volunteers was 25 to 45 years. There were 7 volunteers for each experiment.

Cytofluorometry

Heparinized blood (100 µL) was incubated with FITC-labeled anti-CD66b antibody (as a marker for PMNs) and a PE-labeled antibody for the antigen to be tested (5 µL each). Following incubation for 30 min, erythrocytes were lyzed using fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson, Heidelberg, Germany). Leukocytes were washed in FACS-buffer (phosphate-buffered saline, containing 1% bovine serum albumin and 0.1% sodium azide), and then resuspended in FACS-buffer containing 1% paraformaldehyde. Fluorescence was measured by the FACSCalibur system using CellQuest software (Becton Dickinson). Results were expressed as a percentage of positive cells.

PMNs cultivation and stimulation

In all sets of experiments, PMNs in whole blood were cultured at 37°C with 5% carbon dioxide in air. Heparinized blood was placed into 24-well plates, 2 mL/well, and incubated in the presence or absence of recombinant IL-15 (Sigma, St. Louis, MO, USA) [20 ng/mL] for about 48 h.

Results

When isolated PMNs were cultured with IL-15, a minor effect on the surface expression of the antigen-presenting molecules MHC class II, the costimulatory molecules CD83, the IgG receptors CD64, and the LPS receptor CD14 was seen, although the surface expression of those molecules was markedly enhanced when whole blood was cultured. Data for different donors are summarized in Table 1.

Induction of MHC class II

In freshly prepared PMNs, MHC class II content recorded 11% (Fig. 1A), but was lower in PMNs cultured with medium alone (6%; Fig. 1B). Induction of MHC class II in PMNs co-cultured for 24 h with IL-15 was minor, but after 48 h reached 11% (Fig. 1C).

Upregulation of CD83

In the first set of experiments, CD83 was detected in fresh PMNs (Fig. 2A) and in PMNs cultured with medium only for 48 h (Fig. 2B), at levels of 7% and 16%, respectively. However, in PMNs cultured in IL-15-treated medium, CD83 content reached 40% (Fig. 2C).

Upregulation of CD64

Expression of CD64 on fresh PMNs recorded 16% (Fig. 3A). After 48 h, unstimulated PMNs had maximally 9% positive cells (Fig. 3B), while CD64 expression increased on IL-15-treated PMNs, reaching 38% (Fig. 3C).

Upregulation of CD14

In parallel to CD64, upregulation of CD14 was also seen. With freshly prepared PMNs stimulated with IL-15, the CD14 content measured 34% (Fig. 4A).

Table 1. Summary of results for polymorphonuclear neutrophil surface molecules after 48-h incubation with or without interleukin-15 stimulation

Surface molecules	Donor no.	Percent cells	
		Unstimulated cells	Stimulated cells
MHC class II	1	6	11
	2	4	8
	3	3	8
	4	5	6
	5	4	4
	6	3	2
	7	2	3
CD83	1	16	40
	2	13	31
	3	10	30
	4	12	20
	5	10	13
	6	8	15
	7	9	213
CD64	1	9	38
	2	7	20
	3	8	19
	4	6	10
	5	5	9
	6	7	11
	7	8	8
CD14	1	11	37
	2	15	30
	3	12	25
	4	10	19
	5	12	18
	6	11	18
	7	13	17

Abbreviation: MHC = major histocompatibility complex

In whole blood, CD64 upregulation occurred within 48 h, reaching 11% in PMNs cultured with medium alone (Fig. 4B) and 37% in IL-15-containing medium (Fig. 4C).

Discussion

PMNs are terminally differentiated cells with a short half-life and limited protein synthesis, but their production in large numbers and their continued regeneration from bone marrow indicated that the recruitment of PMNs into local tissue is an important process of biologic significance [17]. Previous studies have demonstrated that PMNs 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 [16]. There is thus strong support for the hypothesis that human PMNs can actively synthesize immunoregulatory molecules [18] and have the potential to act as antigen-presenting cells (APCs) [19].

The current study showed that PMNs could be induced to express MHC class II after activation with IL-15, as shown in previous studies by interferon-γ and granulocyte monocyte-colony stimulating factor [19]. The absence or low detection of MHC class II on human PMNs stimulated with IL-15 in this study could be due to [20]:

- the relatively long time (44 h) required for class II induction, which is beyond the period of PMN survival in most culture systems;
- variation in IL-2 and IL-15 receptor numbers between individuals;
- variation in synthetic capacity of PMNs;
- differing capacities of cytokine receptors to signal; and
- PMNs themselves producing factors that inhibit class II induction.

In addition to MHC class II, cytofluorometry has been used to show the upregulation of CD14 (the major cell surface receptor for LPS on PMNs), CD64, and CD83 (a cell surface marker for dendritic cells) on PMNs after 48-h treatment with IL-15 in 48-h culture.

The major cell surface receptor for LPS on PMNs is CD14, a 55-kDa glycosylphosphatidylinositol-linked membrane protein. PMNs contain a reservoir of preformed CD14 stored in granules, from where it is translocated to the surface by chemotactic agents and by LPS. Synthesis, expression, and release of membrane-bound CD14 in neutrophils and monocytes are regulated differentially by various cytokines [21].

An important role has been clearly demonstrated for CD14 in the interaction between PMNs and LPS or other bacterial components from both Gram-negative and Gram-positive organisms [21-28].

A receptor expression pattern similar to CD64 has been demonstrated on PMNs isolated from patients with bacterial infections. PMNs from patients with staphylococcal pharyngitis, but not from patients with documented urinary tract infection, were found to stain positive. Also, PMNs from patients with leukocyte adhesion deficiency expressed CD64. In vitro, CD64 can be induced on neutrophils by incubation with interferon, but no induction by granulocyte-monocyte stimulating factor was reported, although an additive effect between both cytokines in vitro was recently discussed [29].

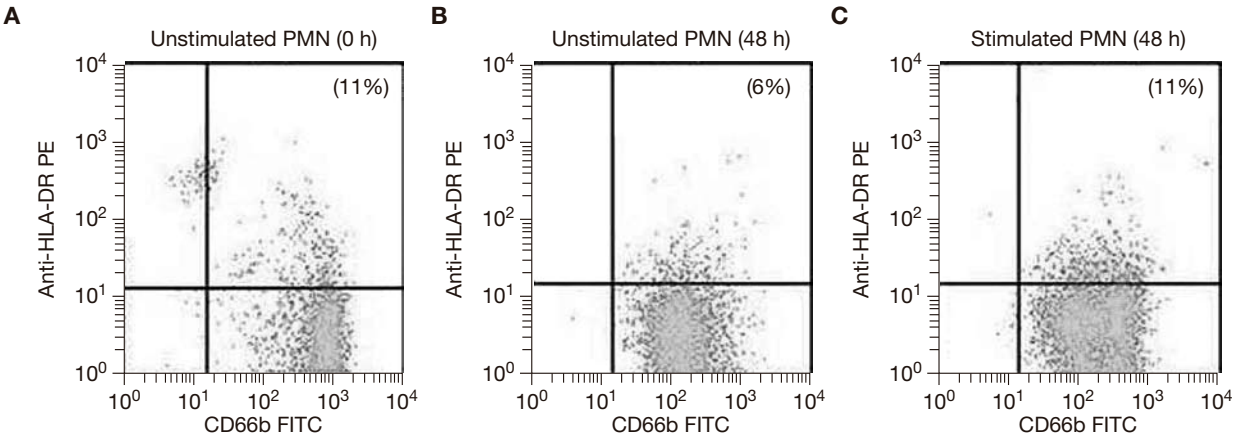

Fig. 1. Cytofluorometric analysis of major histocompatibility complex (MHC) class II expression on polymorphonuclear neutrophils (PMNs) in whole blood labeled with CD66b-fluorescein isothiocyanate (FITC) as a specific marker for PMNs and MHC class II-phycoerythrin (PE). (A) Fresh PMNs. (B) PMNs cultured for 48 h in medium only. (C) PMNs cultured for 48 h in medium containing interleukin-15 (20 ng/mL). HLA-DR = human leukocyte antigen-DR.

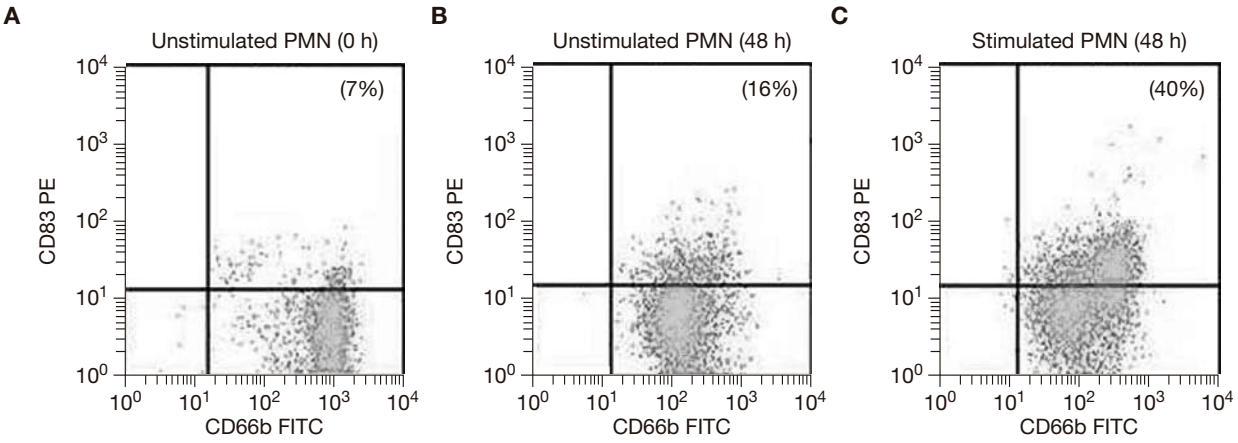

Fig. 2. Cytofluorometric analysis of surface expression of CD83 on polymorphonuclear neutrophils (PMNs) in whole blood labeled with CD66b-fluorescein isothiocyanate (FITC) as a specific marker for PMNs and phycoerythrin (PE)-labeled CD83 antibodies. (A) Fresh PMNs. (B) PMNs cultured for 48 h in medium only. (C) Medium containing interleukin-15 (20 ng/mL).

The expressions of CD14 and CD64 play an important role in PMN activation, as CD14-CD64-positive PMNs interact more efficiently with bacteria because of better recognition and high-affinity binding, and the increased expression of CD64 on monocytes and neutrophils during the first 14 days of septic shock may indicate persistence of leukocyte activation [29].

CD83 is a 45-kDa protein belonging to the immunoglobulin family. Its constitutive expression is restricted to dendritic cells and thymic epithelial cells [30,31]. However, expression of CD83 can be induced in monocytes [32-34], PMNs precursors [35], and even in mature PMNs [36,37]. The functional consequences of CD83 upregulation are not yet known, particularly as the function of CD83 is still elusive. A role in antigen presentation was presumed due to its association with APCs. In addition, a regulatory function for T cells has been described [38,39]. Several lines of evidence argue against a role of CD83 in antigen presentation [19]:

- antibodies to CD83 (at least clones Hb15A and Hb15E, which were tested) did not inhibit MHC class II-restricted T-cell activation;
- high expression of CD83 did not enhance T-cell activation; and
- CD83 is also upregulated in the absence of MHC class II.

Induction of membrane-bound molecules was higher when PMNs were stimulated in whole blood. The explanation for this phenomenon may be the loss of IL-2R and IL-15R by PMNs during the isolation procedure.

In conclusion, knowledge of PMN function has changed in recent years. In addition to their function

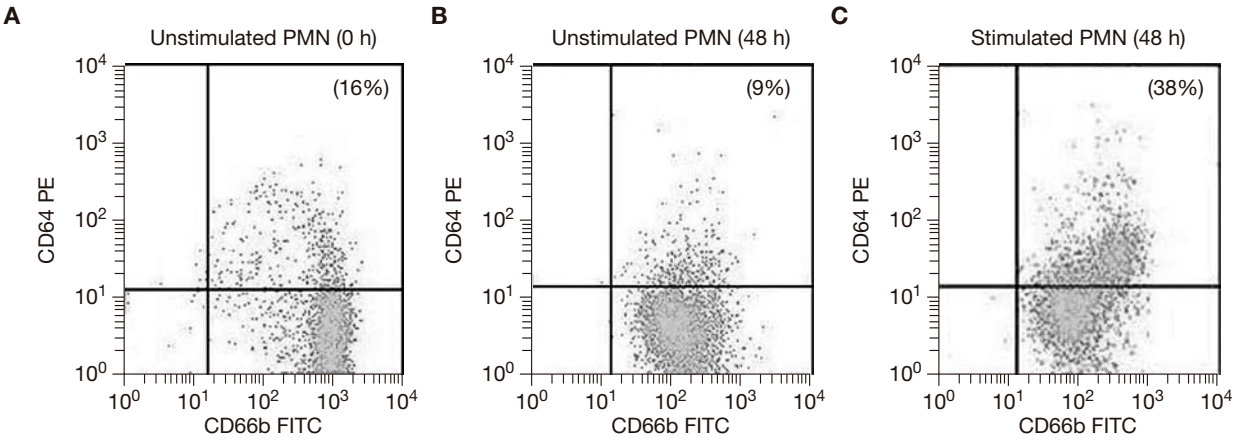

Fig. 3. Cytofluorometric analysis of surface expression of CD64 on polymorphonuclear neutrophils (PMNs) in whole blood labeled with CD66b-fluorescein isothiocyanate (FITC) as a specific marker for PMNs and phycoerythrin (PE)-labeled CD64 antibodies. (A) Fresh PMNs. (B) PMNs cultured for 48 h in medium only. (C) Medium containing interleukin-15 (20 ng/mL).

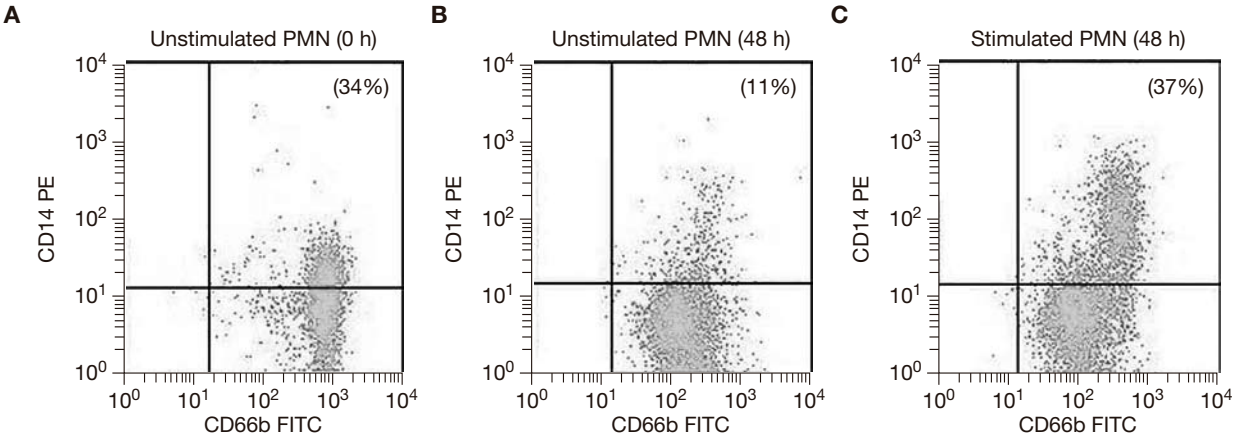

Fig. 4. Cytofluorometric analysis of surface expression of lipopolysaccharide receptor CD14 on polymorphonuclear neutrophils (PMNs) in whole blood labeled with CD66b-fluorescein isothiocyanate (FITC) as a specific marker for PMNs and phycoerythrin (PE)-labeled CD14 antibodies. (A) Fresh PMNs. (B) PMNs cultured for 48 h in medium only. (C) Medium containing interleukin-15 (20 ng/mL).

as the first line of defense against bacterial infection, these cells also act as APCs. This research has confirmed that IL-15-stimulated PMNs acquired APC molecules (MHC class II), LPS receptor (CD14), costimulatory surface molecules (CD83), and CD64.

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References

1. Weber RL, Iacono VJ. The cytokines: a review of interleukins. Periodontal Clin Investig. 1997;19:17-22.
2. Balkwill FR, Burke F. The cytokine network. Immunol Today. 1989;10:299-304.
3. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today. 1996;17:138-46.
4. Bhandari V. Developmental differences in the role of interleukins in hyperoxic lung injury in animal models. Front Biosci. 2002;7:d1624-33.
5. Giri JG, Anderson DM, Kumaki S, Park LS, Grabstein KH, Cosman D. IL-15, a novel T-cell growth factor that shares activities and receptor components with IL-2. J Leukoc Biol. 1995;57:763-6.
6. Girard D, Boiani N, Beaulieu AD. Human neutrophils express the interleukin-15 receptor alpha chain (IL-15Ralpha) but not the IL-9Ralpha component. Clin Immunol Immunopathol. 1998;88:232-40.
7. Nishimura H, Yajima T, Naiki Y, Tsunobuchi H, Umemura M, Itano K, et al. Differential roles of interleukin 15 mRNA

isoforms generated by alternative splicing in immune responses in vivo. J Exp Med. 2000;191:157-70.
8. Cassatella MA, McDonald PP. Interleukin-15 and its impact on neutrophil function. Curr Opin Hematol. 2000;7: 174-7.
9. Girard D, Paquet ME, Paquin R, Beaulieu AD. Differential effects of interleukin-15 (IL-15) and IL-2 on human neutrophils: modulation of phagocytosis, cytoskeleton rearrangement, gene expression, and apoptosis by IL-15. Blood. 1996; 88:3176-84.
10. McDonald PP, Russo MP, Ferrini S, Cassatella MA. Interleukin-15 (IL-15) induces NF-kappaB activation and IL-8 production in human neutrophils. Blood. 1998;92:4828-35.
11. Musso T, Calosso L, Zucca M, Millesimo M, Puliti M, Bulfone-Paus S, et al. Interleukin-15 activates proinflammatory and antimicrobial functions in polymorphonuclear cells. Infect Immun. 1998;66:2640-7.
12. Mastroianni CM, d'Ettorre G, Forcina G, Lichtner M, Mengoni F, D'Agostino C, et al. Interleukin-15 enhances neutrophil functional activity in patients with human immunodeficiency virus infection. Blood. 2000;96:1979-84.
13. Zissel G, Bäumer I, Schlaak M, Müller-Quernheim J. In vitro release of interleukin-15 by broncho-alveolar lavage cells and peripheral blood mononuclear cells from patients with different lung diseases. Eur Cytokine Netw. 2000;11:105-12.
14. Muro S, Taha R, Tsicopoulos A, Olivenstein R, Tonnel AB, Christodoulopoulos P, et al. Expression of IL-15 in inflammatory pulmonary diseases. J Allergy Clin Immunol. 2001; 108:970-5.
15. Cassatella MA. The production of cytokines by polymorphonuclear neutrophils. Immunol Today. 1995;16:21-6.
16. Lloyd AR, Oppenheim JJ. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. Immunol Today. 1992;13:169-72.
17. Wei S, Liu JH, Blanchard DK, Djeu JY. Induction of IL-8 gene expression in human polymorphonuclear neutrophils by recombinant IL-2. J Immunol. 1994;152:3630-6.
18. Newburger PE, Subrahmanyam YV, Weissman SM. Global analysis of neutrophil gene expression. Curr Opin Hematol. 2000;7:16-20.
19. Radsak M, Iking-Konert C, Stegmaier S, Andrassy K, Hänsch GM. Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation. Immunology. 2000;101:521-30.
20. Gosselin EJ, Wardwell K, Rigby WF, Guyre PM. Induction of MHC class II on human polymorphonuclear neutrophils by granulocyte/macrophage colony-stimulating factor, IFN-gamma, and IL-3. J Immunol. 1993;151:1482-90.
21. Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M, et al. CD14 is a pattern recognition receptor. Immunity. 1994;1:509-16.
22. Ferrero E, Jiao D, Tsuberi BZ, Tesio L, Rong GW, Haziot A, et al. Transgenic mice expressing human CD14 are hypersensitive to lipopolysaccharide. Proc Natl Acad Sci USA. 1993;90:2380-4.
23. Pugin J, Schürer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci USA. 1993;90:2744-8.
24. Wright SD. CD14 and innate recognition of bacteria. J Immunol. 1995;155:6-8.
25. Haziot A, Ferrero E, Köntgen F, Hijiya N, Yamamoto S, Silver J, et al. Resistance to endotoxin shock and reduced dissemination of Gram-negative bacteria in CD14-deficient mice. Immunity. 1996;4:407-14.
26. Perera PY, Vogel SN, Detore GR, Haziot A, Goyert SM. CD14-dependent and CD14-independent signaling pathways in murine macrophages from normal and CD14 knockout mice stimulated with lipopolysaccharide or taxol. J Immunol. 1997;158:4422-9.
27. Schimke J, Mathison J, Morgiewicz J, Ulevitch RJ. Anti-CD14 mAb treatment provides therapeutic benefit after in vivo exposure to endotoxin. Proc Natl Acad Sci USA. 1998; 95:13875-80.
28. Verbon A, Dekkers PE, ten Hove T, Hack CE, Pribble JP, Turner T, et al. IC14, an anti-CD14 antibody, inhibits endotoxin-mediated symptoms and inflammatory responses in humans. J Immunol. 2001;166:3599-605.
29. Wagner C, Deppisch R, Denefleh B, Hug F, Andrassy K, Hänsch GM. Expression patterns of the lipopolysaccharide receptor CD14, and the FCgamma receptors CD16 and CD64 on polymorphonuclear neutrophils: data from patients with severe bacterial infections and lipopolysaccharide-exposed cells. Shock. 2003;19:5-12.
30. Zhou LJ, Schwarting R, Smith HM, Tedder TF. A novel cell-surface molecule expressed by human interdigitating reticulum cells, Langerhans cells, and activated lymphocytes is a new member of the Ig superfamily. J Immunol. 1992;149: 735-42.
31. Fujimoto Y, Tu L, Miller AS, Bock C, Fujimoto M, Doyle C, et al. CD83 expression influences CD4+ T cell development in the thymus. Cell. 2002;108:755-67.
32. Pickl WF, Majdic O, Kohl P, Stöckl J, Riedl E, Scheinecker C, et al. Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. J Immunol. 1996;157:3850-9.
33. Zhou LJ, Tedder TF. CD14+ blood monocytes can differentiate into functionally mature CD83+ dendritic cells.

Proc Natl Acad Sci USA. 1996;93:2588-92.
34. Palucka KA, Taquet N, Sanchez-Chapuis F, Gluckman JC. Dendritic cells as the terminal stage of monocyte differentiation. J Immunol. 1998;160:4587-95.
35. Oehler L, Majdic O, Pickl WF, Stöckl J, Riedl E, Drach J, et al. Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. J Exp Med. 1998;187: 1019-28.
36. Yamashiro S, Wang JM, Yang D, Gong WH, Kamohara H, Yoshimura T. Expression of CCR6 and CD83 by cytokine-activated human neutrophils. Blood. 2000;96:3958-63.
37. Iking-Konert C, Csekö C, Wagner C, Stegmaier S, Andrassy K, Hänsch GM. Transdifferentiation of polymorphonuclear neutrophils: acquisition of CD83 and other functional characteristics of dendritic cells. J Mol Med. 2001;79:464-74.
38. Lechmann M, Krooshoop DJ, Dudziak D, Kremmer E, Kuhnt C, Figdor CG, et al. The extracellular domain of CD83 inhibits cell-mediated T cell stimulation and binds to a ligand on dendritic cells. J Exp Med. 2001;194:1813-21.
39. Scholler N, Hayden-Ledbetter M, Dahlin A, Hellström I, Hellström KE, Ledbetter JA. Cutting edge: CD83 regulates the development of cellular immunity. J Immunol. 2002;168:2599-602.