

Neutrophil plasticity: acquisition of phenotype and functionality of antigen-presenting cell

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ABSTRACT

Accumulating lines of evidence now demonstrate that neutrophils can participate in adaptive immune responses directly or indirectly. Even more striking is their potential to acquire phenotypic and functional properties that are typically reserved for professional APCs. These newly emerging concepts of neutrophil heterogeneity and plasticity now challenge the classic view of neutrophils as terminally differentiated leukocytes fully committed to phagocyte functionality. Here, we present a brief overview of our current understanding of neutrophil plasticity by focusing on the acquisition of DC-like properties in culture and at sites of inflammation. Human and murine neutrophils acquire surface expression of MHC II, costimulatory molecules, and other surface markers of DCs when cultured in the presence of selected cytokines. The resulting populations also exhibit potent APC activities to present various antigens to T cells. "Unusual" neutrophils expressing DC markers have been detected in inflammatory lesions in human patients and mouse disease models. These findings imply that acquisition of DC-like properties by neutrophils at the sites of inflammation may represent a key process for linking the innate and adaptive arms of immune responses. *J. Leukoc. Biol.* 98: 489–496; 2015.

Introduction

Neutrophils are the most abundant leukocytes in the human blood circulation and are the first to arrive at sites of infection or injury. They are generally considered to function primarily as ancient "phagocytes" by clearing invading bacterial and fungal pathogens, by releasing granules containing antimicrobial proteins and proteases, and by remodeling the damaged tissue. Over the last 10 years, however, it has become evident that neutrophils can participate in adaptive immune responses as well. Upon sensing invading microbes or intrinsic danger

signals, neutrophils release granule contents, cytokines, chemokines, lipids, and ROS, thereby recruiting and activating APCs, including monocytes, macrophages, and DCs. Moreover, neutrophils can even directly modulate the function of T and B cells. This emerging concept of functional plasticity of neutrophils has been reviewed recently by Amulic et al. [1], Mantovani et al. [2], Mócsai [3], Kalyan and Kabelitz [4], and Scapini and Cassatella [5].

Perhaps even more striking is the notion that neutrophils can acquire unique properties that are typically reserved for professional APCs, as reviewed by Ashtekar and Sara [6], Müller et al. [7], and Nauseef and Borregaard [8]. When cultured in the presence of selected cytokines, human peripheral blood neutrophils begin to express several DC markers (e.g., MHC II and costimulatory molecules) and to exhibit APC capacity to present exogenous antigens to T cells (see below). However, the cellular identity and in vivo function of those unusual neutrophils remain relatively unclear. We reported recently that when cultured with GM-CSF, murine neutrophils can acquire DC markers and APC functionality, while retaining their original properties as phagocytes; these leukocytes with dual properties have been termed "neutrophil-DC hybrids" [9]. Moreover, the hybrid cells become readily detectable in experimentally induced inflammatory lesions in mice [10]. In essence, the hybrid cells share many of the characteristics reported in the literature for the unusual neutrophils expressing DC markers and APC function. Therefore, in this review article, we will address recent advances in our understanding of neutrophil plasticity with an emphasis on the potential to transdifferentiate into DC-like leukocytes.

NEUTROPHIL TRANSDIFFERENTIATION INTO HYBRIDS IN CULTURE

When studying neutrophil plasticity, it is crucial to start from a highly purified, uniform population. In our study, Gr-1^{high}/CD48[−] band cells were FACS sorted (>99.5% purity) from BM of adult C57BL/6 mice (CD45.2⁺); the resulting populations were extremely

Abbreviations: BM = bone marrow, CD62L = cluster of differentiation 62 ligand, DC = dendritic cell, EGFP = enhanced GFP, MMP9 = matrix metalloproteinase 9, MPO = myeloperoxidase, NET = neutrophil extracellular, PEC = peritoneal exudate cell, PMN = polymorphonuclear neutrophils, ROS = reactive oxygen species, SAg = superantigen, TAP = transporter associated with antigen processing, TT = tetanus toxoid

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homogeneous in their morphology characterized by small cell size and ring-shaped nuclei and by surface expression of neutrophil markers (e.g., Ly6G, Ly6C, 7/4, CD62L, CD24, and CD11b) but not markers of DCs (e.g., CD11c and MHC II), macrophages (e.g., CD115), or GM progenitors (e.g., CD34). When tested after 6 days culturing with GM-CSF in the presence of BM feeder cells from CD45.1⁺ mice (added to sustain the survival of band cells), CD45.2⁺/CD45.1⁻ cells displayed DC markers MHC II and CD11c while maintaining the expression of a neutrophil marker Ly6G. The CD45.2⁺/CD45.1⁻ cells also exhibited a DC-like morphology characterized by oval-shaped nuclei and dendritic processes. The resulting population was thus termed the neutrophil-DC hybrid [9]. Although anti-Gr-1 mAb (clone RB6-8C5) is widely used to identify neutrophils, it recognizes Ly6G (expressed exclusively by neutrophils) and Ly6C (expressed by not only neutrophils but also some monocytes and selected DC subsets) [11–14]. Thus, sustained expression of Ly6G, which has not been detected in any of the currently recognized DC subsets, serves as a unique marker for the neutrophil-derived hybrid cells. The Ly6G⁺/CD11c⁺/MHC II⁺ hybrids purified from GM-CSF-supplemented BM cultures indeed exhibited dual features of DCs and neutrophils [9].

To identify a human counterpart, we purified CD15⁺/CD10⁻/CD64⁻/CD14⁻ band cells and CD14⁺ monocytes from human BM samples and cultured them in parallel for 7 days in the presence of GM-CSF, TNF- α , and IL-4. Both populations acquired surface expression of DC markers (MHC II, CD1c, and CD11c) but not a macrophage marker CD163. Importantly, band cells retained surface expression of all tested neutrophil markers (e.g., CD15, CD24, CD66b, and CD89), which were not detectable on a monocyte-derived DC population. These observations indicate the potential of human neutrophils to transdifferentiate into a hybrid population characterized by dual expression of neutrophil and DC markers [9].

HUMAN NEUTROPHILS ACQUIRE APC-LIKE PROPERTIES IN CULTURE

As described briefly in Introduction, human peripheral blood neutrophils have been shown to acquire various properties of APCs when cultured in the presence of selected cytokines. As many of these features were also observed for the neutrophil-DC hybrids, we summarize the key findings reported in the literature in **Table 1** compared with our own observations.

Matsumoto et al. [15] reported in 1987 that human peripheral blood neutrophils began to express MHC class II molecules when cultured for 20 h in the presence of IFN- γ . Unfortunately, this seminal discovery was mostly ignored in the immunology community, as IFN- γ was well known to induce MHC II expression by many cell types [23, 24]. Gosselin et al. [16] demonstrated in 1993 that human neutrophils acquired MHC II mRNA and protein expression after 44 h culturing with GM-CSF. Interestingly, the resulting MHC II⁺ neutrophils retained the morphology of PMNs, characterized by lobulated nuclei, even after 44 h culturing with GM-CSF [16]. This is consistent with the observations by other investigators that human [22] and murine neutrophils [9] began to exhibit DC-like morphology at later

time-points. Smith et al. [17] demonstrated further that IL-3 could augment GM-CSF-induced MHC II expression by triggering the expression of IL-3R α (or CD123). With regard to functional significance, Fanger et al. [18] demonstrated that MHC II⁺ neutrophils, recovered after 44 h culturing in the presence of GM-CSF plus IFN- γ , exhibited markedly augmented accessory cell function, as measured by SAg-induced CD4 T cell activation compared with MHC II⁻ neutrophils recovered from control cultures. On the other hand, those neutrophils failed to present TT protein or even TT peptide to a TT-specific CD4 T cell line; the authors argued that this failure was a result of the lack of surface expression of costimulatory molecules CD80 and CD86. A somewhat conflicting finding was also reported. Iking-Konert et al. [19] cultured CD15⁺ neutrophils purified from peripheral blood for 48 h in the presence of the same cytokines (GM-CSF and IFN- γ); the resulting population expressed CD86 (and also CD83) and showed efficient TT presentation to a TT-specific CD4 T cell line in a MHC II-dependent fashion.

To determine whether CCR6 (expressed by immature DCs) is also inducible by neutrophils, Yamashiro et al. [21] cultured peripheral blood neutrophils in the presence of the supernatant collected from PHA-stimulated PBMC culture. CCR6 mRNA became detectable in those neutrophils at 6 h in culture, and this induction was blocked by mAb against TNF- α . By testing various cytokines (GM-CSF, IFN- γ , TNF- α , IL-1 β , and IL-4), individually and in combinations, they found that addition of TNF- α and IFN- γ to the neutrophil culture was sufficient to trigger rapid (<6 h) CCR6 mRNA and protein expression. The resulting population also showed chemotactic migration toward CCR6 ligand MIP-3 α , and CD83 mRNA and surface expression also became detectable after 18 h culturing in the presence of TNF- α and IFN- γ [21]. Taken together, these reports demonstrate that surface expression of selected DC markers (e.g., MHC II, CD86, CD83, and CCR6) is readily inducible in human neutrophils by exposure to GM-CSF, IFN- γ , IL-3, and/or TNF- α . Furthermore, the resulting populations exhibit limited APC capacity to augment SAg-induced T cell proliferation and to present exogenous antigens to CD4 T cell lines.

Beauvillain et al. [25] reported a striking ability of human neutrophils to cross-present exogenous antigen to CD8 T cells. In brief, when pulsed for 4 h with hepatitis C virus NS3 antigen as an intact, full-length protein, CD13⁺/CD11b⁺ neutrophils, purified from peripheral blood, induced marked activation of a CD8 T cell clone, recognizing a MHC I-restricted NS3 peptide antigen. This implied that neutrophils most likely acquired the antigen cross-presenting capacity during coculturing with CD8 T cells. More recently, Davey et al. [20] demonstrated that upon stimulation by microbe-specific, unconventional T cells, neutrophils acquired APC markers, as well as the ability cross-present exogenous antigens to CD8 T cells. Peripheral blood neutrophils showed prolonged survival and surface expression of MHC II, CD40, and CD83 when cocultured for 48 h with V γ 9⁺/V δ 2⁺ $\gamma\delta$ T cells and V α 7.2⁺/CD161⁺ mucosal-associated invariant T cells (both of which recognized microbial metabolites). These changes were not observed when neutralizing mAb against GM-CSF, TNF- α , and IFN- γ were added to the above cocultures, and the same changes were inducible by exposing neutrophils to the 3 cytokines in the absence of T cells. Thus, it

TABLE 1. Cytokine-induced acquisition of APC-like properties by human neutrophils in culture

Source	Culture conditions	APC-like properties	Neutrophil properties	Ref.
Blood	IFN- γ (20 h)	MHC II		[15]
Blood	GM-CSF (44 h)	MHC II	PMN morphology	[16]
Blood	GM-CSF + IL-3 (40 h)	MHC II		[17]
Blood	GM-CSF + IFN- γ (44 h)	MHC II, SAg presentation		[18]
Blood	GM-CSF + IFN- γ (48 h)	MHC II, CD83, CD86, TT presentation	CD66b, CD15, bacterial uptake	[19]
Blood	GM-CSF + IFN- γ + TNF- α (48 h)	MHC II, CD40, CD83, SAg presentation, cross-presentation	PMN morphology, CD11b, CD66b	[20]
Blood	TNF- α + IFN- γ (6–18 h)	CD83, CCR6, chemotaxis to MIP-3 α		[21]
Blood ^a	GM-CSF + IL-4 + TNF- α (6–9 days)	MHC II, CD1, CD40, CD80, CD86, TT presentation, allogeneic T cell stimulation, DC morphology	MPO, lactoferrin	[22]
BM	GM-CSF + IL-4 + TNF- α (7 days)	MHC II, CD1	CD66b, CD15, CD24, CD89	[9]

References for each report are provided. Note that most studies used neutrophils isolated from peripheral blood from healthy individuals. ^aIn one study, neutrophils were isolated from patients with chronic myeloid leukemia or bacterial infections and from donors receiving G-CSF treatment.

seems reasonable to state that certain T cell populations can induce phenotypic differentiation of neutrophils into APC-like leukocytes by elaborating specific cytokines. With regard to function, neutrophils preincubated for 48 h with GM-CSF, TNF- α , and IFN- γ presented not only *Staphylococcus aureus* SAg to autologous V β 2⁺ CD4 T cells but also influenza M1 antigen in full-length protein and MHC I-restricted peptide forms to M1-specific CD8 T cell lines.

It should be pointed out that in all of the studies cited above, neutrophils were isolated from peripheral blood of healthy individuals by gradient centrifugation and/or magnetic bead separation with varying degrees of purity, ranging from 90% to 99%. By contrast, Oehler et al. [22] purified CD15⁺/lactoferrin⁺ immediate precursors of neutrophils by magnetic beads from patients with chronic myeloid leukemia or bacterial infections and from donors receiving G-CSF treatment; these blood samples showed high neutrophil counts with marked left shifts, mostly composed of band cells and metamyelocytes. When cultured for 5 days in the presence of GM-CSF, IL-4, and TNF- α , those neutrophils exhibited the ability to induce significant proliferation of allogeneic T cells. Moreover, the cells harvested on day 9 further showed an elevated allogeneic-stimulatory capacity, as well as potent APC function to present TT protein to autologous memory T cells isolated from the same individuals. Concomitant to the acquisition of these functional properties, many DC markers, including MHC II, costimulatory molecules (CD80, CD86, and CD40), and members of the CD1 family (CD1a, b, and c) became detectable on days 6–9 in culture. These neutrophils also altered their morphology gradually. Before culture, most cells displayed typical morphology of band cells characterized by rod-shaped nuclei. On days 3–6, they increased the cell size and showed nonlobulated, oval-shaped nuclei. On day 9, a vast majority of the cells showed typical DC-like morphology, characterized by extension of widespread cytoplasmic projections. Importantly, those neutrophils retained MPO and lactoferrin in cytoplasmic granules, even after prolonged culture (up to 9 days) [22]. In our study, we purified neutrophils expressing the typical phenotype of band cells (CD15⁺/CD10[−]/CD64[−]/CD14[−]) [26] by 2-step magnetic bead

separations from BM samples derived from healthy volunteers. We confirmed many of these findings by culturing those relatively immature neutrophils for 7 days in the presence of the same cytokines (GM-CSF, IL-4, and TNF- α) [9]. In addition to apparent differences in culturing periods and cytokine compositions, the variations in terms of relative maturity, source, and purity of the starting neutrophil preparations should be taken into consideration when comparing the results from different studies. Nevertheless, it appears reasonable to state that human neutrophils can acquire surface expression of many DC markers, full APC capacity, and DC-like morphology after long-term (5–9 days) culturing in the presence of GM-CSF, IL-4, and TNF- α . Importantly, they maintain some of the original features of neutrophils.

APC-LIKE PROPERTIES EXHIBITED BY MURINE NEUTROPHILS

Several early studies reported the potential of murine neutrophils to function as APCs [27–30]. In these studies, neutrophils were isolated from acute peritonitis lesions induced by i.p. administration of glycogen, killed bacteria, or thioglycollate (Table 2). This selection of the source for neutrophils was, most likely, for a technical reason—a majority (40–60%) of the PECs includes neutrophils, and large numbers of neutrophils (>10⁷ cells/mouse) can be readily isolated by gradient centrifugation. Okuda et al. [27] were the first to report surface expression of MHC II on PEC neutrophils, as well as their capacity to present an exogenous antigen lysozyme to antigen-primed T cells [28]. Fitzgerald et al. [29] demonstrated that PEC neutrophils induced significant proliferation of allogeneic T cells, and Culshaw et al. [30] showed their capacity to present OVA to OVA-specific, naïve CD4 T cells isolated from OT-II TCR transgenic mice. By contrast, Abi Abdallah et al. [31] reported that Ly6G⁺ neutrophils freshly purified from PEC did not express MHC II or CD86 but acquired both during short-term (2 h) coculturing with CD4 T cells. By first pulsing PEC neutrophils with OVA protein and then coculturing them with OVA-specific CD4 T cells from OT-II mice, these investigators further

TABLE 2. APC-like properties exhibited by murine neutrophils

Source	Culture conditions	APC-like properties	Neutrophil properties	Ref.
PEC ^a	No culturing	MHC II		[27]
PEC ^a	No culturing	Activation of antigen-primed T cells		[28]
PEC ^b	No culturing	Activation of allogeneic T cells		[29]
PEC ^c	No culturing	OVA presentation to naïve CD4 T cells		[30]
PEC ^c	Coculturing with T cells (2 h)	OVA presentation, T cell differentiation to Th1 and Th17		[31]
PEC ^c	No culturing	OVA cross-presentation to CD8 T cell hybridoma		[32]
PEC ^c BM	No culturing GM-CSF (overnight)	OVA cross-presentation to CD8 naïve T cells Antigen cross-presentation to CD8 naïve T cells	Bacterial uptake	[25]
BM	GM-CSF (6–9 days)	MHC II, CD11c, CD1a, CD40, CD80, CD86, CD205, cytokine release, OVA presentation to naïve CD4 T cells, DC morphology, probing motion, podosome formation	7/4, CD62L, CXCR2, MPO, MMP9, NET formation, bacterial uptake and killing	[9]

Note that most studies used neutrophils freshly isolated from PEC after i.p. administration of ^aglycogen, ^bformalin-fixed bacteria, or ^cthioglycollate.

demonstrated that PEC neutrophils triggered not only proliferation but also differentiation of T cells into IFN- γ -producing Th1 and IL-17-producing Th17 populations but not into an IL-4-producing Th2 population. However, these observations do not necessarily imply that neutrophils constitutively exhibit such DC-like properties in the steady state, as neutrophil populations were isolated from inflamed peritoneal cavity. In fact, we failed to detect MHC II expression on Ly6G⁺ neutrophils freshly isolated from BM or peripheral blood, whereas significant fractions (2–10%) of Ly6G⁺ PEC neutrophils, isolated from peritonitis lesions induced by thioglycollate injection or bacterial inoculation, expressed MHC II at high levels [10]. These Ly6G⁺/MHC II⁺ cells also exhibited phenotypic and functional features of neutrophil-DC hybrids (see below). Thus, we speculated that emergence of the hybrid cells may account for the early observations that murine neutrophils isolated from PEC samples exhibited DC-like properties in the absence of culturing in the presence of cytokines.

Potter and Harding [32] showed that PEC neutrophils incorporated fluorescein-labeled *Escherichia coli* efficiently. Importantly, the PEC neutrophils that had phagocytosed OVA-producing *E. coli* processed OVA antigen into MHC I-restricted OVA peptides for presentation to an OVA-specific CD8 T cell hybridoma. Interestingly, this process was not inhibited by lactacystin (a proteasome inhibitor) or brefeldin A (an inhibitor of anterograde endoplasmic reticulum-Golgi transport), suggesting that neutrophils processed bacterial OVA antigen in a vacuolar pathway. Beauvillain et al. [25] also demonstrated that PEC neutrophils were capable of cross-presenting OVA protein to OVA-specific, naïve CD8 T cells isolated from OT-I TCR transgenic mice. In marked contrast to the study cited above, they found that lactacystin significantly inhibited this capacity and that PEC neutrophils isolated from the TAP-deficient mice exhibited a markedly reduced ability to present OVA to CD8 T cells, indicating that cross-presentation occurred in a mechanism requiring proteasomes and TAP machinery. These

investigators also confirmed the same capacity for Ly6G⁺ neutrophils purified from BM. However, it should be pointed out that those BM neutrophils were tested for APC function after overnight culturing in the presence of GM-CSF. Nevertheless, these 2 reports indicate a striking ability of neutrophils to cross-present exogenous antigen to CD8 T cells by 2 mechanisms, i.e., a proteasome-independent, vacuolar alternate MHC I pathway and a proteasome- and TAP-dependent pathway.

In our study, Gr-1^{high}/CD48⁺ band cells, purified from the BM of CD45.2⁺ mice (>99.5% purity), were cultured with GM-CSF in the presence of BM feeder cells from CD45.1⁺ mice, and CD45.2⁺/CD45.1⁺ populations isolated at different time-points were examined for morphology, surface phenotype, and function [9]. Gradual increase in cell size and change of nuclear shape (from ring shape into oval shape) were observed on days 2–4, and the CD45.2⁺/CD45.1⁺ cells harvested on days 6–8 showed a typical DC-like morphology characterized by extension of dendritic processes. At the same time, they uniformly displayed MHC II and CD11c on the surface, while maintaining Ly6G expression. Importantly, only small fractions (15–30%) of the starting neutrophils remained viable for 6–8 days in these cultures, suggesting heterogeneity in neutrophils. A key question concerned whether mature neutrophils would also acquire the same DC-like phenotype. To test this, we purified Gr-1^{high}/CD11b^{low} immature neutrophils and Gr-1^{high}/CD11b^{high} mature neutrophils from BM of CD45.2⁺ mice; both populations acquired MHC II and CD11c when cultured in the presence of GM-CSF and BM feeder cells from CD45.1⁺ mice. It remains to be determined whether only a particular subset of neutrophils (or neutrophils in a specific activation status) can acquire DC-like properties.

Neutrophil-DC hybrid cells could be generated in a larger quantity by culturing crude BM cells in the presence of GM-CSF [9]. It should be emphasized that floating cells (primarily neutrophils) and adherent cells were kept in our culture, as opposed to the protocol by Inaba et al. [33] for generating

BM-derived DC cultures, in which floating neutrophils are discarded repeatedly. The number of Ly6G⁺/CD11c⁺/MHC II⁺ hybrids increased time dependently with a peak on day 9 when they accounted for 3–5% of total cells. No hybrid cells were found when BM cells were cultured with GM-CSF by use of the protocol by Inaba et al. [33]. Neutrophil-DC hybrids, purified from BM cultures, exhibited a number of DC-like properties summarized in Table 2. They displayed CD1d, CD11a, CD18, CD40, CD54, CD80, CD86, and CD205 on the surface; formed podosome-like structures at the ventral surface; and showed probing motion of dendrites in time-lapse imaging. With regard to APC function, the hybrid cells elaborated several cytokines (e.g., IL-12p70, TNF- α and IL-6) upon stimulation with TLR agonists and presented various forms of OVA (OVA peptide, soluble and immobilized OVA protein, and OVA-expressing *E. coli*) to naïve CD4 T cells isolated from OT-II mice. Those T cells stimulated by the hybrid cells elaborated IFN- γ and IL-17 but not IL-4. It should be stated that they exhibited only a modest ability to cross-present OVA to CD8 T cells from OT-I mice. Importantly, the hybrids were found to maintain surface expression of neutrophil markers 7/4, CD62L, and CXCR2. Furthermore, they produced MPO and MMP9, extruded NETs upon stimulation, exhibited efficient uptake of soluble and particular materials and living bacteria, and killed internalized bacteria rapidly in a cathelicidin-dependent fashion. As illustrated in **Fig. 1**, the hybrid cells exhibit dual properties of neutrophils and DCs [9].

No neutrophil-DC hybrids emerged in BM cultures supplemented with G-CSF, M-CSF, or Flt3 ligand [9]. To study cytokine requirement more systematically, we compared a total of 61 cytokines in parallel [34]. When tested individually, GM-CSF exhibited the most potent ability to promote the generation of neutrophil-DC hybrids in BM culture. When tested in combination with GM-CSF, IL-4 was found to augment hybrid cell generation in a synergistic fashion. Interestingly, IFN- γ suppressed GM-CSF-induced development of hybrid cells [34].

These observations suggest that neutrophil transdifferentiation into hybrids is tightly regulated by cytokines.

EMERGENCE OF UNUSUAL NEUTROPHILS WITH APC PROPERTIES AT INFLAMMATORY LESIONS

Emergence of unusual neutrophils exhibiting APC-like properties is probably not an artificial phenomenon associated with in vitro culturing processes, as similar neutrophil populations have been detected in patients (**Table 3**). Corroborating with the in vitro findings that human neutrophils can acquire MHC II expression when cultured in the presence of IFN- γ and/or GM-CSF (see Table 1), Reinisch et al. [35] and Mudzinski et al. [36] reported the emergence of MHC II⁺ neutrophils in patients receiving IFN- γ and GM-CSF, respectively. More specifically, MHC II expression, which was not detected on neutrophils before treatment, was observed in 22–38% of circulating blood neutrophils after 2 wk of IFN- γ treatment or in 47–63% after 3 days of GM-CSF treatment. These studies demonstrated the ability of circulating neutrophils to acquire MHC II expression in response to cytokine stimulation. Hänsch et al. [37] reported that MHC II expression was detected in a significant fraction (up to 15%) of peripheral blood neutrophils in patients with Wegener's granulomatosis, a rare autoimmune disease characterized by necrotizing granuloma, necrotizing vasculitis, and progressive glomerulonephritis, whereas control neutrophils from healthy individuals showed no or little MHC II expression. The same group demonstrated further that neutrophils from patients with Wegener's granulomatosis also expressed CD80 and CD86 [38]. Interestingly, CD83 expression was detected on neutrophils from patients with acute bacterial infection but not those from patients with Wegener's granulomatosis [39]. Cross et al. [40] observed marked MHC II mRNA expression by neutrophils isolated from synovial fluid of patients with rheumatoid arthritis. Iking-Konert et al. [41] demonstrated further that neutrophils in synovial fluid but not in peripheral blood of rheumatoid arthritis patients express MHC II, CD64 (high-affinity Fc γ R), and CD83 on the cell surface. Most recently, Davey et al. [20] demonstrated that circulating neutrophils in sepsis patients showed elevated surface expression of CD40, CD64, and CD86 compared with control neutrophils in healthy individuals. Furthermore, those neutrophils isolated from sepsis patients exhibited the ability to cross-present full-length M1 protein to M1-specific CD8 T cell lines. These observations imply that human neutrophils can acquire surface expression of DC markers and APC functionality under inflammatory conditions.

Working with a mouse model of inflammatory bowel disease, Ostanin et al. [42] demonstrated that neutrophils isolated from inflamed colon lesions expressed MHC II and CD86 at high levels and that those colonic neutrophils presented OVA antigen to naïve CD4 T cells from OT-II mice in an MHC II-dependent fashion. We observed that Ly6G⁺/CD11c⁺/MHC II⁺ hybrids emerged in thioglycollate-induced acute peritonitis lesions in a time-dependent manner with a peak on day 2 [10]. It should be stated that neutrophil-DC hybrids represented a relatively minor population, accounting for <1% of total PEC numbers.

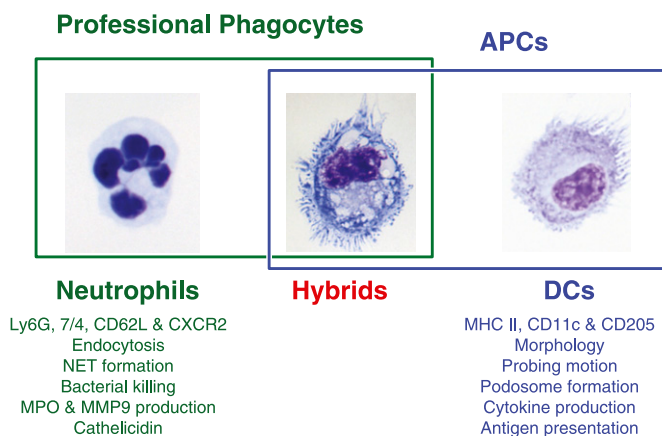


Figure 1. Dual properties of neutrophil-DC hybrids. Neutrophil-DC hybrids (in red) exhibit the surface markers and functional properties of neutrophils as shown in green. At the same time, hybrid cells resemble conventional DCs by surface phenotype, morphology, and function, as shown in blue.

TABLE 3. Emergence of unusual neutrophils with APC-like properties at inflammatory lesions

Species	Tissue/disease	APC-like properties	Neutrophil properties	Ref.
Human	Blood/IFN- γ treatment	MHC II		[35]
Human	Blood/GM-CSF treatment	MHC II		[36]
Human	Blood/Wegener's granulomatosis	MHC II		[37]
Human	Blood/Wegener's granulomatosis	CD80, CD86		[38]
Human	Blood/bacterial infection	CD86		[39]
Human	Synovial fluid/rheumatoid arthritis	MHC II mRNA		[40]
Human	Synovial fluid/rheumatoid arthritis	MHC II, CD64, CD83		[41]
Mouse	Colon/inflammatory bowel disease	MHC II, CD86, OVA presentation to naïve CD4 T cells		[42]
Mouse	Peritoneal cavity/sterile inflammation; peritoneal cavity/bacterial infection; lymph node/bacterial infection; skin/chronic inflammation; lung/acute inflammation	MHC II, CD80, CD86, DC morphology, OVA presentation to naïve CD4 T cells, lymph node-directed homing	Ly6G, 7/4, CD62L, CXCR2, bacterial uptake and killing	[34]
Human	Blood/sepsis	CD40, CD64, CD86, cross-presentation	CD15, CD66b	[20]

Nevertheless, those hybrid cells exhibited DC-like morphology and expressed additional markers for DC (CD80 and CD86) and neutrophils (7/4, CD62L, and CXCR2). Emergence of Ly6G⁺/CD11c⁺/MHC II⁺ hybrids was also observed in various inflammatory disease models, including allergen-induced acute lung inflammation, chronic skin inflammation, bacterial peritonitis, and bacterial lymphadenitis. To study the function of neutrophil-DC hybrids, we injected GFP-expressing *E. coli* or OVA-producing *E. coli* into thioglycollate-induced peritonitis lesions and harvested PEC shortly (1 h) thereafter. The hybrid cells purified from these PEC samples showed a large number of GFP⁺ bacteria and triggered robust proliferation of OVA-reactive, native CD4 T cells from OT-II mice. However, the same hybrid cells induced only modest proliferation of OVA-reactive, naïve CD8 T cells from OT-I mice. Upon s.c. injection, the hybrid cells migrated to the T cell area of draining lymph nodes. These findings imply that neutrophil-DC hybrids, emerging at various inflammatory sites, resemble in vitro-generated hybrid cells by exhibiting dual phenotypic and functional properties of neutrophils and DCs.

To determine the origin of the in vivo-emerging hybrids, BM neutrophils, freshly purified from CD45.2 donor mice, were adoptively transferred to CD45.1 recipient mice that had received thioglycollate treatment. Surface expression of CD11c and MHC II was observed in 20–30% of the CD45.2⁺/CD45.1[−] cells recovered 3 days later from the inflamed peritoneal cavity. Neutrophils are known to acquire passively membrane lipids and membrane proteins from apoptotic or necrotic cells during phagocytosis [43]. To exclude this possibility, the above experiments were repeated by purifying BM neutrophils from CD11c promoter-driven diphtheria toxin receptor-EGFP transgenic mice (in which EGFP fluorescence signals are expressed by all CD11c⁺ populations) and from I- β -EGFP knock-in mice (in which EGFP is expressed by all MHC II⁺ populations). BM neutrophils, freshly purified from these reporter strains, showed no EGFP signals, but strong EGFP signals were found in ~20% of the CD45.2⁺/CD45.1[−] neutrophils recovered from peritonitis lesions after adoptive transfer

[10]. Importantly, a majority (70–80%) of CD45.2⁺/CD45.1[−] neutrophils recovered from inflammatory lesions had failed to acquire surface expression of DC markers or EGFP signals. In fact, only ~25% of those neutrophils displayed DC-like morphology, whereas the remaining cells maintained band cell morphology or matured into PMNs characterized by segmented nuclei. This implies remarkable heterogeneity among neutrophils in their potential to acquire DC-like properties. Nevertheless, these observations indicate that a fraction of neutrophils can transdifferentiate into neutrophil-DC hybrids after extravasation at inflammatory sites.

CONCLUDING REMARKS

As described above, a number of studies have demonstrated the potential of human and murine neutrophils to acquire DC-like phenotype and function in vitro after exposure to selected cytokines, as well as in vivo upon recruitment to the sites of inflammation. Although beyond the scope of this review, neutrophils can also acquire macrophage-like properties depending on the cytokine milieu. Araki et al. [44] purified CD15⁺/CD14[−] immature neutrophils from blood of healthy volunteers after G-CSF treatment and cultured them for 11 days in the presence of GM-CSF, TNF- α , IFN- γ , and IL-4 and for an additional 7 days with M-CSF alone. The resulting population expressed MHC II, M-CSFR (CD115), and macrophage mannose receptor (CD206) on the surface; displayed macrophage-like morphology; and exhibited macrophage-lineage mRNA expression profiles. Chakravarti et al. [45] reported an interesting phenomenon, termed “inflammatory reprogramming” of neutrophils. When cultured for 3 days with GM-CSF, IL-4, and TNF- α (which are locally present at various inflammatory lesions), not only did human blood neutrophils acquire MHC II and CD80 surface expression, but they also showed dramatic changes in diverse functional properties, including increased production of ROS, leukotrienes, and cytokines; augmented phagocytosis; increased adhesion to stromal cells; decreased exocytosis of

primary and secondary granules; and reduced chemotaxis. Taken together, these reports challenge the classic view of neutrophils as terminally differentiated, short-lived leukocytes destined to function only as phagocytes.

It is tempting to speculate that those neutrophils exhibiting DC-like properties may play protective roles against infection by clearing bacterial pathogens and presenting bacterial antigens to immunologically naïve T cells. Conversely, they may play pathogenic roles in autoimmune diseases by capturing and digesting tissue debris at inflammatory sites and presenting tissue-associated self-antigens to naïve T cells. Although attractive, these possibilities cannot be tested with any of the currently available experimental systems. Although systemic injection of anti-Gr-1 mAb had been widely used to deplete neutrophils in mice, we now know that anti-Ly6G mAb offers more specific depletion of neutrophils, as it does not recognize monocytes or T cells [46–48]. However, even this approach will not allow us to distinguish relative contributions by conventional neutrophils versus neutrophil-DC hybrids (which retain Ly6G expression) to the immunologic abnormalities inducible by anti-Ly6G mAb administration. Thus, a major challenge in this field will be the identification of unique surface molecule(s) selectively expressed by neutrophil-DC hybrids. Although neutrophils acquire APC-like properties when cultured in the presence of GM-CSF, IFN- γ , IL-3, IL-4, and/or TNF- α , it remains unclear whether these cytokines are required and sufficient for their *in vivo* transdifferentiation at inflammatory sites. It is evident that not all neutrophils can uniformly give rise to neutrophil-DC hybrids; only small fractions (20–30%) of the starting cells acquired DC markers in culture or upon recruitment to inflammatory sites. Thus, an obvious question concerns the identity of such neutrophil subpopulation(s) with the potential to acquire DC-like properties. Moreover, no information is available with regard to reversibility of this phenomenon. Molecular mechanisms regulating neutrophil plasticity represent a second important area for future studies. We anticipate that these lines of future investigation would ultimately lead to the development of new therapeutic approaches for many human diseases characterized by neutrophil infiltration and immune dysfunction.

AUTHORSHIP

A.T. and Y.Y. outlined the review and wrote the manuscript.

DISCLOSURES

The authors declare no conflict of interest.

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