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COMMENT

The functional diversity of neutrophils and clustered polarization of immunity

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Neutrophils, bone marrow-derived innate immune cells, are among the first defensive and inflammatory responses of the host to infection and other danger signals. Increasing evidence indicates that neutrophils are critically involved in shaping adaptive immunity as professional antigen-presenting cells (APCs).[1–3] However, neutrophils also negatively regulate inflammatory and immune responses or promote tissue repair and wound healing through multiple approaches.[3–5] With the widely diverse roles of neutrophils in host homeostasis and diseases, it is necessary to identify new subsets of functionally different neutrophils. Interestingly and importantly, Li et al.[6] and Sun et al.[7] recently identified N(IL-23) and N(IL-33) subsets, respectively. These findings are of importance for us to fully understand the biological diversity of neutrophils and, even more importantly, could reveal the wider functional polarization of neutrophils beyond the traditional N1/N2 bidirectional polarization.

Accumulating evidence has revealed that neutrophils exhibit considerable functional plasticity and diversity and that there may be distinct neutrophil subsets. Tsuda et al. reported that type 1 neutrophils (N1) with the $CD49d^{+}CD11b^{-}$ phenotype predominately produce IL-12 and CCL3, type 2 neutrophils (N2) display the $CD49d^{-}CD11b^{+}$ phenotype and mainly produce IL-10 and CCL2, and $CD49d^{-}CD11b^{-}$ resting neutrophils (N0) have no significant cytokine and chemokine productions.[8] Tumor-associated neutrophils are proposed to display an antitumorigenic N1 phenotype versus a protumorigenic N2 phenotype.[9–11] N1 neutrophils express high levels of immune-activating cytokines and chemokines, low levels of arginase, and have a strong tumor cell-killing capability in vitro, presenting antitumor activity, whereas N2 neutrophils induced by TGF-β in tumors display a protumor phenotype. In contrast to circulating neutrophils, splenic neutrophils express B-cell-stimulating factors, such as BAFF, APRIL, and IL-21, as well as B-cell-attracting chemokines, such as CXCL12 and CXCL13, to promote immunoglobulin class switching, somatic hypermutation, and antibody production.[12] In recent studies reported by Li et al., after stimulation with more than ten cytokines, only IL-23 treatment promoted Th17-like neutrophil polarization (referred to as N(IL-23) cells); these neutrophils selectively produce IL-17A, IL-17F, and IL-22 at the mRNA and protein levels and displays a distinctive gene expression profile compared with those of N0 and LPS-treated neutrophils (referred to as N(LPS) cells). IL-23 induces N(IL-23) polarization via STAT3-dependent RORγt and BATF pathways.[6] Importantly, these N(IL-23) cells are detectable in DSS-induced colitis and are involved in the pathogenesis of DSS-induced colitis in an IL-17-dependent manner.[6] In contrast, IL-33 induces Th9-like neutrophil polarization (referred to as the N(IL-33) cell subset).[7] These N(IL-33) cells express and produce high levels of IL-9 and certain quantities of IL-4, IL-5, and IL-13 by activating c-Jun N-terminal kinase- and NF-κB-dependent pathways.[7] Compared with N0, N(LPS), and N(IL-23) cells, these N(IL-33) cells display a unique gene expression profile, as determined by RNA-seq assays[7] (unpublished data). Importantly, N(IL-33) neutrophils are found in the lungs of OVA-induced allergic inflammation mice, as detected by flow cytometry. The adoptive transfer of the induced N(IL-33) neutrophils markedly enhances the severity of the lung pathology in these allergic inflammation mice.[7] The discovery of functionally different neutrophil subsets, such as N(IL-23) and N(IL-33), in both in vitro and in vivo systems strongly supports the hypothesis that neutrophil functional diversity and differential polarization occurs in various microenvironments. The potential ability of neutrophils to polarize to functionally distinct subpopulations may be dependent on their developmental stage, surrounding cytokines and microenvironment. However, our understanding of the induction factors and intracellular signals and the pathogenesis contribution of these newly identified neutrophil subpopulations is still very limited and needs to be explored in the future. The contribution of the newly identified neutrophil subsets, such as N(IL-23) and N(IL-33), to different diseases and graft rejection, must be determined. We should also investigate whether human neutrophils have the same subpopulations as mice and, if so, what their roles are in inflammatory disease and organ allograft rejection. We believe that with the development of single-cell RNA sequencing technology, an increasing number of neutrophil subpopulations will be uncovered in the coming days. Considering the wide range of roles neutrophils play in host homeostasis and disease, it is essential to comprehensively understand the regulatory roles of different neutrophil subpopulations in physiological and pathological situations and to explore novel therapeutic approaches for selectively targeting these subpopulations to treat neutrophil-related diseases.

It should be noted that although neutrophils predominately participate in the inflammatory response and regulate adaptive immunity in a positive way, neutrophils can also promote inflammation resolution by releasing soluble mediators, apoptotic bodies and neutrophil-derived microvesicles into the microenvironment, which can be transmitted as a passive anti-inflammatory signal to other immune cells.[13] Reber et al. reported that neutrophils are essential to protect the host from LPS-induced inflammation through neutrophil-derived myeloperoxidase.[14] The

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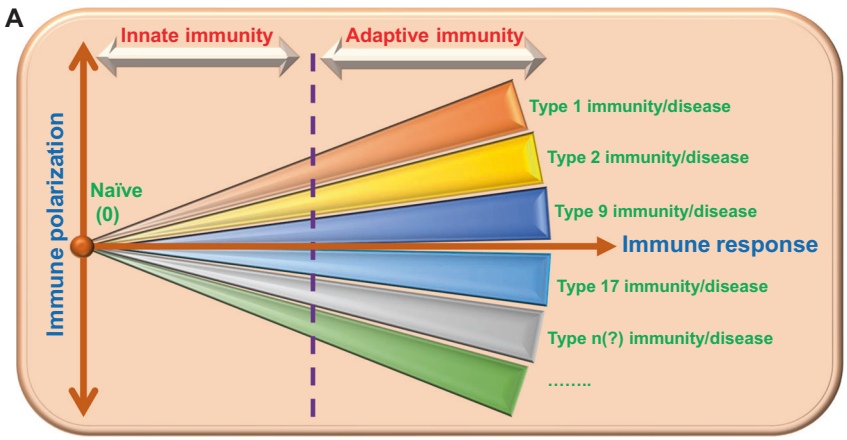
injection of neutrophils into CCl_4-treated mice ameliorates liver fibrosis, whereas neutrophil depletion by injection of an anti-Ly6G antibody enhances CCl_4-induced fibrosis.[15] It was recently reported that neutrophils play a crucial role in the resolution of inflammation and liver repair by promoting the phenotypic conversion of proinflammatory $Ly6C^{high}CX3CR1^{low}$ macrophages to proresolving $Ly6C^{low}CX3CR1^{high}$ macrophages.[16] A subset of neutrophils inhibits T cell proliferation mainly through the expression of Mac-1 and ROS during human acute systemic inflammation.[4] A recent study showed that neutrophils inhibited IL-1β and tumor necrosis factor-alpha (TNF-α) production by $CD14^+$ monocytes in cell–cell contact-dependent and independent manners.[17] Neutrophils reduce acute graft-versus-host disease in a manner dependent on IL-10 and Tregs.[18] Neutrophil apoptosis and their subsequent phagocytosis by macrophages promote anti-inflammatory M2 polarization, which might contribute to kidney repair after injury.[19] It is well known that granulocytic myeloid-derived suppressive cells play an important role in the induction of graft tolerance.[20] Thus, neutrophils indeed have the ability to suppress inflammation and adaptive immune response by multiple approaches. The identification of immunosuppressive neutrophil subsets and their relevant mechanisms will definitely hasten the application of these neutrophil subsets to downregulate redundant proinflammatory activity and inappropriate adaptive immunity in human diseases and transplant settings.

The complicated interaction of neutrophils with other immune cells and the dual roles neutrophils play in innate and adaptive immunity have recently been appreciated.[2] One study using two-photon microscopy found that neutrophils interact with donor DCs in lung grafts to promote T cell differentiation toward the Th1 alloimmune response.[21] Neutrophils act as APCs to stimulate adaptive immunity partially by expressing MHC-II and costimulatory molecules.[22] Importantly, studies using intravital two-photon microscopy discovered that neutrophils directly interact with CD4+ T cells in vivo.[23] The recruited neutrophils in lymph nodes and spleens secrete B-cell-activating factor (BAFF) to accelerate plasma cell generation and antigen-specific antibody production.[24] In contrast, certain splenic neutrophils restrict the autoreactive B-cell response by interacting with invariant natural killer T cells through the FasL pathway.[25] The detailed molecular mechanisms involved in the modulation of other immune cells by neutrophils should be explored. It is well known that different diseases involve different types of immune response, especially Th cell subset differentiation. For example, rheumatoid arthritis is associated with Th1 and Th17 subsets and allergic asthma involves Th2 and Th9 subsets. We also know that innate immune cells trigger and drive the subsequent cellular and humoral adaptive immune response via antigen presentation, costimulation and cytokine production. Thus, it is reasonable to hypothesize that the different types of Th cell-mediated diseases are originally orchestrated by identical types of informatory microenvironment that mainly offers consistently polarized innate immune cells, such as dendritic cells, macrophages, neutrophils, NK cells, ILCs and others (Fig. 1). For example, the Th1-type immune response is coordinately driven by the type 1 innate immune response, and the Th2-type immune response is consistently driven by type 1 innate inflammation. Thus, innate and adaptive immunity is coordinately clustered (Fig. 1). This hypothesis requires us to detect the polarization tendency of innate immune cells for the prognosis of certain types of immune disease in which an individual likely suffers from consistent type Th cell-mediated disease during the early stage. The reversion or rebalance of innate immune cell polarization might have preventive and therapeutic significance for immune diseases and graft rejection.



B

Innate immune cells					Adaptive immune cells
Neutrophils (N)	Macrophages (M)	Dendirtic cells (DC)	NK cells	ILCs	Th cells
N0 N1 N2 N(IL-23) N(IL-33) …	M0 M1 M2 M9(?) M17 Mreg …	DC0 DC1 DC2 DC9(?) DC17 (?) DCreg …	NK0 NK1 NK2 NK22 Nkreg …	ILC0 (?) ILC1 ILC2 ILC3 …	Th0 Th1 Th2 Th9 Th17 Th22 Tfh Treg …

Fig. 1 The clustered polarization of innate and adaptive immune responses is coordinately driven by innate and adaptive immune cell subpopulations. **a** The different types of adaptive immune response are coordinately driven by the consistent polarization of innate and adaptive immune cell subpopulations. The clusters of polarized innate and adaptive immune cells are simply shown. **b** The brief summary of the different subpopulations of innate and adaptive immune cells, including neutrophils, macrophages, DCs, NK cells, ILCs, and Th cells. The unprimed cells were defined as type 0

Neutrophils may be one of the target cell types for this purpose. The newly identified N(IL-23) and N(IL-33) subsets may be used for cellular therapy in clinics after systemic studies in animal models and clinical samples are performed.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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