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Review

When neutrophils meet T cells: Beginnings of a tumultuous relationship with underappreciated potential

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Neutrophils play a key role in the innate immune system's response to infection. They eliminate microbes through phagocytosis, the production of ROS, and the secretion of various proteases and antimicrobial peptides. In addition, they influence adaptive immune responses by modulating B-cell antibody production, dendritic cell activation and antimicrobial $CD4^+$ and $CD8^+$ T-cell responses. Here we discuss the current knowledge of the reciprocal interactions between neutrophils and T cells. A special emphasis is put on their interaction with γδ T cells, which respond in the early stages of infection to produce a pivotal source of neutrophil-recruiting IL-17. Human peripheral blood γδ T cells are activated by microbe-derived and endogenous isoprenoid pyrophosphate antigens, the levels of which can be enhanced by the therapeutic application of aminobisphosphonates. We specifically discuss intriguing new evidence showing how pyrophosphates and aminobisphosphonates modulate the interplay between neutrophils and human γδ T cells.

Keywords: Immune regulation · Innate immunity · Neutrophils · T cells · γδ T cells

Introduction

Neutrophils are short-lived primordial effector cells of the innate immune system. They eliminate extracellular microbes through the phagocytosis of pathogens, the release of proteolytic enzymes from intracellular granules (e.g. lysozyme, serine proteases, metalloproteases), and produce antimicrobial peptides (e.g. α-defensins, cathelicidin) and ROS [1, 2]. In recent years it has become evident that neutrophils do not constitute a homogeneous cell population, but rather, comprise subpopulations which can be identified on the basis of surface markers and function [2, 3]. Many effector functions are rapidly induced in neutrophils upon activation by contact with microbes or bacterial products including LPS or formyl-methionyl-leucyl-phenylalanine (fMLP), a chemotactic peptide, which leads to further alteration of surface marker expression. Increasing evidence indicates that neutrophils also play important roles far beyond simply being innate effector cells. Neutrophils communicate extensively with other innate cells including NK cells and DCs [4, 5]. It is now better recognized that neutrophils share a sophisticated relationship with the stalwarts of adaptive immunity, B cells and T cells [6, 7]. Recent excellent reviews have covered many aspects of the ever- increasing known functions of neutrophils as executors of innate immunity and modulators of adaptive immunity [8–10]. In this review we specifically focus on the pathways regulating the neutrophil to T-cell cross-talk, highlighting in particular new findings on the modulation of γδ T-cell activity.

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The regulatory arsenal of neutrophils

Activation of neutrophils is mainly driven by proinflammatory signals initiated through TLR and chemoattractants. Phagocytosis of microbes, the release of cargo from different types of granules and the formation of neutrophil extracellular traps (NETs) containing extracellular DNA, enzymes and antimicrobial peptides are key antibacterial strategies of neutrophils [2, 10]. Many molecules

induced or released by activated neutrophils have a direct or indirect impact on T cells [2, 8–10]. Upon activation, neutrophils produce ROS including H_2O_2, which are not only important for antimicrobial activity, but also have the potential to potently inhibit T-cell activation [11, 12]. Furthermore, serine proteases can digest molecules involved in T-cell activation such as CD25 and thereby impact T-cell activation [13]. Neutrophil-derived myeloperoxidase (MPO) inhibits activation and migration of DCs, thereby reducing inflammatory T-cell responses [14]. Release of arginase-1 from gelatinase-containing granules depletes the extracellular levels of L-arginine which is essentially required for T-cell activation. Amongst other effects, arginase-1 inhibits T-cell activation by downregulating CD3ξ expression [15, 16]. Additionally, neutrophils can also produce cytokines with known inhibitory activity on T cells such as IL-10 and TGF-β [8, 17]. On the other hand, neutrophils can also contribute to the activation of T cells in multiple ways, for example, through modulation and recruitment of DCs and via the release of various initiatory chemokines and cytokines [5, 7, 18, 19]. Moreover, recent evidence indicates that neutrophils can transport antigens to sites of T-cell activation [20, 21] and even act themselves as APCs [22]. An additional level of plasticity of murine neutrophils was recently revealed by Matsushima et al. [23]. These investigators demonstrated that BM-derived neutrophils can acquire a hybrid phenotype exhibiting phenotypic markers and functional properties of both neutrophils and DCs when stimulated by GM-CSF. Neutrophil-DC hybrid cells retained neutrophil functions such as extrusion of NETs while simultaneously displaying DC morphology and functions including Ag uptake and presentation, and secretion of IL-12 [23]. Thus, multiple junctions of cross-regulation between neutrophils and T cells exist that shape the nature and tempo of the adaptive immune response at any given moment of time (Table 1).

Table 1. Effector pathways of neutrophils regulating T-cell-dependent immunity[a)]

	Impact on T-cell activation
Suppressive pathways	
ROS	Inhibition
arginase-1	Inhibition (depletion of arginine)
serine proteases	Inhibition (cleavage of cytokine receptors)
IL-10	Inhibition
TGF-β	Inhibition
Stimulatory pathways	
IL-12	Activation
Chemokines (e.g. CCL20, CCL1, MCP-1)	T-cell recruitment
MHC class II expression and Ag presentation	Activation of naïve $CD4^+$ T cells
Antigen cross-presentation	Activation of naïve $CD8^+$ T cells
NETs	(co)stimulation of human T cells

[a)] Compiled from [1, 2, 7, 8, 9, 17, 43]. As briefly discussed in the text, additional indirect effects on T-cell activation by neutrophils results from regulatory interactions of neutrophils with DCs.

Neutrophils suppress T-cell activation

As mentioned above, it has been appreciated for some time that neutrophils can suppress human T-cell activation in vitro [11]. Major effector mechanisms include the release of ROS, MPO, and arginase-1 [7, 14, 24]. Recent studies have uncovered more details of these mechanisms, also with respect to possible (patho)physiological relevance. Since human studies are mostly performed using in vitro culture of PBMCs with neutrophils added back, it is important to consider the possible impact of different isolation methods in light of the relative fragility of neutrophils [25]. Moreover, when using polyclonal T-cell activation with anti-CD3 mAb, neutrophils can inhibit T-cell activation by interfering with the anti-CD3 mAb rather than with T cells [26]. This notwithstanding, it is clear that purified (and usually unavoidably activated) neutrophils can inhibit human T-cell responses to polyclonal and antigen-specific stimuli [3, 26–29]. Based on inhibitor studies of arginase-1 and H_2O_2 production (using N^G-Hydroxy-L-arginine (NOHA) and catalase, respectively), these agents have been identified as suppressive molecules released by neutrophils. Induction of T-cell death by activated neutrophils might also contribute to T-cell suppression in some circumstances [26, 28]. Suppressive neutrophils have been identified as being $CD62L^{dim}/CD11c^{bright}/CD16^{bright}$ [3, 29]. Importantly, the suppressive action of neutrophils appears to be integrin-dependent, requires close contact with T cells [28], and involves the highly localized release of H_2O_2 into the immunological synapse [3]. Two clinically relevant examples for neutrophil mediated T-cell inhibition are observed in cancer patients and in normal pregnancy. Ficoll-Hypaque-separated PBMCs from cancer patients are significantly contaminated with granulocytes which inhibit T-cell activation via H_2O_2 production [27]. Increased proportions of arginase-1 producing neutrophils have been observed in glioblastoma patients, associated with reduced T-cell function [30]. Again, targeting arginase-1 by NOHA was found to restore the defective T-cell responsiveness [30]. Interestingly, regulation of L-arginine levels by neutrophils might also contribute to tolerance of the semiallogeneic fetus. Thus, during normal human pregnancy, neutrophils in the blood and placenta were found to express increased levels of arginase-1, again associated with transient T-cell hyporesponsiveness [31].

Various lines of evidence indicate that neutrophils similarly control T-cell responses in mice. $CD4^+$ T-cell and B-cell responses to protein antigens are inhibited by neutrophils at the level of antigen presentation in LNs, both through competition with DCs for antigen and additional direct effects on DCs [32]. A suppressive function of neutrophils has also been noted in several infection models. As an example, elimination of the intracellular microbe *Brucella abortus* was more efficient in neutropenic as compared with WT mice, due to enhanced B-cell and Th1-cell responses [33]. The impact of neutrophils on adaptive immune responses has also

been studied in mycobacterial infections [34]. Here, neutrophils were shown to exert differential suppressive activity on Th1 versus Th17 cells in vivo, with only Th17 cells being selectively inhibited in an IL-10-dependent manner [34].

Neutrophils can also enhance T-cell responses

Reflecting the surprisingly large plasticity of their functional repertoire, neutrophils can also enhance various T-cell responses either directly or indirectly through activation of DCs. Neutrophils induce maturation of DCs in a cell contact- and cytokine-dependent manner and thus contribute to subsequent T-cell activation [5, 18], which has been studied in various infection models. In a mouse influenza infection model, the depletion of neutrophils by Ly6G-specific mAb reduced the overall virus-specific $CD8^+$ T-cell response at the level of cytokine production and killer activity while having no impact on antigen presentation and expansion of naïve $CD8^+$ T-cells in secondary LNs [35]. The role of neutrophils in mycobacterial infection appears to be complex: while IL-10 producing neutrophils shut down Th17 $CD4^+$ T cells [32], they were found to promote activation and proliferation of *Mycobacterium tuberculosis*-specific $CD4^+$ T cells in lung-draining mediastinal LNs [36]. Thus, in the presence of neutrophils, lung DCs take up *M. tuberculosis*-infected neutrophils and can then efficiently migrate to the LNs, whereas in the case of neutrophil depletion, DCs are directly infected with *M. tuberculosis* which reduces their migratory capacity and subsequently the T-cell response in the LN [36]. Furthermore, neutrophils support T-cell responses through the secretion of chemokines that attract DCs or T cells to specific sites of inflammation. This has been documented in murine models of *Leishmania major* infection (CCL3; [19]), contact hypersensitivity (CCL1, CCL2, CCL5; [37]), and allospecific $CD8^+$ responses (MCP-1; [38]) as well as in human in vitro studies (CCL2, CCL20 for Th17; CCL20, CXCL10 for Th1; [39]). Under in vitro conditions, this appears to be a bidirectional phenomenon as Th17 cells also attract neutrophils via the secretion of the neutrophil chemotactic factor, CXCL8 (IL-8) [39]. Intriguingly, neutrophils can additionally promote T-cell responses through ameliorating the inhibitory capacity of other suppressive cell populations. In a murine model of sterile systemic chronic inflammation, Ryan et al. demonstrated that IFN-γ-secreting neutrophils confer T-cell resistance to the antiinflammatory activity of myeloid derived suppressor cells (MDSCs), thereby promoting the maintenance of chronic inflammation [40]. Moreover, neutrophils stimulate T-cell responses at the level of antigen presentation. In this respect, it is interesting to note that neutrophils themselves can pick up and transport antigens to the LNs as the site of T-cell priming [20] and to the BM as a site for maintaining memory $CD8^+$ T cells [21]. In addition, neutrophils can also act as APCs, expressing typical APC markers including MHC class II, CD80, and CD86, and thereby driving antigen-specific murine and human T-cell activation [22, 41]. Importantly, neutrophils can even cross-present exogenous antigens to naïve $CD8^+$ T cells, as has been nicely shown in the OT-1 in vivo model by Beauvillain et al. [42]. Last but not the least, NETs decorated with extracellular DNA and antimicrobial peptides have been shown to directly prime human T-cell activation in vitro. In the presence of NETs, the proliferation and cytokine production of T cells in response to specific antigen or suboptimal TCR stimulation was shown to be significantly enhanced [43]. Taken together, neutrophils can utilize multiple pathways to directly or indirectly support adaptive T-cell responses.

T cells reciprocally modulate neutrophil activation and function

In a reciprocal manner, T cells can also modulate neutrophil activation and function. Th17 cells contribute to the recruitment of neutrophils to sites of infection through secretion of IL-17 [44], although early sources of IL-17 are innate lymphoid cells (ILCs) and γδ T cells rather than Th17 $CD4^+$ T cells (discussed below). Quite surprisingly, human Treg cells can also recruit neutrophils through the secretion of CXCL8 [45]. Furthermore, activated human $CD4^+$ and $CD8^+$ T cells have been shown to upregulate CD11b, CD64, and CD62L on neutrophils and delay neutrophil apoptosis, mainly via the secretion of IFN-γ and GM-CSF [46]. Murine NKT cells expressing invariant TCR trigger IFN-γ production in neutrophils and thereby promote their infiltration into ischemic kidneys [47], and modulate neutrophil suppressive activity by inhibiting their IL-10 production and stimulating IL-12 secretion [48]. There is less published evidence for inhibitory effects of T cells on neutrophils. Given that neutrophils express death receptors [49] and are susceptible to TNF-triggered apoptosis [50], it is conceivable, however, that activated T cells also exert negative effects on neutrophils via secreted or cell surface expressed death receptor ligands.

A whirling dance: neutrophils and γδ T cells

γδ T cells have a prominent role in immunosurveillance, given their recognition of stress-induced molecules and production of cytokines regulating local immune responses and tissue repair [51]. Studies based on the comparison of WT and TCR-γδ KO mice have revealed an important role of γδ T cells in recruiting neutrophils to sites of bacterial infection and epithelial wound healing. As an example, γδ T cells were found to govern neutrophil-mediated host defense against *Streptococcus pneumoniae* lung infection by promoting the production of TNF-α and CXCL2 in the infected tissue [52]. Recruitment of neutrophils required for epithelial wound healing in a model of corneal epithelial abrasion-induced inflammation was similarly found to depend on the presence of γδ T cells [53]. In recent years, IL-17 has been recognized as a major driving factor for the recruitment of neutrophils to sites of infection and inflammation. Together with ILCs, γδ T cells have been identified as early producers of IL-17, thus stimulating the recruitment of neutrophils. IL-17-producing γδ T cells have been shown to control the neutrophil influx in different infection

models including intraperitoneal inoculation of *Escherichia coli* and systemic infection with *Candida albicans* [54, 55]. γδ T cells were also shown to regulate the accumulation of neutrophils in the lung and small intestine in a thermal injury model where neutrophils are responsible for the secondary tissue damage; the helper activity of γδ T cells could be related to their secretion of CCL4 and CXCL1 [56]. While these results indicate that γδ T cells support neutrophil recruitment, there is also evidence that neutrophils can actually inhibit or limit γδ T-cell functions. Wozniak et al. [57] studied γδ T cells and IL-17 production in mice infected with the opportunistic fungal pathogen *Cryptococcus neoformans*, which leads to pulmonary cryptococcosis. They identified both neutrophils and pulmonary γδ T cells as a source of IL-17A [57]. Following depletion of neutrophils, they observed increased proportions of IL-17-producing γδ T cells in the lung coinciding with increased levels of CXCL1 and IL-6, suggesting that neutrophils exert a regulatory effect on γδ T cells [57].

In humans, the dominant population of peripheral blood γδ T cells expresses a Vγ9Vδ2 TCR. Instead of peptides bound to MHC molecules, Vγ9Vδ2 T cells recognize pyrophosphate intermediates of the microbial or eukaryotic isoprenoid synthesis pathways. Microbial pyrophosphates such as (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP) activate human γδ T cells at pico- to nanomolar concentrations, whereas endogenous isopentenyl pyrophosphate (IPP) requires 3-log higher (micromolar range) concentrations [51, 58]. Such high concentrations are not produced by healthy cells but are an accumulated product of the dysregulated metabolism of tumor cells, which then forms the basis for the MHC nonrestricted antitumor reactivity of human Vγ9Vδ2 T cells [58]. Once activated, Vγ9Vδ2 T cells can stimulate neutrophil phagocytosis, chemotaxis, and α-defensin release via the production of CCL8 [59] and further promote neutrophil survival [60]. On the other hand, both human and murine γδ T cells can kill neutrophils that have been induced to express HSP72 in response to proinflammatory stimuli [61]. There are two scenarios where pyrophosphates dictate the interaction between human neutrophils and γδ T cells. In the context of infection, neutrophils take up bacteria by phagocytosis. This leads to the release of microbial HMB-PP which then selectively activates Vγ9Vδ2 T cells [60]. On the other hand, the levels of endogenous pyrophosphate IPP can be manipulated by nitrogen-containing aminobisphosphonates (N-BP), drugs that are in clinical use for the treatment of osteoporosis and related bone fragility disorders. N-BP such as zoledronate inhibit an enzyme downstream of IPP synthesis in the mevalonate pathway resulting in increased accumulation of IPP and subsequent selective Vγ9Vδ2 T-cell activation and expansion in PBMC responder cells [62]. Utilizing fluorophore-conjugated zoledronate to study the uptake in various cell populations, we observed efficient ingestion by not only monocytes but also by neutrophils — a consequence that has been largely ignored previously. When analyzing zoledronate stimulation of Ficoll-Hypaque-separated PBMCa (devoid of neutrophils) versus RBC-lysed whole leukocytes, we found strong expansion of Vγ9Vδ2 T cells within PBMCs but very little within whole leukocyte responder cells, particularly if $CD14^+$ monocytes were depleted from those leukocytes [63]. Further studies revealed that zoledronate-activated neutrophils potently inhibit the response of Vγ9Vδ2 T cells to pyrophosphate antigens and zoledronate itself. In searching for the underlying mechanism of suppression, we found that the inhibitory activity of neutrophils on γδ T-cell expansion could be fully overcome by collectively blocking ROS, arginase-1 and serine proteases [63]. We have recently reported that the proportion of peripheral blood Vγ9Vδ2 T cells strongly declines in osteoporosis patients who are on continuous intravenous N-BP treatment [64]. The underlying mechanism of this loss of Vγ9Vδ2 T cells in patients on N-BP treatment is unknown, but our in vitro studies suggest it may very well be related to the zoledronate-induced inhibitory effect of neutrophils. An important but as yet not unambiguously settled issue is the impact of the neutrophil lifespan on the interplay between neutrophils and (γδ) T cells. While it is commonly believed that neutrophils are short-lived cells with a circulatory half-life in the range of about 6 to 8 h, some studies (summarized in [65]) suggest a much longer (up to several days) half-life. Obviously, this would be highly relevant in the context of the above discussed N-BP-induced inhibitory effect of neutrophils on γδ T-cell activation, an issue that clearly deserves further investigation.

Figure 1 summarizes the emerging cross-regulation of the two relatively enigmatic players of the innate immune system, neutrophils and γδ T cells, in their attempts to guard against the threat of infection while brokering a semblance of homeostasis.

Concluding remarks

A substantial body of evidence now exists for reciprocal interactions between neutrophils and T cells, both in vivo and in vitro, across mouse and human studies. Manipulation of the neutrophil compartment or targeting their regulatory products (e.g. enzymes, cytokines, ROS) modulates T-cell responses and might provide further avenues for immunotherapeutic approaches in infection and tumor immunity. However, we would also like to stress some practical implications. The standard procedure for in vitro analysis of human T-cell/B-cell activation is to isolate PBMCs via Ficoll-Hypaque density gradient centrifugation. This method deliberately removes granulocytes, and the subsequent in vitro studies with PBMCs are performed in the absence of those cells that account for roughly 50% of blood leukocytes. In view of the potent immunoregulatory activity of neutrophils we raise a caveat on the interpretation of experiments performed with PBMCs in vitro: that the potential influence of neutrophils should be considered when extrapolating their possible significance in vivo. To address these issues, it might be advisable to include experiments with whole leukocyte cultures [63] and/or to add back purified neutrophils to PBMCs. This is perhaps best exemplified with the numerous studies showing the efficacy of N-BP in expanding and activating the effector functions of human peripheral blood Vγ9Vδ2 T cells for cancer immunotherapy in vitro using PBMC cell culture systems [66, 67]; clinical efficacy has been difficult to achieve due in part to the in vivo loss of these effector T cells [67]. As

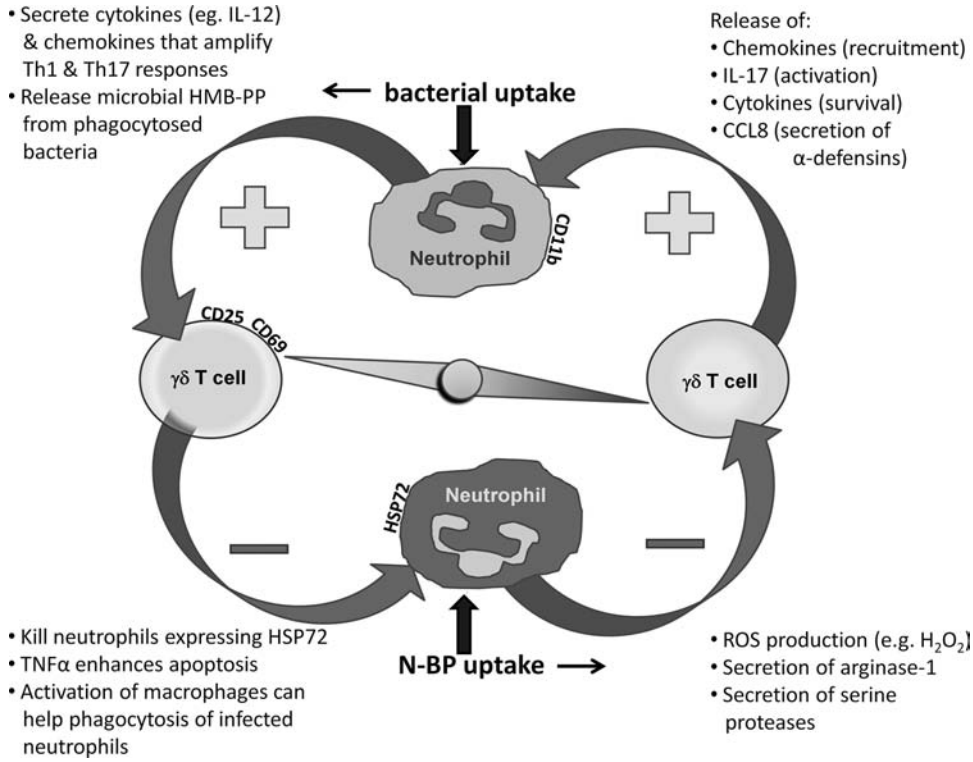

Figure 1. The reciprocal interplay between neutrophils and γδ T cells. Both in vitro experiments using human cells and various murine in vivo models comparing WT and TCRδ KO mice suggest that neutrophils interact with γδ T cells in a cross-regulatory manner. The particular characteristics of the initiatory stimulant or stress determines whether their effects on each other are ultimately "activating" ("+", top half) or "inhibiting" ("−", bottom half) in nature. N-BP, aminobisphosphonate; HMB-PP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (microbial phosphoantigen for human peripheral blood γδ T cells).

discussed in this article and elsewhere [63, 64], inhibitory effects of N-BP-activated neutrophils might contribute to the loss and/or "silencing" of γδ T cells in vivo. We conclude that the regulatory interplay between neutrophils and various subsets of T cells requires further investigation, with the potential of delineating new avenues for modulating T-cell responses in vivo.

Note: Independent of our report [63], Sabbione et al. very recently also observed suppression of human γδ T-cell function by neutrophils. In their studies, ROS was identified as the major inhibitory principle [68], in line with our own results that catalase partially reverts the suppression.

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References

1 **Galli, S. J, Borregaard, N. and Wynn, T. A.**, Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nat. Immunol.* 2011. **12**: 1035–1044.

2 **Amulic, B., Cazalet, C., Hayes, G. L., Metzler, K. D. and Zychlinsky, A.**, Neutrophil function: from mechanisms to disease. *Annu. Rev. Immunol.* 2012. **30**: 459–489.

3 **Pillay, J., Kamp, V. M., van Hoffen, E., Visser, T., Tak, T., Lammers, J. W., Ulfman, L. H. et al.**, A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. *J. Clin. Invest.* 2012. **122**: 327–336.

4 **Costantini, C., Calzetti, F., Perbellini, O., Micheletti, A., Scarponi, C., Lonardi, S., Pelletier, M. et al.**, Human neutrophils interact with both 6-sulfo LacNAc$^+$ DC and NK cells to amplify NK-derived IFNγ: role of CD18, ICAM-1, and ICAM-3. *Blood* 2011. **117**: 1677–1686.

5 **van Gisbergen, K. P., Sanchez-Hernandez, M., Geijtenbeek, T. B. and van Kooyk, Y.**, Neutrophils mediate immune modulation of dendritic cells through glycosylation-dependent interactions between Mac-1 and DC-SIGN. *J. Exp. Med.* 2005. **201**: 1281–1292.

6 **Cerutti, A., Puga, I. and Cols, M.**, New helping friends for B cells. *Eur. J. Immunol.* 2012. **42**: 1956–1968.

7 **Müller, I., Munder, M., Kropf, P. and Hänsch, G. M.**, Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? *Trends Immunol.* 2009. **30**: 522–530.

8 **Mantovani, A., Cassatella, M. A., Costantini, C. and Jaillon, S.**, Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.* 2011. **11**: 519–531.

9 **Mócsai, A.**, Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J. Exp. Med.* 2013. **210**: 1283–1299.

10 **Mayadas, T. N., Cullere, X. and Lowell, C. A.**, The multifaceted functions of neutrophils. *Annu. Rev. Pathol.* 2014. **9**: 181–218.

11 **El-Hag, A., Lipsky, P. E., Bennett, M. and Clark, R. A.**, Immunomodulation by neutrophil myeloperoxidase and hydrogen peroxide: differential susceptibility of human lymphocyte functions. *J. Immunol.* 1986. **36**: 3420–3426.

12 **Cemerski, S., Cantagrel, A., Van Meerwijk, J. P. and Romagnoli, P.**, Reactive oxygen species differentially affect T cell receptor-signaling pathways. *J. Biol. Chem.* 2002. **277**: 19585–19593.

13 **Bank, U., Reinhold, D., Schneemilch, C., Kunz, D., Synowitz, H. J. and Ansorge, S.**, Selective proteolytic cleavage of IL-2 receptor and IL-6 receptor ligand binding chains by neutrophil-derived serine proteases at foci of inflammation. *J. Interferon. Cytokine Res.* 1999. **19**: 1277–1287.

14 **Odobasic, D., Kitching, A. R., Yang, Y., O'Sullivan, K. M., Muljadi, R. C., Edgtton, K. L., Tan, D. S. et al.**, Neutrophil myeloperoxidase regulates T-cell-driven tissue inflammation in mice by inhibiting dendritic cell function. *Blood* 2013. **121**: 4195–4204.

15 **Munder, M., Schneider, H., Luckner, C., Giese, T., Langhans, C. D., Fuentes, J. M., Kropf, P. et al.**, Suppression of T-cell functions by human granulocyte arginase. *Blood* 2006. **108**: 1627–1634.

16 **Rotondo, R., Bertolotto, M., Barisione, G., Astigiano, S., Mandruzzato, S., Ottonello, L., Dallegri, F. et al.**, Exocytosis of azurophil and arginase 1-containing granules by activated polymorphonuclear neutrophils is required to inhibit T lymphocyte proliferation. *J. Leukoc. Biol.* 2011. **89**: 721–727.

17 **Borregaard, N., Sørensen, O. E. and Theilgaard-Mönch, K.**, Neutrophil granules: a library of innate immunity proteins. *Trends Immunol.* 2007. **28**: 340–345.

18 **Bennouna, S. and Denkers, E. Y.**, Microbial antigen triggers rapid mobilization of TNF-α to the surface of mouse neutrophils transforming them into inducers of high-level dendritic cell TNF-α production. *J. Immunol.* 2005. **174**: 4845–4851.

19 **Charmoy, M., Brunner-Agten, S., Aebischer, D., Auderset, F., Launois, P., Milon, G., Proudfoot, A. E. and Tacchini-Cottier, F.**, Neutrophil-derived CCL3 is essential for the rapid recruitment of dendritic cells to the site of *Leishmania major* inoculation in resistant mice. *PLoS. Pathog.* 2010. **6**: e1000755.

20 **Maletto, B. A., Ropolo, A. S., Alignani, D. O., Liscovsky, M. V., Ranocchia, R. P., Moron, V. G. and Pistoresi-Palencia, M. C.**, Presence of neutrophil-bearing antigen in lymphoid organs of immune mice. *Blood* 2006. **108**: 3094–3102.

21 **Duffy, D., Perrin, H., Abadie, V., Benhabiles, N., Boissonnas, A., Liard, C., Descours, B. et al.**, Neutrophils transport antigen from the dermis to the bone marrow, initiating a source of memory $CD8^+$ T cells. *Immunity* 2012. **37**: 917–929.

22 **Abi Abdallah, D. S., Egan, C. E., Butcher, B. A. and Denkers, E. Y.**, Mouse neutrophils are professional antigen-presenting cells programmed to instruct T_h1 and T_h17 T-cell differentiation. *Int. Immunol.* 2011. **23**: 317–326.

23 **Matsushima, H., Geng, S., Lu, R., Okamoto, T., Yao, Y., Mayuzumi, N., Kotol, P. F. et al.**, Neutrophil differentiation into a unique hybrid population exhibiting dual phenotype and functionality of neutrophils and dendritic cells. *Blood* 2013. **121**: 1677–1689.

24 **Pillay, J., Tak, T., Kamp, V. M. and Koenderman, L.**, Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. *Cell. Mol. Life Sci.* 2013. **70**: 3813–3827.

25 **Zhou, L., Somasundaram, R., Nederhof, R. F., Dijkstra, G., Faber, K. N., Peppelenbosch, M. P. and Fuhler, G. M.**, Impact of human granulocyte and monocyte isolation procedures on functional studies. *Clin. Vaccine Immunol.* 2012. **19**: 1065–1074.

26 **Hock, B. D., Taylor, K. G., Cross, N. B., Kettle, A. J., Hampton, M. B. and McKenzie, J. L.**, Effect of activated human polymorphonuclear leucocytes on T lymphocyte proliferation and viability. *Immunology* 2012. **137**: 249–258.

27 **Schmielau, J. and Finn, O. J.**, Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Cancer Res.* 2001. **61**: 4756–4760.

28 **Thewissen, M., Damoiseaux, J., van de Gaar, J. and Tervaert, J. W.**, Neutrophils and T cells: bidirectional effects and functional interferences. *Mol. Immunol.* 2011. **48**: 2094–2101.

29 **Kamp, V. M., Pillay, J., Lammers, J. W., Pickkers, P., Ulfman, L. H. and Koenderman, L.**, Human suppressive neutrophils $CD16^{bright}/CD62L^{dim}$ exhibit decreased adhesion. *J. Leukoc. Biol.* 2012. **92**: 1011–1020.

30 **Sippel, T. R., White, J., Nag, K., Tsvankin, V., Klaassen, M., Kleinschmidt-DeMasters, B. K. and Waziri, A.**, Neutrophil degranulation and immunosuppression in patients with GBM: restoration of cellular immune function by targeting arginase I. *Clin. Cancer Res.* 2011. **17**: 6992–7002.

31 **Kropf, P., Baud, D., Marshall, S. E., Munder, M., Mosley, A., Fuentes, J. M., Bangham, C. R. et al.**, Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. *Eur. J. Immunol.* 2007. **37**: 935–945.

32 **Doz, E., Lombard, R., Carreras, F., Buzoni-Gatel, D. and Winter, N.**, Mycobacteria-infected dendritic cells attract neutrophils that produce IL-10 and specifically shut down Th17 CD4 T cells through their IL-10 receptor. *J. Immunol.* 2013. **191**: 3818–3826.

33 **Barquero-Calvo, E., Martirosyan, A., Ordoñez-Rueda, D., Arce-Gorvel, V., Alfaro-Alarcón, A., Lepidi, H., Malissen, B. et al.**, Neutrophils exert a suppressive effect on Th1 responses to intracellular pathogen Brucella abortus. *PLoS Pathog.* 2013. **9**: e1003167.

34 **Yang, C. W. and Unanue, E. R.**, Neutrophils control the magnitude and spread of the immune response in a thromboxane A2-mediated process. *J. Exp. Med.* 2013. **210**: 375–387.

35 **Tate, M. D., Brooks, A. G., Reading, P. C. and Mintern, J. D.** Neutrophils sustain effective $CD8^+$ T-cell responses in the respiratory tract following influenza infection. *Immunol. Cell. Biol.* 2012. **90**: 197–205.

36 **Blomgran, R. and Ernst, J. D.** Lung neutrophils facilitate activation of naive antigen-specific $CD4^+$ T cells during *Mycobacterium tuberculosis* infection. *J. Immunol.* 2011. **186**: 7110–7119.

37 **Kish, D. D., Gorbachev, A. V., Parameswaran, N., Gupta, N. and Fairchild, R. L.**, Neutrophil expression of Fas ligand and perforin directs effector CD8 T cell infiltration into antigen-challenged skin. *J. Immunol.* 2012. **189**: 2191–2202.

38 **Shiratsuchi, Y., Iyoda, T., Tanimoto, N., Kegai, D., Nagata, K. and Kobayashi, Y.**, Infiltrating neutrophils induce allospecific CTL in response to immunization with apoptotic cells via MCP-1 production. *J. Leukoc. Biol.* 2007. **81**: 412–420.

39 **Pelletier, M., Maggi, L., Micheletti, A., Lazzeri, E., Tamassia, N., Costantini, C., Cosmi, L. et al.**, Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010. **115**: 335–343.

40 **Ryan, S. O., Johnson, J. L. and Cobb, B. A.**, Neutrophils confer T cell resistance to myeloid-derived suppressor cell-mediated suppression to promote chronic inflammation. *J. Immunol.* 2013. **190**: 5037–5047.

41 **Radsak, M., Iking-Konert, C., Stegmaier, S., Andrassy, K. and Hänsch, G. M.**, 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**: 521–530.

42 **Beauvillain, C., Delneste, Y., Scotet, M., Peres, A., Gascan, H., Guermonprez, P., Barnaba, V. and Jeannin, P.**, Neutrophils efficiently cross-prime naive T cells in vivo. *Blood* 2007. **110**: 2965–2973.

43 **Tillack, K., Breiden, P., Martin, R. and Sospedra, M.**, T. lymphocyte priming by neutrophil extracellular traps links innate and adaptive immune responses. *J. Immunol.* 2012. **188**: 3150–3159.

44 **Mills, K. H.**, Induction, function and regulation of IL-17-producing T cells. *Eur. J. Immunol.* 2008. **38**: 2636–2649.

45 **Himmel, M. E., Crome, S. Q., Ivison, S., Piccirillo, C., Steiner, T. S. and Levings, M. K.**, Human $CD4^+$ $FOXP3^+$ regulatory T cells produce CXCL8 and recruit neutrophils. *Eur. J. Immunol.* 2011. **41**: 306–312.

46 **Pelletier, M., Micheletti, A. and Cassatella, M. A.**, Modulation of human neutrophil survival and antigen expression by activated $CD4^+$ and $CD8^+$ T cells. *J. Leukoc. Biol.* 2010. **88**: 1163–1170.

47 **Li, L., Huang, L., Sung, S. S., Lobo, P. I., Brown, M. G., Gregg, R. K., Engelhard, V. H. and Okusa, M. D.**, NKT cell activation mediates neutrophil IFN-γ production and renal ischemia-reperfusion injury. *J. Immunol.* 2007. **178**: 5899–5911.

48 **De Santo, C., Arscott, R., Booth, S., Karydis, I., Jones, M., Asher, R., Salio, M. et al.**, Invariant NKT cells modulate the suppressive activity of IL-10-secreting neutrophils differentiated with serum amyloid A. *Nat. Immunol.* 2010. **11**: 1039–1046.

49 **Akgul, C. and Edwards, S. W.**, Regulation of neutrophil apoptosis via death receptors. *Cell. Mol. Life Sci.* 2003. **60**: 2402–2408.

50 **Salamone, G., Giordano, M., Trevani, A. S., Gamberale, R., Vermeulen, M., Schettinni, J. and Geffner, J. R.**, Promotion of neutrophil apoptosis by TNF-α. *J. Immunol.* 2001. **166**: 3476–3483.

51 **Vantourout, P. and Hayday, A.**, Six-of-the-best: unique contributions of γδ T cells to immunology. *Nat. Rev. Immunol.* 2013. **13**: 88–100.

52 **Nakasone, C., Yamamoto, N., Nakamatsu, M., Kinjo, T., Miyagi, K., Uezu, K., Nakamura, K. et al.**, Accumulation of gamma/delta T cells in the lungs and their roles in neutrophil-mediated host defense against pneumococcal infection. *Microbes Infect.* 2007. **9**: 251–258.

53 **Li, Z., Burns, A. R., Rumbaut, R. E. and Smith, C. W.**, γδ T cells are necessary for platelet and neutrophil accumulation in limbal vessels and efficient epithelial repair after corneal abrasion. *Am. J. Pathol.* 2007. **171**: 838–845.

54 **Shibata, K., Yamada, H., Hara, H., Kishihara, K. and Yoshikai, Y.**, Resident $V\delta1^+$ γδ T cells control early infiltration of neutrophils after *Escherichia coli* infection via IL-17 production. *J. Immunol.* 2007. **178**: 4466–4472.

55 **Dejima, T., Shibata, K., Yamada, H., Hara, H., Iwakura, Y., Naito, S. and Yoshikai, Y.**, Protective role of naturally occurring interleukin-17A-producing γδ T cells in the lung at the early stage of systemic candidiasis in mice. *Infect. Immun.* 2011. **79**: 4503–4510.

56 **Toth, B., Alexander, M., Daniel, T., Chaudry, I. H., Hubbard, W. J. and Schwacha, M. G.**, The role of γδ T cells in the regulation of neutrophil-mediated tissue damage after thermal injury. *J. Leukoc. Biol.* 2004. **76**: 545–552.

57 **Wozniak, K. L., Kolls, J. K. and Wormley, F. L Jr.**, Depletion of neutrophils in a protective model of pulmonary cryptococcosis results in increased IL-17A production by gamma/delta T cells. *BMC Immunol.* 2012. **13**: 65. DOI: **10.1186/1471-2172-13-65**.

58 **Kalyan, S. and Kabelitz, D.**, Defining the nature of human γδ T cells: a biographical sketch of the highly empathetic. *Cell. Mol. Immunol.* 2013. **10**: 21–29.

59 **Agrati, C., Cimini, E., Sacchi, A., Bordoni, V., Gioia, C., Casetti, R., Turchi, F. et al.**, Activated $V\gamma9V\delta2$ T cells trigger granulocyte functions via MCP-2 release. *J. Immunol.* 2009. **182**: 522–529.

60 **Davey, M. S., Lin, C. Y., Roberts, G. W., Heuston, S., Brown, A. C., Chess, J. A, Toleman, M. A. et al.**, Human neutrophil clearance of bacterial pathogens triggers anti-microbial γδ T cell responses in early infection. *PLoS. Pathog.* 2011. **7**: e1002040.

61 **Hirsh, M. I., Hashiguchi, N., Chen, Y., Yip, L. and Junger, W. G.**, Surface expression of HSP72 by LPS-stimulated neutrophils facilitates γδT cell-mediated killing. *Eur. J. Immunol.* 2006. **36**: 712–721.

62 **Kabelitz, D.**, Small molecules for the activation of human γδ T cell responses against infection. *Recent. Pat. Antiinfect. Drug. Discov.* 2008. **3**: 1–9.

63 **Kalyan, S., Chandrasekaran, V., Quabius, E. S., Lindhorst, T. K. and Kabelitz, D.**, Neutrophil uptake of nitrogen-bisphosphonates leads to the suppression of human peripheral blood γδ T cells. *Cell. Mol. Life Sci.* 2013. In press. DOI: 10.1007/s00018-013-1495-x

64 **Kalyan, S., Quabius, E. S., Wiltfang, J., Mönig, H. and Kabelitz, D.**, Can peripheral blood γδ T cells predict osteonecrosis of the jaw? An immunological perspective on the adverse drug effects of aminobisphosphonate therapy. *J. Bone Miner. Res.* 2013. **28**: 728–735.

65 **Tak, T., Tesselaar, K., Pillay, J., Borghans, J. A. and Koenderman, L.**, What's your age again? Determination of human neutrophil half-lives revisited. *J. Leukoc. Biol.* 2013. **94**: 595–601.

66 **Hannani, D., Ma, Y., Yamazaki, T., Déchanet-Merville, J., Kroemer, G. and Zitvogel, L.**, Harnessing γδ T cells in anticancer immunotherapy. *Trends Immunol.* 2012. **33**: 199–206.

67 **Dieli, F., Vermijlen, D., Fulfaro, F., Caccamo, N., Meraviglia, S., Cicero, G., Roberts, A. et al.**, Targeting human γδ T cells with zoledronate and interleukin-2 for immunotherapy of hormone-refractory prostate cancer. *Cancer Res.* 2007. **67**: 7450–7457.

68 **Sabbione, F., Gabelloni, M. L., Ernst, G., Gori, M. S., Salamone, G., Oleastro, M., Trevani, A. et al.**, Neutrophils suppress γδ T-cell function. *Eur. J. Immunol.* 2013. In press. DOI: 10.1002/eji.201343664.

Abbreviation: **HMB-PP**: (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate · **IPP**: isopentenyl pyrophosphate · **N-BP**: nitrogen-containing aminobisphosphonate · **NET**: neutrophil extracellular trap

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