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Unneutral Neutrophils in Late Phase Allergic Reactions

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Editorial Title: Unneutral Neutrophils in Late Phase Allergic Reactions

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Neutrophils are the most abundant leukocyte in the body, and their role in both innate and adaptive immunity continues to unfold. In the context of immediate hypersensitivity allergic reactions, neutrophils have been observed in cutaneous, nasal, and asthmatic late phase allergic reactions (LPAR). However, as the complexities of immediate hypersensitivity reactions and LPAR have been elucidated over the past 30 years, the role of neutrophils in these reactions remains poorly understood. The clinical and histologic evolution of LPAR following allergen challenge is usually evident over 4-12 hours, but may persist longer, and is characterized by a mixed cellular influx of neutrophils, eosinophils, basophils, monocytes, and CD4⁺ and CD8⁺ T cells (1). However, what promotes the LPAR in some individuals and not others following an immediate hypersensitivity reaction, manifest by mast cell activation and degranulation, remains unknown.

Recently, Polak et al (2) provided evidence that neutrophils, activated by GM-CSF, IL-3, and IFN γ , have the potential to endocytose the prototypic birch pollen allergen, Bet v 1, where it was rapidly degraded in the endolysosomes into peptide fragments that were loaded into HLA-DR molecules which were transported to the cell surface and efficiently stimulated Bet v 1-specific Th2 cell clones in an epitope-specific manner to proliferate and produce IL-4, IL-5, and IL-13, but little IFN γ . A characteristic of the activated neutrophils was the absence of CD80 or CD86 expression, suggesting their capacity to stimulate T cells was limited to memory T cells, not naïve T cells. Neutrophils that were not cytokine activated were unable to stimulate the T cell clones. Comparison of cytokine-activated neutrophils with cytokine-activated monocytes revealed that the cytokine-activated neutrophils had accelerated kinetics of antigen processing, while the monocytes were more potent stimulators of the T cell clones and were CD80⁺. Cytokine stimulated eosinophils did not function as antigen-presenting cells. Although basophils were not studied, they are also well characterized as unable to present antigen. These in vitro co-culture experiments were complemented by ex vivo analysis of blister fluid from intradermal

grass pollen-induced LPAR 20-24 hours post allergen injection. The mixed cellularity included neutrophils, of which 11-25% were HLA-DR⁺, while peripheral blood neutrophils were HLA-DR⁻, which was consistent with the in situ activation of neutrophils in the context of a LPAR. Likewise, blister fluids from control diluent injection sites contained few T cells and no granulocytes or monocytes. The authors concluded from their studies that neutrophils act as magnifiers of LPAR by acting as antigen presenting cells. Polak et al (3) address the conundrum regarding the mechanism by which GM-CSF, IL-3, and IFN γ activated, but CD80/CD86 negative, neutrophils could stimulate T cell activation and proliferation, and provided evidence that neutrophil CD58 engagement of CD2 on T cells provided signal 2 following TCR engagement of allergen peptide presented by the neutrophil HLA-DR.

While the authors confirmed and extended previous studies demonstrating the capacity of GM-CSF, IL-3, and IFN γ stimulated neutrophils to upregulate HLA-DR expression and serve as antigen presenting cells to memory CD4⁺ T cells, their conclusion regarding the role of activated neutrophils in the pathobiology of LPAR deserved additional considerations. Since the LPAR usually evolves over 4-12 hours and the cytokine stimulated neutrophils used in the in vitro studies were cultured for 48 hours prior to co-culture with the birch allergen-specific T cell clones that were also in vitro expanded, the extrapolation of in vitro results to in vivo events must be cautiously considered. Since the LPAR influx of leukocytes following mast cell degranulation usually peaks by 12 hours, the in vivo kinetics of cytokine activation of neutrophils becomes important. Equally relevant is that the in vitro studies were done with purified neutrophils and birch pollen-specific T cell clones, in the absence of the complex milieu of the in vivo mixed cellular, chemokine, and cytokine response that occurs during the immediate allergic response and the evolution of the LPAR. A key aspect of their studies was the detailed analysis of the ability of cytokine-activated neutrophils to endocytosis and degrade the allergen Bet v 1

into peptides that could be loaded into HLA-DR molecules and expressed as a broad spectrum of epitopes recognized by Bet v 1-specific CD4⁺ T cell clones.

The dynamics of neutrophil participation in the LPAR may also extend beyond serving as antigen presenting cells. IL-4 has been shown in mice to antagonize neutrophil expansion and migration by signaling through the type 2 IL-4R to decrease responsiveness to G-CSF and downregulate expression of chemokine receptors (4). In this issue, Impellizzieri et al (5) demonstrate that engagement of the human neutrophil type 1 or type 2 IL-4R by either IL-4 or IL-13 respectively, can downregulate neutrophil CXCR1 and CXCR2 expression and thereby impairs their chemotaxis to CXCL8. Hence, in the LPAR, cytokine-activated neutrophils can serve as antigen presenting cells capable of stimulating the proliferation of influxing CD4⁺ T cells and their production of Th2 cytokines (IL-4, IL-5, IL-13), which serve as an agonists for promotion of Th2 immunity. Furthermore, the induced IL-4 and IL-13 can act in a negative feedback loop to neutrophils to antagonize their further migration, thereby limiting the local immune reaction. This model is consistent with the minor presence of neutrophils in atopic dermatitis Th2/Th22 axis lesions versus an abundance of neutrophils in psoriasis Th1/Th17 driven lesions (6). Such a negative feedback loop is also consistent with the ability of anti-IL-4 mAb to increase lung neutrophils in a dust mite sensitization model of airway inflammation (7).

An additional consideration in the role of neutrophils in the LPAR is the emerging appreciation of phenotypic diversity and plasticity among neutrophil subpopulations. Precise identification of the various functional subpopulations is not yet clearly established, but the demonstration by Polak et al (2) that less than 25% of the cytokine-stimulated neutrophils expressed HLA-DR raises the possibility of neutrophil heterogeneity within the LPAR. The concept of pro-atopic neutrophil subsets has been suggested by the association of CD49d⁺ cysteinyl leukotriene receptor 1⁺ neutrophils in acute viral respiratory infections with post-viral atopic airway disease (8). Further evidence for neutrophil heterogeneity is suggested in allergic rhinitis patients wherein the

activated neutrophils in atopic nasal tissue were predominantly CD16^{high} CD62L^{dim}, in comparison with normal nasal mucosa neutrophils that were CD16^{high} CD62L^{high}, with the activated neutrophils capable of stimulating T cells (9).

Neutrophil extracellular DNA traps (NETs), as well as eosinophil extracellular DNA traps, have been described in neutrophilic inflammation and eosinophilic inflammation, respectively, of the respiratory tract and nasal sinus tract of atopic subjects, and have been implicated in exacerbation of the inflammatory response as a mechanism of host defense against microbes (10). Herein, Impellizzieri et al (5) extended their studies to demonstrate that IL-4 or IL-13 stimulation of neutrophils decreased NETs formation. In aggregate, the recent studies by Polak (2,3) and by Impellizzieri (5) implicate the neutrophil as a dynamic player in LPAR (Figure).

There is still much to learn regarding the role of neutrophils in the initiation and promotion of immune responses for both host defense and atopy. Since the early descriptions of neutrophils as part of the cutaneous LPAR, neutrophils took a back seat to atopy mechanistic studies focused on Th2 responses. However, the recent literature is replete with evidence that the neutrophil is far more than a short-lived innate cell relegated to anti-microbial activity. Insight into the partisan role of activated neutrophils for promoting Th2 immunity, and the potential for Th2 immunity to modulate neutrophil function, may provide the mechanistic basis for therapeutic manipulation of neutrophils.

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Figure Legend.

Figure 1. Complexities of immediate hypersensitivity and late phase allergic reactions. MC =
mast cell; N = neutrophil; MO = monocyte; BAS = bas; EOS = eosinophil; T = T cell

