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Neutrophils—the unexpected helpers of B-cell activation

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The effective elimination of pathogens requires cooperation between the innate and adaptive branches of the immune system. The innate branch mediates rapid inflammatory responses after infection, whereas highly specific adaptive responses emerge within a few days. The involvement of innate cells in mediating B-cell responses has been traditionally limited to the opsonization and destruction of antigen-coated pathogens (Fig 1A). However, both basophils (Chen *et al*, 2009) and eosinophils (Chu *et al*, 2011) have recently been shown to secrete B-cell stimulatory factors—such as BAFF, APRIL and IL-6—suggesting that innate cells can also influence B-cell activation. Similarly, although neutrophils are traditionally considered to be innate immune cells, they have been shown to influence adaptive responses during infection through the regulation of dendritic cell activation via alarmins (Yang *et al*, 2009) or IL-10 (Zhang *et al*, 2009). Moreover, in response to microbial products, murine neutrophils relocalize to the white pulp of the spleen, where they can encounter resident populations of lymphocytes (Kesteman *et al*, 2008). However, whether neutrophils regulate humoral immune responses was unknown. An impressive *tour de force* led by Andrea Cerutti and published this month in *Nature Immunology*, reveals that splenic neutrophils can function as professional helper cells for marginal zone B cells, leading to the generation of affinity-matured antibodies (Puga *et al*, 2011; Fig 1B).

The study begins by analysing the distribution of neutrophils in secondary lymphoid tissue sections from individuals without inflammation or infection. Under these conditions, although neutrophils are predominantly excluded from follicles, they are relatively abundant in regions proximal to the splenic marginal zone (MZ). The fact that such a distribution is conserved in both macaques and mice suggested that neutrophils in the peri-MZ might be functionally significant during homeostasis. Furthermore, this distribution is altered in pathological spleens, such that neutrophils infiltrate the follicular mantle and germinal centres.

Interestingly, the peri-MZ localization of neutrophils not only means that they are in an ideal location to respond to blood-borne antigens, but also renders them in close proximity to MZ B cells, which are classically associated with T-cell-independent antibody responses. In view of this, Puga and colleagues went on to show that this splenic neutrophil population—unlike those in general circulation (N_c)—are able to mediate the activation of IgM secretion from MZ B cells (Fig 1B). As a result, these cells were named B-helper neutrophils (N_{BH}), and a detailed characterization of this population revealed the potential molecular mechanism underlying their capacity to mediate MZ B-cell activation. N_{BH} have a higher expression of B-cell-stimulating molecules—such as BAFF, APRIL, IL-21 and CD40L—than do N_c cells. In line with this, N_{BH}-cell-conditioned medium can activate MZ B cells, an effect that is abrogated when signalling through these receptors is blocked. However, as the extent of antibody secretion is greater after incubation with the N_{BH} cells, contact-dependent mechanisms seem to also participate in MZ B-cell activation. Intriguingly, unlike N_c cells, the N_{BH} population spontaneously forms DNA-containing neutrophil extracellular trap (NET)-like projections. Although similar structures have recently been associated with the ability to trigger Toll-like receptor 9 (TLR9)-mediated activation of dendritic cells and B cells in systemic lupus erythematosus (SLE; Lande *et al*, 2011), it is not clear whether NETs are involved in N_{BH}-mediated MZ B-cell activation. In particular, it will be interesting to investigate the role of NETs as a potential source of immune complexes containing TLR9 ligands, which might facilitate B-cell activation (Leadbetter *et al*, 2002). Regardless, the identification of a population of neutrophils able to function as professional helper cells for MZ B cells uncovers an exciting new avenue for communication between the innate and adaptive immune networks.

But what is the consequence of N_{BH}-mediated assistance on the MZ B-cell population? Follicular B-cell activation in response to T-cell-dependent antigen has been relatively well characterized and is often accompanied by the formation of germinal centres (MacLennan, 1994). Germinal centres have been traditionally associated with the diversification of the Ig genes through somatic hypermutation and subsequent selection of high-affinity clones, as well as the generation of immunological memory. However, although it has been reported that $CD11c^{lo}$ dendritic cells promote the formation of plasmablasts from MZ B cells during systemic infection (Balázs *et al*, 2002), much less is understood about the impact of accessory cell help on the induction of T-cell-independent responses. Puga and colleagues showed that N_{BH} cells trigger the expression of the Blimp 1 and XBP1 transcription factors and the surface marker CD38 in MZ B cells, which is indicative of plasmablast formation. Furthermore, in line with the upregulation of AID expression in MZ B cells in close proximity to N_{BH} cells, the secreted antibodies were shown to have undergone class switch, favouring the generation of IgG2 and IgA. Importantly, in spite of normal levels of class-switched antibodies to T-cell-dependent antigens, patients with severe congenital neutropenia have

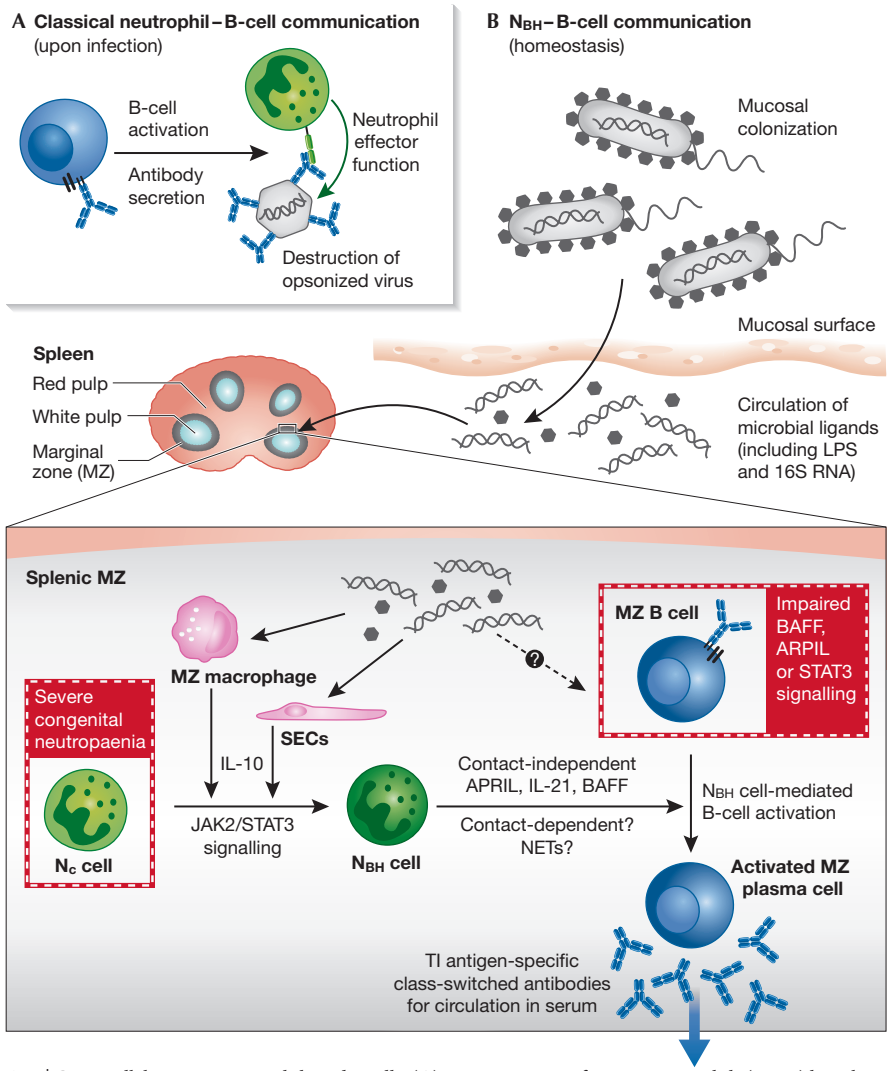

Fig 1 | Cross-talk between neutrophils and B cells. (**A**) In response to infection, neutrophils (green) have been traditionally thought to opsonize pathogens that are coated with antibodies secreted by B cells (blue). (**B**) The newly identified B-helper neutrophil population (N_{BH}, dark green) in the splenic marginal zone (MZ, grey) can activate MZ B cells (dark blue) to secrete antibodies against TI antigens. This probably occurs through the secretion of APRIL, BAFF and IL-21 in a contact-independent mechanism, although contact-dependent and/or neutrophil extracellular traps (NETs) might also play a role. Secreted antibodies are often class-switched and might enter the general circulation to provide basal innate immunity against microbial pathogens. N_{BH} cells probably arise from circulatory neutrophils (N_c) as a result of JAK2 and STAT3 signalling, in response to IL-10 secretion by sinusoidal endothelial cells (SECs) and/or macrophages. This might be triggered by microbial ligands present in the general circulation that are translocated across mucosal surfaces after bacterial colonization. Patients with severe congenital neutropenia have reduced levels of antibodies against TI antigen, and patients with altered signalling in response to BAFF, APRIL and IL-21 have impaired MZ B-cell development (both highlighted in red boxes). LPS, lipopolysaccharide; TI, T-cell-independent.

decreased levels of IgA and IgG to microbial T-cell-independent antigens such as lipopolysaccharide. Interestingly, sequencing the antibodies secreted by N_{BH}-activated MZ B cells also indicated that, at least in humans, they accumulate mutations as observed during somatic hypermutation. Thus, surprisingly, N_{BH} cell assistance seems to trigger the diversification of antibodies from the MZ B-cell population, similarly to the influence of $CD4^+$ T cells on follicular B cells.

The ability of N_{BH} cells to mediate the secretion of class-switched antibodies from MZ B cells raises questions as to the origin of this population. When N_C cells are exposed to IL-10, they upregulate the expression of mRNA encoding BAFF and APRIL, and become inducible N_{BH}-like cells. The generation of this inducible population requires signalling through JAK2 and STAT3. N_{BH} in the splenic MZ are in close proximity to sinusoidal endothelial cells, which secrete IL-10 and various neutrophil-attracting chemokines in response to microbial ligands. On this basis, Puga and colleagues postulate that microbial ligands—which might enter general circulation after systemic translocation across mucosal surfaces (Clarke *et al*, 2010)—trigger both reprogramming and chemotactic signals to N_c cells, resulting in the formation of N_{BH} cells. In line with this concept, the splenic N_{BH} population is established early in fetal life, but is greatly enhanced two days after birth, coincident with mucosal colonization by bacteria. Moreover, mice that are either germ-free or unable to mediate TLR signalling, have fewer N_{BH} cells. In the light of these observations, N_{BH} cells are suggested to stimulate the generation of class-switched antibodies to T-cell-independent antigens from MZ B cells in the steady state, providing individuals with an innate layer of antimicrobial antibody defence.

Several intriguing questions are raised by this study that will remain the challenge of future work. Such issues include the identification of the source of the initial signal that triggers the generation of the N_{BH} cell population and uncovering the mechanism(s) by which N_{BH}-mediated MZ B-cell activation is regulated. Nonetheless, this exciting study not only defines new communications between branches of the immune system, but also opens potential therapeutic avenues involving the manipulation of neutrophil populations to enhance basal immunity.

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