MHC class I is complicated by the existence of two types of proteasomes. Time will tell whether constitutive expression of immunoproteasomes has evolved as a solution toward stronger CTL responses to infection and cancer.

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A helping hand from neutrophils in T cell–independent antibody responses?

Stuart G Tangye & Robert Brink

Neutrophils and B cells are thought to specialize in driving innate and adaptive immune responses to infection. New data suggest that these cells may also communicate in the splenic marginal zone to drive T cell–independent antibody responses.

Neutrophils, together with eosinophils and basophils, constitute the granulocyte family of white blood cells. Neutrophils are the most abundant of the granulocytes and are in fact the most plentiful of all white blood cells in the body[1]. Although neutrophils reside mainly in the bloodstream, they are rapidly mobilized to areas of infection in response to migratory cues as a first line of immune defense. Their main role is to rapidly neutralize microorganisms at sites of infection. This is achieved by the release of antimicrobial molecules as well as the ingestion of antibody and/or complement-coated microbes and killing of the microbes with intracellular toxins such as myeloperoxidase-generated free radicals[1]. However, neutrophils can also produce molecules such as BAFF[2], which has no known direct antimicrobial activity but instead sustains the survival and responsiveness of B lymphocytes (B cells)[3]. In this issue of *Nature Immunology*, Puga and colleagues investigate the potential of neutrophils to deliver signals to B cells[4]. They suggest that the function of at least a subpopulation of neutrophils may include a role in driving the production of antibodies secreted by marginal zone (MZ) B cells.

Unlike neutrophils, B cells are guided fundamentally by the interaction of their clonally defined antigen receptors with foreign and self antigen[5]. After interaction of the B cell antigen receptor with foreign antigen, B cells become activated and then differentiate into antibody-secreting plasma cells. The antibody responses to most foreign antigens require that B cells directly act together with and receive help from $CD4^+$ T cells. As a result of this, T cell–dependent antigens induce B cells to differentiate into long-lived memory and plasma cells that provide long-term serological protection against infectious pathogens, as well as after vaccination[6]. Interestingly, long-lived plasma cell are maintained by signals provided by a diverse array of cell types that localize together in plasma-cell survival niches mainly in the bone marrow[3]. In contrast to T cell–dependent responses, which require that B cells directly act together with T cells[5], T cell–independent responses occur by the provision of second signals to B cells through receptors for a variety of ligands, including complement fragments (such as C3b), microbial products (such as CpG, double-stranded RNA and flagellin) and members of the tumor necrosis factor superfamily (such as BAFF and APRIL). T cell–independent antigens are usually unable to induce immunological memory. Although they are independent of T cells, these responses often require input from other cell types. Indeed, B cell responses to T cell–independent antigens have been found to be induced or enhanced by signals provided by monocytes, macrophages, dendritic cells and natural killer cells[7,8].

MZ B cells are found mainly in the spleen. There they show distinct sublocalization, being distributed around the edge of the predominant follicular B cell population[9] (**Fig. 1**). A chief function of MZ B cells seems to be in the rapid initiation of mainly T cell–independent antibody responses to blood-borne antigens such as bacteria. This is due to an intrinsic semi-activated state that allows them to respond to stimuli more quickly than do follicular B cells and to their localization adjacent to the marginal sinus of the spleen, where they have ready access to particles carried in the blood[9]. In humans, the follicle is composed mainly of naive B cells, whereas memory B cells—including those that express immunoglobulin M (IgM) and IgD or switched isotypes (IgG and IgA)—localize in the MZ[10]. A key feature of memory B cells is their ability to rapidly respond to subsequent exposure to their specific antigen; thus, their localization to the splenic MZ provides them with the spatial and geographical opportunity to also encounter cognate antigens that drain into the spleen via the blood[6].

Puga *et al.* begin their investigation by examining spleens from humans, monkeys and mice[4]. They observe that in each case, cells expressing the neutrophil markers myeloperoxidase and elastase are present in large numbers adjacent to the MZ area of the spleen (**Fig. 1**). In contrast, neutrophils are rare in the tonsils, lymph nodes and other lymphoid organs that lack an MZ[4]. Although the colocalization of neutrophils with MZ B cells in the spleen is provocative, further evidence is needed to establish whether this phenomenon is anything more than coincidental. To determine whether there is a potential functional connection between these two cell types, Puga *et al.* take the reductionist approach of culturing MZ B cells and splenic neutrophils together and observing the effect on B cell function. Intriguingly, coculture with splenic neutrophils results in MZ B cells' secreting IgM antibody and undergoing class-switch recombination to produce IgG and IgA antibodies, as well as somatic hypermutation. These properties are not shared by blood-derived

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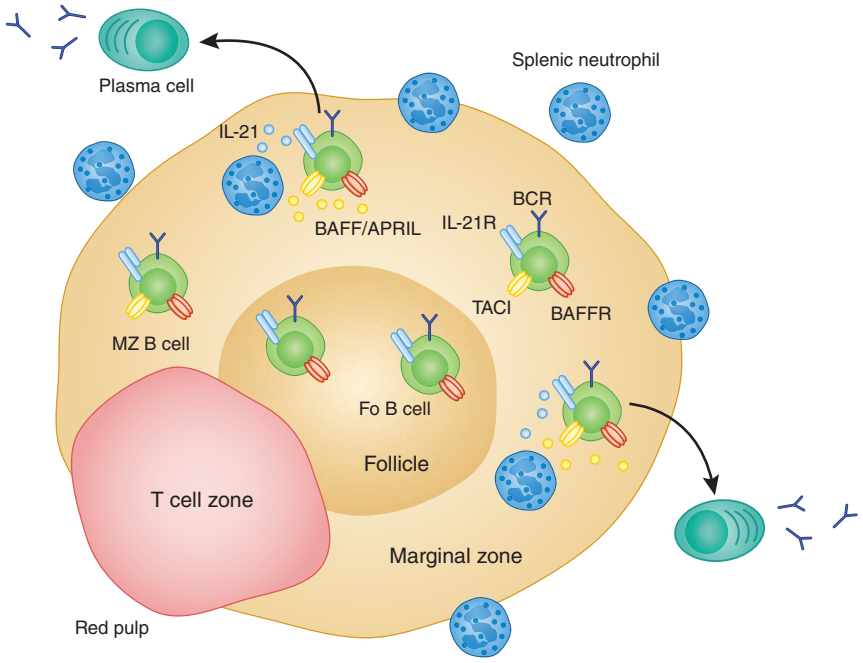

Figure 1 Proposed model for the mechanisms underlying neutrophil-mediated, T cell–independent B cell differentiation. Neutrophils surround the MZ of the spleen. After infection, neutrophils rapidly migrate into the MZ. In humans, the MZ is composed mainly of memory cells. Interactions between neutrophils and MZ B cells results in the activation and differentiation of MZ-resident B cells into plasma cells able to secrete IgM, IgG and IgA. The stimulatory effects of neutrophils result from production of BAFF, APRIL and IL-21; BAFF and APRIL bind the transmembrane activator TACI, which is constitutively expressed by all human memory B cells. Although granulocyte colony-stimulating factor (G-CSF) induces BAFF in neutrophils[2], it is unclear which *in vivo* cues induce the production of APRIL and IL-21 by neutrophils.

neutrophils, and the effects on splenic follicular B cells are minor, which suggests that splenic neutrophils are specialized in helping MZ B cells to differentiate into antibody-secreting plasma cells (**Fig. 1**).

Splenic neutrophils are found to be phenotypically distinct from those in the blood. Interestingly, the authors identify two distinct populations of neutrophils in the spleen, which they call 'N_{BH1}' and 'N_{BH2}' cells. N_{BH1} neutrophils seem to be the more activated subset, as shown by their higher expression of CD40L, CD86 and CD95 and of transcripts encoding molecules involved in B cell activation, such as *TNFSF13A* (BAFF), *TNFSF13* (APRIL), *IL6* (interleukin 6 (IL-6)) and *IL21* (IL-21)[4]. Despite higher expression of these mediators by N_{BH1} neutrophils, it is the N_{BH2} subset that induces enhanced responses in human MZ B cells in terms of expression of activation-induced cytidine deaminase, required for class switching, and antibody secretion. The ability of splenic neutrophils to promote the differentiation of MZ B cells into IgM-secreting cell is abolished by the inclusion of antagonists of BAFF, APRIL and IL-21 in the cocultures, which suggests that these stimuli might trigger the same process *in vivo* (**Fig. 1**). Notably, these same reagents result in only somewhat less secretion of IgG and IgA (50–70%) by MZ B cells cultured together with neutrophils[4], which suggests that factors other than BAFF, APRIL and IL-21, such as CD40L, might contribute to neutrophil-induced class switching. BAFF and IL-21 have been shown to induce antibody secretion by human splenic MZ B cells[11]. Although it has been proposed that BAFF and IL-21 would be derived from dendritic cells and helper T cells *in vivo*[11], the present findings indicate that neutrophils can also provide these factors[4].

The importance of the *in vitro* effects of splenic neutrophils on human MZ B cell responses are extrapolated to an *in vivo* setting by study of specific cohorts of immunodeficient patients. These people were selected on the basis of their neutropenia; curiously, they are also found to have fewer circulating MZ-like (IgD^+CD27^+) B cells and lower titers of antibodies to T cell–independent antigens. This provides correlative data that neutrophil deficiency *in vivo* compromises B cell differentiation, which may manifest as impaired immunity to T cell–independent antigens (such as encapsulated bacteria) secondary to the impaired generation of MZ-like B cells.

Although several tantalizing results are presented by Puga *et al.*[4], many questions remain unanswered. Although BAFF, APRIL and IL-21 seem to mediate the B cell–helper function of splenic neutrophils, the mechanism underlying the greater potency of N_{BH2} neutrophils remains unclear. Although the authors attribute this to more production of BAFF, APRIL and IL-21 protein by N_{BH2} neutrophils than by N_{BH1} neutrophils, possibly as a result of the differentiation of N_{BH1} cells into functional N_{BH2} cells, both populations seem to produce similar amounts of BAFF and APRIL, and the preferential production of IL-21 by these subsets differs according to the assay used (for example, microarray and quantitative PCR versus enzyme-linked immunosorbent assay). Although the findings obtained by the study of immunodeficient patients potentially support the case for the importance of neutrophils in promoting T cell–independent responses, impaired humoral immunity in some of these conditions could result from primary B cell defects rather than being secondary to neutropenia or neutrophil dysfunction. An example of this is Wiskott-Aldrich syndrome, in which the impaired generation of MZ B cells and immune responses to T cell–independent antigens are B cell intrinsic[12]. This point highlights one of the most important challenges arising from this study[4]: how to establish that the ability of splenic neutrophils to drive MZ B cell activation and differentiation *in vitro* reflects a true *in vivo* function. Understandably, it would be extremely difficult to provide a definitive answer to this for humans. However, the multifunctional nature of neutrophils also makes this an important challenge even in mouse models that can be manipulated more easily. One possibility may be to induce specific genetic modifications in neutrophils so they lack the ability to help B cells (for example, deficiency in BAFF or IL-21) but retain their more conventional innate immune functions. It is also plausible that the interaction between neutrophils and MZ B cells *in vivo* may differ quantitatively or qualitatively from the interaction that is apparent under the *in vitro* culture conditions used by Puga *et al.*[4]. For example, weaker stimulation of MZ B cells by neutrophils than by T cells may contribute to the semi-activated state of MZ B cells without necessarily driving antibody production *per se*. Despite such uncertainties, the results of Puga *et al.*[4] provide a platform for further investigation of the increasingly common examples of interactions between the innate and adaptive immune systems.

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A new therapeutic strategy for malaria: targeting T cell exhaustion

Gordon J Freeman & Arlene H Sharpe

Boosting immune responses during malaria remains a challenge. Overcoming T cell exhaustion by blocking coinhibitory receptors offers a promising lead.

Malaria is an increasing global problem, and resistance to the present antimalarial drugs has necessitated new therapeutic approaches[1]. Understanding host responses to the *Plasmodium* species that cause malaria is critical for the rational design of effective antimalarial therapies and vaccines. The malaria parasite has evolved many mechanisms with which to evade immune responses, including a period of obligate intracellular growth in immune-privileged liver cells, plasmodial antigen switching among the 60 variations of the *Plasmodium falciparum* erythrocyte membrane protein PfEMP1, and inhibition of immune responses. The erythrocytic (blood) stage of malaria accounts for most of the immunopathology and mortality of malaria. Human and rodent studies have shown that $CD4^+$ T cells and antibodies are important for protective immunity to blood-stage malaria[1]. In this issue of *Nature Immunology*, Butler *et al.* examine immune responses during malaria infection and find that *Plasmodium*-specific T cells show features of T cell exhaustion[2].

Exhausted T cells develop in the setting of persistent antigen exposure, which drives a program of gene expression distinct from that of naive, memory or activated T cells and causes pathogen-specific T cells to lose functional activity[3,4]. First described in $CD8^+$ T cells in the mouse model of chronic infection with lymphocytic choriomeningitis virus, T cell exhaustion is now recognized as a general characteristic of chronic viral infections, including infection with human immunodeficiency virus, hepatitis C virus or hepatitis B virus. Exhausted T cells can express many coinhibitory receptors[5] (**Fig. 1**). PD-1 (CD279) is the best-characterized coinhibitory receptor expressed during chronic infection; it is a mediator of immune dysfunction and disease progression[6,7]. PD-1 has two ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-DC or CD273). PD-L1 expression is upregulated by interferons and is broadly induced on hematopoietic and nonhematopoietic cells. Signaling through PD-1 attenuates T cell antigen receptor signals and inhibits the cytokine production and cytolytic function of T cells. Blockade of PD-1 or PD-L1 during chronic viral infection can restore T cell function and diminish the viral load. The identification of exhausted T cells in malaria provides novel mechanistic insights and suggests a new therapeutic approach for malaria.

Using *Plasmodium yoelii*, which causes a relatively acute infection that can be detected in the blood for about 5 weeks but is eventually cleared, Butler *et al.* study *Plasmodium*-specific antibody and T cell responses[2]. Butler *et al.* show that $CD4^+$ T cells and antibody responses are critical for a successful antimalaria immune response, but $CD8^+$ T cells are not. Because tetramers of major histocompatibility complex (MHC) are not available for plasmodial antigens, the authors confirm the utility of surrogate markers ($CD49^{hi}CD11a^{hi}$ T cells) and use these to examine activated *Plasmodium*-specific T cells. *Plasmodium*-specific T cells show functional T cell exhaustion with less production of cytokines in response to mitogens. Although exhaustion was initially described in $CD8^+$ T cells, subsequent work has shown that $CD4^+$ T cells also can become exhausted. Exhausted *Plasmodium*-specific $CD4^+$ T cells have high expression of the coinhibitory receptors PD-1 and LAG-3 (CD223)[8] but not 2B4 or CD160 (**Fig. 1**). LAG-3 binds to MHC class II proteins with a higher affinity than does CD4. Expressed on activated T cells, regulatory T cells, plasmacytoid dendritic cells and some natural killer cells, LAG-3 negatively regulates T cell activation and proliferation. In contrast, exhausted *Plasmodium*-specific $CD8^+$ T cells have high expression of PD-1, LAG-3, 2B4 and CD160. A report examining human immunodeficiency virus–specific exhausted T cells has found similar differences between exhausted $CD4^+$ and $CD8^+$ T cells in coinhibitory receptor expression[9].

As exhausted *Plasmodium*-specific $CD4^+$ T cells have abundant expression of PD-1 and LAG-3, and $CD4^+$ T cells are important for an effective anti-*Plasmodium* response, Butler *et al.* examine whether blockade of LAG-3 and PD-L1 (the main ligand for PD-1), alone or together, enhances antimalarial immunity[2]. They quantify *P. yoelii* parasites in blood and find that combined blockade of PD-L1 and LAG-3 leads to an immediate halt to the increase in blood parasites and accelerates parasite clearance. Blockade of PD-L1 alone is moderately effective, but blockade of LAG-3 alone has little effect. Dual blockade leads to more *Plasmodium*-specific $CD4^+$ T cells and $CD8^+$ T cells, which produce more cytokines and enhance parasite control during blood-stage infection in both inbred and outbred mice. Dual blockade also leads to much more protective antibody, as demonstrated by experiments showing that transfer of serum from treated mice accelerates parasite clearance in naive recipients. *Plasmodium*-specific MSP1 immunoglobulin G titers are 2.5-fold higher. Consistent with that enhanced antibody response, the number of follicular helper T cells is increased by sevenfold, and

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