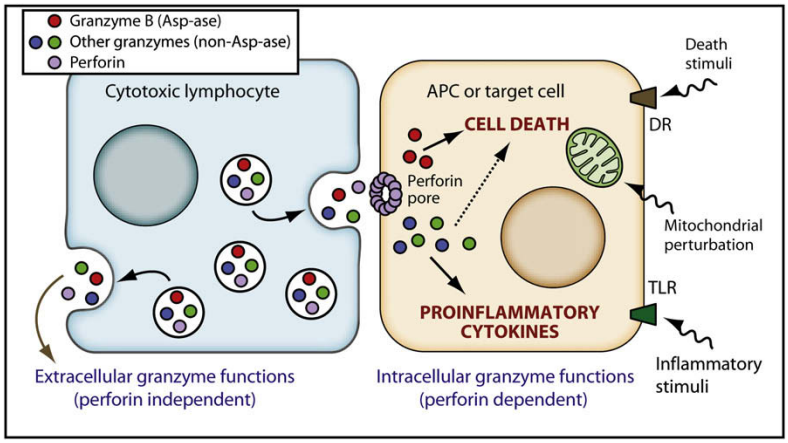


Figure 1. Granzymes: Extrinsic Activators of Cell Death and Inflammation that Mimic Intrinsic Caspase Pathways

Granzyme B, which like the proapoptotic caspases cleaves after specific Asp residues, is transferred from a cytotoxic lymphocyte into the cytoplasm of a target or antigen-presenting cell to impose death in response to virus infection or malignant transformation. Granzyme B (red dots) mediates this process in a perforin-dependent manner, as perforin (purple dots) provides access to its cytoplasmic substrates. The principal function of other (non-Asp-ase) granzymes (green and blue dots) within the target cell may be to mimic the proinflammatory caspases that are critical for inflammasome formation and release of cytokines such as IL-1β, IL-6, and TNF. It is presumed that this function of granzymes is also perforin dependent but this remains to be formally demonstrated. Other intrinsic cell-death and proinflammatory pathways can be activated by stimuli other than the granzymes, for example inflammatory stimuli operating through Toll-like receptors (TLR), cell death through ligation of death receptors (DR), or mitochondrial perturbation. In some situations, it might also be possible for granzymes other than B to activate target cell death, but far less efficiently than granzyme B. Other granzyme functions (cell detachment, chemotaxis, lymphocyte migration through the extracellular matrix) occur in the extracellular space and are independent of perforin.

nonapoptotic roles of granzymes, in accord with the prescient predictions made by Kramer and Simon (1987) more than twenty years ago.

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An Innate Path to Human B Cell Tolerance

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Self-reactive B cells are eliminated during development by antibody-affinity selection and receptor-editing mechanisms. Work by Isnardi et al. (2008) in this issue of *Immunity* suggests that removal of autoreactivity from the immature B cell pool also requires innate immunity pathways.

The random nature of antibody diversification processes guarantees that a large number of newly generated antibodies will recognize self-antigens. These potentially harmful antibodies with self-specificities are eliminated in large numbers at various steps of B cell development so that the mature B cell repertoire of a healthy individual is largely devoid of self-reactive antibodies. Knowledge of this process has greatly advanced ever since the Nussenzweig group developed a combination of single-cell polymerase chain reaction (PCR) and antibody-cloning techniques to investigate single B cell specificities in humans (Wardemann and Nussenzweig, 2007). This technique is now utilized by Isnardi et al. to characterize alterations in the naive B cell repertoire of patients with deficiencies in innate immune pathways (Isnardi et al., 2008).

The analysis of B cells from healthy individuals detects several checkpoints against autoreactive B cells in bone

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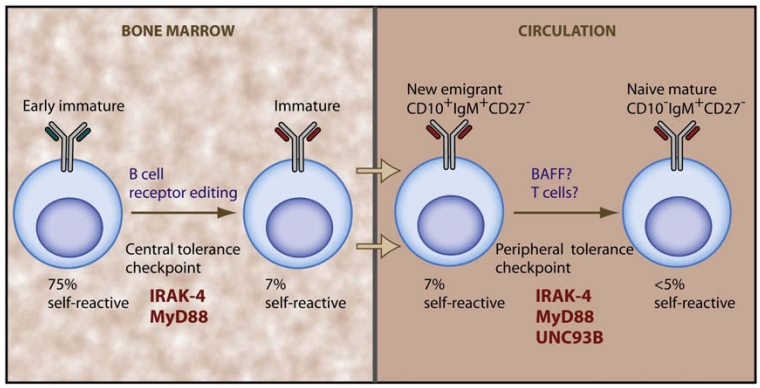


Figure 1. Alterations in Both Central and Peripheral B Cell Tolerance Checkpoints in Humans with Deficiencies in Innate Immunity

Healthy controls generate highly autoreactive repertoires in the early immature stage. A central-tolerance checkpoint removes a large proportion of autoreactive B cells in the bone marrow, primarily through receptor editing. Immature B cells exit from the bone marrow as new emigrant B cells, normally with a low degree (7%) of autoreactivity. A second tolerance checkpoint occurs during B cell maturation, although its molecular mechanism is largely unknown and might depend on BAFF or T cell help. Work by Isnardi et al. (2008) now shows that deficiencies in IRAK-4 and MyD88 in humans result in alterations of the central- and peripheral-tolerance checkpoints and a defect in B cell receptor editing. In contrast, UNC93B mutations in humans change the peripheral-tolerance checkpoint, but not the central-tolerance checkpoint.

marrow and peripheral blood of healthy humans (Wardemann and Nussenzweig, 2007). Three quarters of the newly formed B cells are estimated to be self-reactive, and a large proportion of those autoantibodies recognize multiple specificities. The transition from early immature to immature B cells in the bone marrow is considered to be a major checkpoint that eliminates a large part of the originally self-reactive antibodies (Figure 1). The most likely molecular event underlying this checkpoint of B cell central tolerance is receptor editing, a mechanism in which random Ig light-chain replacement silences self-reactive antibodies. Once immature B cells are released from the bone marrow, they are detected in the periphery as new emigrant B cells ($CD10^+IgM^+CD27^-$) that still harbor a substantial number of self-reactive clones (approximately 7%). A second tolerance checkpoint occurs in their transition into the mature naive B cell ($CD10^-IgM^+CD27^-$) compartment. At this point, less than 5% of the peripheral naive compartment consists of autoreactive B cells. Overall, failures in any of these two major B cell tolerance checkpoints would lead to higher numbers of mature autoreactive B cells and would predispose to the development of autoimmunity. This hypothesis has been confirmed by the analysis of B cells from patients with autoimmune disease in which autoantibodies have a prominent role: The naive mature B cell compartments of both systemic lupus erythematosus (SLE) and rheumatoid arthritis patients contain a large number of autoreactive B cells irrespective of their disease treatment course.

Antibody-affinity selection seems to underlie the removal of autoreactive B cells from the repertoire in the first tolerance checkpoint. First, antibodies with very high affinity for self are removed by clonal deletion in early development. Next, antigen-receptor editing by light-chain replacement occurs in many of the remaining self-reactive B cells and depends largely on the strength of B cell receptor (BCR) signaling. This idea has been cited as a means of explaining the fact that X-linked agammaglobulinemia (XLA) patients with defective BCR signals exhibit signs of extensive secondary Ig light-chain recombination and are frequently self-reactive (Ng et al., 2004). Following this line of thinking, any other alteration in the strength of the BCR would be expected to greatly modify B cell central-tolerance outcomes. In fact, lower responsiveness to antigen-receptor stimulation is now invoked as a possible explanation for the augmented autoreactivity in systemic autoimmune diseases like SLE (Grimaldi et al., 2007).

The second checkpoint for self-tolerance occurring in the periphery at the B cell maturation stage is less mechanistically defined and could be affected by signals other than the BCR. For example, changes in sensitivity to the B cell-activating factor BAFF or overall amounts of BAFF in the periphery have been proposed to affect this selection process. Additionally, analysis of patients with defects in CD40L or major histocompatibility complex class II (MHC-II) supports a tolerogenic effect of T cell help in this peripheral-tolerance checkpoint: CD40L- and MHC-II-deficient patients express a higher proportion of autoreactive antibodies in their naive mature B cell compartments, whereas their central tolerance seems unaltered (Herve et al., 2007). How selection of the naive B cell compartment is regulated by T cell help is at the moment unclear. It nevertheless highlights the fact that B cell tolerance might be regulated by additional pathways yet untested.

Current work by Isnardi et al. expands our view of genetic factors that alter the human B cell repertoire by analyzing the effect of deficiencies in innate immune pathways. Specifically, B cells from patients with complete defects in the genes encoding interleukin-1 receptor-associated kinase 4 (IRAK-4), myeloid differentiation factor 88 (MyD88), and UNC-93B were evaluated for autoreactive specificities (Isnardi et al., 2008). In humans, mutations in the genes encoding MyD88 and IRAK-4 predispose to recurrent bacterial infections, but they appear to be less critical in the prevention of viral infections. In contrast, mutations in the *UNC93B* gene predispose to herpes simplex encephalitis (Zhang et al., 2007). These defects are generally consistent with the role that these three molecules play in transmitting signals initiated by interleukin-1 (IL-1) receptors and Toll-like receptors (TLRs), all important pathways in the fight against pathogens. IRAK-4 and MyD88 associate and mediate signaling of all known TLRs except TLR3. Both molecules also associate with the IL-1 family of receptors. Meanwhile, UNC93B's function is to deliver TLRs from the endoplasmic reticulum to endolysosomes and is essential for signaling of TLR3, TLR7, and TLR9, all three TLRs with intracellular localization (Kim et al., 2008; Tabeta et al., 2006).

The analysis of B cells from patients with defects in the IRAK-4, MyD88, and

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UNC93B pathways reported by Isnardi et al. (2008) provides some intriguing results that support an expanded role of innate receptors in shaping the antibody repertoire at early stages of B cell development. When tested for B cell specificities by the single B cell antibody-cloning technique, IRAK-4-deficient patients showed the most unambiguous differences in B cell repertoire compared to healthy controls: Half of the new emigrant immature B cells in IRAK-4-deficient individuals were still self-reactive against multiple antigens, and most of them were still autoreactive at the mature naive stage, compared to less than 5% autoreactive B cells present in healthy controls. Moreover, a large proportion of those autoantibodies present in IRAK-4-deficient patients were reactive against double-stranded DNA or other nuclear antigens. The single MyD88-deficient patient analyzed showed a similar trend as in the IRAK-4 case, albeit of lower magnitude. On the other hand, mutations in the *UNC93B* gene did not change the proportion of autoreactive immature B cells but did slightly increase the number of autoreactive B cells in the periphery (Isnardi et al., 2008). These results imply that IRAK-4 and MyD88 are essential for the establishment of both the central and peripheral checkpoints for B cell tolerance, whereas UNC93B would only be strictly required for the peripheral-tolerance checkpoint. These divergent results can be interpreted in various ways. They could mean that IRAK-4 and MyD88 deficiencies affect early B cell populations through pathways that do not depend on intracellular TLRs. However, human B cells seemed to be mostly devoid of the common TLRs with extracellular expression, and they also lack receptors for IL-1. Another possibility is that UNC93B, which has low expression in human B cells, could be somewhat redundant in its function. Consistent with this view, UNC93B deficiency in humans is not completely penetrant (Zhang et al., 2007). Further analysis of more of these rare patients with innate immunity deficiencies and, more specifically, those with mutations on the TLR receptors themselves will be helpful in clarifying these questions.

In terms of the molecular events underlying IRAK-4- and MyD88-deficient patients' defect in central B cell tolerance, sequence analysis of autoantibodies present in these patients showed that they lack most signs of receptor editing that are observed in autoreactive antibodies of healthy individuals. This is in clear contrast with what was observed in cases of hyporesponsive BCR, such as in XLA patients, in which extensive recombination events were observed in self-reactive antibodies. Thus, it can be argued that the main effect of the IRAK-4 and MyD88 deficiencies in B cell tolerance could be unrelated to the antibody-affinity selection process.

The augmented autoreactivity of the naive B cell repertoire in IRAK and MyD88 deficiencies in humans seems counterintuitive, considering the current understanding of the role of TLRs in autoimmune responses, which is primarily based on in vitro and mouse work. The general view is that TLRs play a key role in the expansion of autoreactive B cells because certain TLRs can synergize with nucleic-acid-binding BCRs to stimulate B cell proliferation and differentiation (Marshak-Rothstein and Rifkin, 2007). Two intracellularly located TLRs have been most prominently implicated in promoting autoreactivity: the single-stranded RNA-binding TLR7 and the CpG DNA-binding TLR9. Both TLR7 and TLR9 require IRAK-4, MyD88, and UNC93B for the induction of proper cell activation in humans and in mice. Our lesson from the analysis of human B cells is that TLRs may not just regulate B cell activation in the periphery, but they may also shape the naive B cell repertoire. Of course, this effect of TLRs on immature B cell populations could be extrinsic to the B cells and just a consequence of an altered environment in the bone marrow. This possibility cannot be easily assessed in the human system, but it could be easily tested in MyD88- and IRAK-4-deficient mice.

Would the IRAK-4 and MyD88 deficiencies predispose to autoimmune disease, considering the large number of autoreactive B cells in the naive compartment? The answer seems to be no, because no autoantibodies were detected in the serum of these patients. Thus, even though autoreactive B cells persisted in the repertoire, these cells were not activated further in the periphery because of a lack of TLR activation signals. TLR inhibitors are now proposed as therapeutic treatment of autoimmune disease. This analysis of IRAK-4- and MyD88-deficient patients suggests that this kind of treatment not only will inhibit B cell activation in the periphery, but that it can also potentially alter the antibody repertoire as a whole.

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