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Published in:
IMMUNOL TODAY

DOI:
10.1016/S0167-5699(97)01160-2

Link to publication

Citation for published version (APA):

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Triple check for antigen specificity of B cells during germinal centre reactions

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Germinal centres (GCs) are specialized microenvironments in peripheral lymphoid organs where B cells undergo affinity maturation and immunoglobulin (Ig) isotype switching (Fig. 1). This leads to the formation of memory B cells and plasma cells. GC development occurs within a few days after the start of a T-cell-dependent B-cell response and involves the coordinated action of several different cell types: B cells, T cells, antigen (Ag)-presenting cells (APCs), follicular dendritic cells (FDCs) and macrophages (reviewed in Ref. 1).

During the GC reaction, the generation of memory B cells that have increased affinity of their B-cell Ag receptors (BCRs) is achieved by a process that includes: (1) generation of BCR diversity via B-cell proliferation and somatic hypermutation of the Ig variable (V) gene; and (2) selection of the 'best-fitting' (i.e. highest-affinity) B cells. However, implicit in this selection process is the danger that unselected auto-reactive B-cell clones might be generated. This article will propose that GC reactions include three different checkpoints to ensure appropriate Ag specificity of memory B cells (Fig. 1, insert).

Checkpoints for Ag specificity

Checkpoint 1: Ag-specific initiation of the GC reaction

The first step of Ag-specific B-cell activation takes place outside the lymphoid follicle (Fig. 1), when they bind native Ags and receive accessory signals provided by T cells activated by APCs. These signals critically involve CD40 binding to CD40 ligand (CDWl) and CD28-CD86 interactions; indeed, experiments in mice have shown that blocking of either pathway by anti-CDWl, soluble CD40 or anti-CD86) completely inhibits GC reactions and/or the formation of memory B cells2,3. CD28 involvement at the initiation of GC reactions was recently illustrated in CD28-knockout mice, which failed to develop detectable GCs (Ref. 3). The importance of CD40 ligation at this checkpoint was demonstrated by the finding that patients with X-linked hyper-IgM syndrome (due to mutated and consequently ineffective CD40L) do not develop GCs (Ref. 6). Although ligation of CD40 is important for B cells to become activated, T cells may also depend on the CD40-CD40L interaction, since it has been shown recently that inhibition of this interaction results in the inability of T cells to support further B-cell differentiation4.

Once activated, B cells may either differentiate into antibody-forming plasma cells or localize into primary follicles to undergo affinity maturation. These GC immigrants differentiate into centroblasts that start to proliferate at a very high rate (with a cell cycle time in the order of 7 h). During this proliferation phase, random somatic mutation occurs in the Ig heavy- and light-chain V regions. At an estimated mutation rate of one mutation per 1000 base pairs per generation, nucleotide exchange in the V regions of the Ig gene will occur in nearly every cell division. Consequently, the GC reaction will lead to the generation of clones expressing highly diverse BCRs.

Recruitment of these B-cell clones into the subsequent steps of the maturation process includes selection based on the affinity of their BCRs for the original Ag. For successful selection, it is of crucial importance that GC B cells that have finished their clonal expansion and somatic hypermutation become sensitive to apoptosis5,6. This prepares the cells for affinity selection at the second checkpoint.

Checkpoint 2: selection of the best-fitting BCR by FDCs

FDCs sequester Ags in the form of immune complexes via their complement receptors. Immune complexes formed by antibodies, complement and circulating antigens will be available a few days after initiation of the GC reaction7, but supposedly well before the moment that mutated centroblasts differentiate into centrocytes and become sensitive to apoptosis8. It is generally assumed that the BCR on centrocytes will compete for binding to the Ag present in immune complexes on the FDC surface (Fig. 2). It is conceivable that this binding can only be successful if the affinity of the BCR exceeds that of the antibody present in the immune complex. Of the hypermutated B cells, only those with the highest affinity BCRs will succeed in binding Ag on FDCs and thus escape programmed cell death5,6,9,10 (Fig. 2). Low-affinity B cells with unligated BCRs will die by apoptosis.

FDC-mediated inhibition of apoptosis in GC B cells is a rapid process, occurring within 4 h as the apoptotic machinery (endonuclease activity) in the B cell nucleus is blocked by the FDC (Ref. 14). More recent data suggest that as little as 1-2 h is sufficient. The mechanisms by which the FDC mediates the rapid and irreversible inhibition of apoptosis have not yet been fully elucidated, but signalling via several different routes appears to be involved. It is
possible that interaction of the BCR with immune-complexed Ag on the HX, immediately followed by tight adhesion via lymphocyte function-associated molecule 1 (LFA-1) bound to intercellular adhesion molecule 1 (ICAM-1), and very late antigen 4 (VLA-4) bound to vascular cell adhesion molecule 1 (VCAM-1), initiates the interaction between centrocytes and FDCs. It was previously shown that adhesion molecules can be activated within a few minutes after BCR triggering. Certainly, LFA-1-ICAM-1 and VLA-4-VCAM-1 interactions are important to establish firm contacts between GC B cells and FDCs in vitro. The molecular basis for this shut-off is presently unclear: although apoptosis of GC B cells can be postponed for some time by crosslinking of LFA-1, VLA-4, CD21, CD40, BCR molecules, or co-crosslinking of CD40 and the BCR (Refs. 10, 14, 16–20), none of these signals can shut down the apoptotic endonuclease activity in GC B cells.

It has also been suggested that CD40 ligation is a major signal for preventing apoptosis. However, as shown by Liu et al., although CD40 crosslinking delays apoptosis in GC B cells for 48–72 h, it does not actually prevent apoptosis. Furthermore, blocking of
CD40-CD40L interactions during ongoing GC reactions does not result in increased numbers of apoptotic cells. Taken together, these results indicate that the CD40-CD40L interaction is not involved in the FDC-mediated rescue of GC B cells at the second checkpoint.

If FDCs do not rescue their associated GC B cells through a direct molecular mechanism, as described above, what other events might cause the apoptotic machinery to switch off? Recent experiments have shed some light on this. First, the presence has been demonstrated of functional connexin 43 (Cx43) gap junctions between FDCs and GC B cells. These data suggest transmission of low-molecular-weight substances during FDC–B-cell contact. Second, recent evidence suggests that cysteine proteases, including members of the interleukin-1B-converting enzyme (ICE) family (caspases), are crucial to execute apoptosis in GC B cells. Furthermore, a specific cysteine proteinase inhibitor is possibly translated from the FDC to GC B cells, resulting in a blockade at the very start of the apoptotic cascade.

### Checkpoint 3: Isotype Switching and Maturation of Appropriate B Cells

The mechanisms described above guarantee selection of memory B cells with a high-affinity BCR from a pool of diverse precursors. However, B cells with irrelevant or unwanted (e.g. autoreactive) specificities might still survive and receive further maturation signals as a result of binding of their BCRs to different Ags on the FDCs.

At the post-apoptotic rescue state, T cells might again become critically involved in the maturation of selected/rescued B cells. These B cells will retain some of the Ag from the immune complexes on the FDC, which may then be processed and presented by major histocompatibility complex (MHC) class II molecules to helper T cells. Such T cells would then provide costimulatory signals for further differentiation into memory cells or plasma cells. Thus, irrelevant B cells would not encounter their appropriate Ag-specific helper T cells and, consequently, would not be activated and amplified.

This model brings together data from several different groups. Liu et al. have shown recently that isotype switching occurs in GCs long after the somatic hypermutation step. The phenotype of the cells involved in isotype switching—surface sIgD, CD38^+, CD77^-—fits with the phenotype of GC B cells after FDC-mediated rescue. This implies that a trigger for isotype switching, presumably through CD40-CD40L interaction, must be given to the rescued B cells, suggestive of a renewed interaction with T cells. Indeed, CD4^+ T cells have been found at the site where this post-rescue step should take place: the apical light zone of the GC (Ref. 27). Other studies have shown that these T cells are specific for the Ag that has initiated the GC reaction. Moreover, the presence has recently been demonstrated of CD4^+ memory T cells containing preformed CD40L in the outer zone of GCs (Ref. 27). It was also shown that culture of these T cells with GC B cells rapidly induced a memory phenotype in the B cells.

Furthermore, according to the hypothesis described above, Ag-specific B cells will meet their appropriate helper T cell and become re-activated by cognate T-B-cell interaction, including focal signalling through the CD40 pathway. By contrast, bystander B cells that have made it through checkpoint 2 will lack such help and die. The finding that GC B cells abundantly express Fas (CD95)^+ implicates Fas-mediated programmed cell death of these cells, an idea that is supported by data from lpr mice, which lack functional Fas expression. These mice develop strong autoimmune responses, including autoantibody production, suggesting that the normal Ag-induced GC reaction is functional, but that deletion of autoreactive B cells generated within the GC is not effective, presumably due to the impaired Fas pathway. Own experiments have shown that GC B cells in contact with FDCs are resistant to Fas-mediated...
apoptosis. However, in the absence of FDCs, Fas-mediated signals were found to induce apoptosis in activated B cells. CD40 ligation can prevent this Fas effect for approximately 24 h; however, subsequently, CD40 crosslinking is no longer effective. These data show that FDC-rescued GC B cells can either be deleted via Fas-mediated signals, or expanded by CD40-mediated signals, presumably given by a local CD4+ T cell.

The idea that T cells can choose either Fas triggering or CD40 triggering to determine deletion or expansion of the selected B cells was recently illustrated by Rathmell et al., who showed that the Fas pathway was not operational if the B cell was strongly activated. The next checkpoint is after the proliferation and mutation phase, when the GC B cells compete for Ag binding on FDCs. Here, the high-affinity B cells are selected. Finally, the selected B cells are tested again for Ag specificity by GC T cells. If the selected B cells (for instance, when they would express irrelevant or autoactive BCRs gained during the mutation phase) fail to present the ‘initiating’ Ag, they will be deleted via Fas-mediated apoptosis. This last checkpoint is important to prevent autoactive B cells from entering the memory B-cell pool. Further experiments are now required to dissect the precise steps in the last checkpoint, in order to understand fully the processes occurring in a GC reaction.

Concluding remarks

The three-step checkpoint model described above guarantees Ag specificity throughout a GC reaction. The first checkpoint is at the initiation of the response, when only Ag-specific B cells are able to provoke CD40-CD40L interaction from T cells in order to become activated. The next checkpoint is after the proliferation and mutation phase, when the GC B cells compete for Ag binding on FDCs. Here, the high-affinity B cells are selected. Finally, the selected B cells are tested again for Ag specificity by GC T cells. If the selected B cells (for instance, when they would express irrelevant or autoactive BCRs gained during the mutation phase) fail to present the ‘initiating’ Ag, they will be deleted via Fas-mediated apoptosis. This last checkpoint is important to prevent autoactive B cells from entering the memory B-cell pool. Further experiments are now required to dissect the precise steps in the last checkpoint, in order to understand fully the processes occurring in a GC reaction.

References

Considerable attention has focused recently on the interaction between the innate and acquired arm of the immune system in the induction phase of immune responses, as well as on the better-understood links in the effector phase. For example, a recent review in Immunology Today1 proposed a reciprocal interaction between cytotoxic cells from the innate immune system (natural killer cells) and the cytotoxic component of adaptive immunity (cytotoxic T lymphocytes: CD8+ T cells) in controlling the outcome of the immune response. Several other examples of such interactions have been discussed elsewhere.2,4

A second well-established dichotomy within the immune system is that between an acute and a delayed inflammatory cellular response. Polymorphonuclear leukocytes (PMNs; neutrophils) and macrophages (Mφs) are both professional phagocytes, whose major effector function is killing microorganisms.5 However, PMNs respond to inflammatory stimuli within minutes to hours, appearing rapidly in large numbers within the tissue and being largely responsible for the acute phase of inflammation. By contrast, Mφs are fewer in number, with the peak of infiltration occurring later after challenge compared with PMNs (hours to days), and they participate in both acute and chronic inflammation.6

Communication between PMNs and Mφs

It is widely recognized that Mφs, in addition to their role as phagocytic effector cells, play a central role in regulating both adaptive and innate immunity by acting as antigen-presenting cells (APCs) and by their complex pattern of cytokine secretion. Pathogens that enter the body are trapped by widely distributed resident Mφs, such as alveolar Mφs within the respiratory tract or Kupffer cells within the liver.8,9 The interaction between these cells and a pathogen may initiate inflammation (the innate type of immune reaction) or the cells can act as APCs in the amplification phase of either a humoral or a cellular immune reaction.8 Furthermore, Mφs can directly influence the development of a PMN-dependent inflammatory response via the release of chemotactic factors, such as interleukin 8.