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Memory in the B-cell compartment: antibody affinity maturation

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In the humoral arm of the immune system, the memory response is not only more quickly elicited and of greater magnitude than the primary response, but it is also different in quality. In the recall response to antigen, the antibodies produced are of higher affinity and of different isotype (typically immunoglobulin G rather than immunoglobulin M). This maturation rests on the antigen dependence of B-cell maturation and is effected by programmed genetic modifications of the immunoglobulin gene loci. Here we consider how the B-cell response to antigen depends on the affinity of the antigen–receptor interaction. We also compare and draw parallels between the two processes, which underpin the generation of secondary-response antibodies: V gene somatic hypermutation and immunoglobulin heavy-chain class switching.

Keywords: affinity maturation; antibody; B lymphocyte; immunoglobulin; isotype switching

1. INTRODUCTION

Encounter with antigen leads to the generation of B-cell memory. The specific humoral immune response elicited following the first encounter with an antigen takes a few days to develop and is predominantly composed of IgM antibody of relatively low affinity. The quantity and quality of the antibodies produced change during the course of this primary response (Jerne 1951; Eisen & Siskind 1964) such that later during the primary response, the antigen-specific antibodies usually exhibit improved affinity for the antigen and have also switched from an IgM isotype to one of the downstream isotypes (IgG, IgE or IgA). If the animal encounters the same antigen again, a memory antibody response is produced. This secondary response develops somewhat more rapidly and is predominantly composed of IgG antibody that exhibits an improved affinity for the antigen.

The memory response is more rapid than the primary response, in part because the immunized animal will contain an increased number of B cells specific for the antigen. This is exactly as envisioned by the clonal selection theory (Burnet 1957), where initial antigen challenge is anticipated to lead to clonal expansion of those B cells that produce a cognate antibody specificity. However, quite apart from this, there is also a qualitative difference in the nature of the antibodies produced during the memory response and those made in the immediate primary response. Part of this qualitative difference can be simply understood in terms of the original clonal selection theory. The different cells in the primary repertoire triggered by the antigen will not all recognize the antigen with equal affinity. If there is selective expansion of the high-affinity as opposed to low-affinity clones, then affinity maturation will ensue. Indeed, if the various B-cell clones are present at differing precursor frequencies prior to antigen encounter, then a repertoire shift from an abundantly represented low-affinity clone to a rarely represented high-affinity clone could well result (Berek & Milstein 1987).

However, over and above any repertoire shift ascribable to the selective amplification of different B-cell clones, there are qualitative differences to the antibodies made during the course of the immune response. These changes are due to molecular modifications of the immunoglobulin genes themselves and these are triggered by the antigen encounter.

These molecular modifications can be divided into two components. There is a shift in the C_H region expressed from Cμ to that of a downstream isotype (immunoglobulin heavy-chain class switching). There is an alteration in the repertoire of antibody-combining sites available achieved by V gene somatic hypermutation. Thus, in the B-cell compartment (as opposed to the T-cell compartment), the memory response and the primary response do not differ just in the size and clonal distribution of distinct but pre-existing binding specificities. Rather, there are two specific types of genetic alteration that are used to generate a memory response whose components are different in nature from that in the primary response.

2. INDUCTION OF THE MEMORY RESPONSE

The generation of the memory response is obviously triggered by the antigen itself. However, the shaping of this response is in part regulated by antibody present in the host prior to antigen encounter. We can consider this pre-immune antibody as natural antibody (Jerne 1955; Boyden 1966). It is probable that, upon entering the body, foreign antigen can be bound by serum IgM of low

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affinity ('natural IgM'). The importance of natural antibody in assisting the development of the immune response was suggested many years ago, based on studies of immune responses in colostrum-deprived piglets (Hoerlin 1956; Segre & Kaeberle 1962). This has been shown to be true using gene-targeting technology. Mice have been derived that express surface IgM but are deficient in serum IgM. Such animals retain normal B-lymphocyte populations and the serum levels of immunoglobulins of other isotypes are essentially unaffected. However, when challenged with antigen, they indeed reveal delayed development of the memory response (Ehrenstein *et al.* 1998). Thus, loss of the IgM component of the natural antibody is sufficient to delay development of the specific immune response. This makes sense in view of the fact that natural antibody probably mediates its effect by complexing the incoming antigen to form low-affinity antigen–antibody complexes that recruit complement. Such complexes could assist maturation of the immune response by either accelerating antigen deposition at sites suitable for immune responses (e.g. see Klaus *et al.* 1980) or by co-stimulation of antigen-receptor signalling through co-engagement of the CD19–CD21 complex (Carter & Fearon 1992).

3. ANTIGEN SELECTION: AFFINITY THRESHOLDS AND CEILINGS

The nature of the antibody response is obviously a function of both the distribution of specificities within the available B-cell repertoire and the way in which these specificities are selectively triggered by antigen. This triggering must ultimately depend on the quality of antigen–antibody binding. There must be an affinity threshold for B-cell triggering. If this threshold is set too high, there are likely to be holes in the B-cell repertoire. If set too low, one might anticipate problems associated with excessive B-cell activation and difficulties in the discrimination between self and foreign antigens.

Transgenic models give good support to the idea that B cells recognizing abundant self-antigens with high affinity are deleted early during maturation (Goodnow 1992). Perhaps B-cell triggering thresholds are raised during B-cell maturation with a low threshold being set for deletion in immature B cells whereas, in mature B cells, a higher threshold could be set with the intracellular wiring altered such that triggering leads to cell activation (rather than deletion). Such a difference in affinity thresholds might protect from initiation of autoimmune responses. Indeed, CD22 is a B-cell-specific transmembrane protein associated with the antigen receptor on B cells that is expressed at higher levels during B-cell maturation and serves to dampen antigen-receptor signalling. Deletion of CD22 not only leads to somewhat hyperactive B cells but is also sufficient to predispose to the development of high-affinity autoantibodies (O'Keefe *et al.* 1999). This observation strongly supports the contention that B-cell triggering thresholds must be tightly controlled to avoid autoimmunity: the regulated provision of T-cell help may not yield sufficient protection in the face of a hyperactive B-cell compartment.

Once triggered, the B-cell response matures and there is a shift towards increased affinity. This shift must be mediated by selection based on the quality of antigen–B-cell interaction. Experiments in a model system using a monomeric antigen have charted the affinity-dependence of B-cell triggering with the results supporting the idea that, once a sufficiently high affinity is reached (around 10^{10}–10^{11} M^{-1}), further improvements might not readily prove selectable (Batista & Neuberger 1998). Such limits of selectability could act *in vivo* to mediate the avoidance of clonal dominance and aid the maintenance of diversity in the memory B-cell repertoire.

4. PROCESSES INVOLVED IN THE GENERATION OF THE PRIMARY ANTIBODY REPERTOIRE

Before considering the genetic modifications required to generate a secondary antibody response, it is worth reviewing the gene rearrangements involved in creating the primary repertoire. The functional repertoire of V gene segments is assembled through a process of joining of germline V, D and J gene segments. This occurs during early B-cell development and is achieved by a specific recombination mechanism. There are three lymphoid-specific proteins that appear to have evolved specifically to perform this rearrangement programme. The RAG1 and RAG2 polypeptides are implicated in the recognition and cutting at the rearrangement signal sequences flanking the V, D and J gene segments. Another enzyme, terminal deoxynucleotidyl transferase, adds nucleotides in a non-templated fashion to the coding junctions of the V, D and J segments, thereby achieving substantial diversification of the antibody CDR3 regions. These rearrangement processes have been studied in some detail at the biochemical level (reviewed in Gellert 1997). In addition to these three lymphoid-specific proteins, the programme of DNA cutting and joining uses some ubiquitously expressed enzymes that have other, more general, roles in DNA joining and repair (XRCC4, DNA ligase 4, etc.).

5. PROCESSES INVOLVED IN THE GENERATION OF THE SECONDARY ANTIBODY REPERTOIRE

(a) *Somatic hypermutation*

In contrast to our understanding of the mechanism of immunoglobulin V gene rearrangement, our knowledge of the biochemical basis of somatic hypermutation and heavy-chain class switching is rudimentary.

Following antigen encounter and during the B-cell proliferation in germinal centres, nucleotide substitutions are introduced into a short region of DNA (about 2000 base pairs), including the productively rearranged immunoglobulin V gene segments. This hypermutation is localized not only in respect of its preferential targeting to a small section of the genome but it is also restricted in time, occurring only during a narrow window of B-cell development. Two main sources of information provide the foundation for our knowledge of somatic mutation (see Neuberger *et al.* (1998) for a review). First, it has been possible to compare the expressed immunoglobulin V gene sequences to their germline counterparts. This has given a picture of the types of mutations that have been created and selected during antigen-driven immune responses. Second, the analysis of mutation of immunoglobulin transgenes integrated into the mouse germline

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has allowed information to be gleaned about the DNA sequences necessary for hypermutation recruitment based on studies of modified transgenes. By analysing hypermutation of immunoglobulin transgenes that carry modifications preventing them from expressing functional immunoglobulin, it has also been possible to analyse the intrinsic pattern of mutation creation without being confused by the skewing effects of antigenic selection. The results of such studies have revealed that the transcription enhancer elements play a major role in the recruitment of hypermutation, with the location of the mutation domain being defined by the position of the promoter. The immunoglobulin V gene, although the physiological target of the mutation, is not required for mutation recruitment. Indeed, heterologous sequences can be positioned in its place and thereby become targets for the hypermutation mechanism. So the picture emerges of a transcription-linked mutation creation programme that results in nucleotide substitutions being inserted in a domain located downstream of the promoter.

The mutations, which exhibit a preference for transitions, are not distributed randomly throughout the mutation domain; rather, there are specific hot spots and cold spots and the sequences of the major hot spots conform to a consensus. Analysis of the codon usage among immunoglobulin germline genes indicates that the sequences of V genes have probably evolved to allow mutational hot spots to be created in strategically useful places (Wagner *et al*. 1995) A major hot spot is evident at the edge of CDR1 in both heavy- and light-chain V genes (codon position 31). Intriguingly, another hot spot is often found at the edge of a loop at the base of framework 3 (Jolly *et al*. 1996). Perhaps these hot spots are strategically positioned such that amino-acid substitutions have a particularly good prospect of yielding subtle structural changes in the antigen-binding loops suitable for affinity maturation.

The mechanism of hypermutation remains unidentified, although recent observations made using a cell-line model of hypermutation indicate that the process is accompanied by DNA breaks scattered over the mutation domain (Sale & Neuberger 1998). Perhaps such breaks are necessary intermediates in the hypermutation process, with their generation or detection being associated with transcription.

(b) *Class-switch recombination*

The maturation of the immune response is associated with switching from IgM to one of the downstream immunoglobulin isotypes (see Stavnezer (1996) for review). This class-switch recombination is achieved by the creation of a DNA deletion whose 5′-border is located within or adjacent to Sμ (a region largely composed of tandemly reiterated pentamer motifs, which is found within the major intron separating the J_H cluster from the exons of Cμ) and whose 3′-border is in the vicinity of a Sγ, Sε or Sα, which are composed of repetitive sequences and are found just upstream of the C_H exons of the downstream isotypes. The recombination is neither between homologous sequences nor is it site specific; rather, it is region specific. However, it is a transcription-linked process and is probably initiated through the creation of double-strand DNA breaks (Rolink *et al*. 1996; Casellas *et al*. 1998; Manis *et al*. 1998; Wuerffel *et al*. 1997).

6. PARALLELS BETWEEN HYPERMUTATION AND CLASS SWITCHING

Hypermutation and class switching are the two genetic modifications peculiar to the generation of the antibody memory repertoire. The molecular mechanism of both processes is unknown. While they take place at a similar stage of differentiation, it is clear that they do not need to occur (although they may occur) in the same cell and that they target distinct (albeit closely linked in the case of the heavy-chain locus) regions of the genome.

Recent results prompt us to speculate that there may be mechanistic links between the two processes. We have found that both hypermutation and class switching are affected by deficiency in Msh2, a protein that recognizes DNA mismatches and aberrant structures. Hypermutation in Msh2-deficient mice takes place with diminished efficiency, with the mutations that do occur being strikingly focused on intrinsic mutational hot spots (Rada *et al*. 1998; Frey *et al*. 1998). Class switching in Msh2-deficient mice also occurs with diminished efficiency, with the break-points of the switch recombination showing an altered distribution; those in the vicinity of Sμ reveal greatly increased focusing on the GAGCT pentamer motif (Ehrenstein & Neuberger 2000).

In the case of hypermutation, we have interpreted these findings in favour of a two-stage process—the first being Msh2 independent and associated with the intrinsic hot spots (Rada *et al*. 1998). As DNA breaks scattered over the mutation domain are associated with hypermutation, and these breaks occur in the vicinity of the intrinsic hot spots (Sale & Neuberger 1998), it is possible that the first stage of hypermutation is indeed the creation of a DNA break at a hot spot. The Msh2-independent second stage (which could be dependent on the first stage) allows a more even spread of mutation fixation—presumably by facilitating a mutational process which works away from the targeted hot spots.

Switching is also likely to be initiated by a DNA break. The results from the Msh2-deficient mice are most readily interpreted in terms of the breaks preferentially occurring at the GAGCT consenus motifs; Msh2, if present, then allows migration of the point of synapsis away from the original breakage site.

The parallels between the two processes become even more striking when one compares the sequences of the consensus motifs. The reiterated Sμ pentamer to which switching is preferentially targeted in Msh2-deficient mice, not only fits the RGYW consensus that has previously been proposed for intrinsic mutational hot spots (Rogozin & Kolchanov 1992; Betz *et al*. 1993), but is in fact identical to the refined mutational consensus sequence (GAGCT) deduced in a recent triplet analysis (Milstein *et al*. 1998). Given that both switching and hypermutation are transcription-linked processes (Stavnezer 1996; Neuberger & Milstein 1995), it is tempting to suggest that the initiation of both is associated with the detection or creation of breaks at the hot spot and/or consensus motifs by the transcription machinery. Migration of the mutation creation or

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end-joining machinery away from the original site of DNA breakage would then be dependent on Msh2—either by mediating recognition of the aberrant DNA structure created at the break or by assisting branch migration of mismatched DNA duplexes.

7. CONCLUSION

Hypermutation and switching appear peculiar to the B-cell compartment. Memory in the T-cell compartment can be achieved by modulating the balance in clonal representation of different pre-existing specificities that were available in the primary repertoire, as well as by regulating the ease of T-cell activation. Furthermore, the affinity of a T cell for its target cell can be regulated by modulating the expression and function of other T-cell proteins apart from the antigen receptor itself. However, protective immunity in the humoral compartment is not simply a question of providing sufficient numbers of readily activatable antigen-specific B cells. Protective immunity is in good part conferred by the antibody present in serum immediately on antigen encounter. Thus a major drive for memory in the B-cell compartment must be the production of high titres of functional antigen-specific antibody—not simply for readily activatable antigen-specific B cells. It is for this reason presumably that the B-cell compartment has evolved specific mechanisms that facilitate the production of antibodies (as well as cells) which recognize the antigen with high affinity and which facilitate its clearance.

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