Antibody responses revisited

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Humoral responses are complicated, multi-step processes involving initial antigen driven activation of B cells, selection, amplification and finally differentiation into either antibody secreting plasma cells or memory B cells. While the initial activation is of low-stringency resulting in a heterogenous population of antigen-activated B cells, selection criteria are subsequently enforced which limit the number of B cells that persist during maturation of the antibody response. This article discusses our attempts at using designed antigens to unravel the processes governing B cells selection.

THE immune system consists of a wide range of distinct cell types, each with important roles to play. The lymphocytes occupy central stage as they are the cells that determine the specificity of immunity, and it is their response that orchestrates the effector arms of the immune system. Individual lymphocytes are specialized in that they are committed to respond to a limited set of structurally related antigens. This commitment, which predates the first contact of the immune system with a given antigen, is expressed by the presence on the lymphocyte's surface membrane of receptors specific for determinants (epitopes) on the antigen. Each lymphocyte possesses a population of receptors, all of which have identical combining sites. One set, or clone, of lymphocyte differs from another clone in the structure of the combining region of its receptors and thus differs in the epitopes, expressed on immunogenic substances, that it can recognize. The ability of an organism to respond to virtually any 'non self' antigen is achieved by the existence of a very large number of different clones of lymphocytes, each bearing receptors for a distinct epitope[1]. As a consequence, lymphocytes are an enormously heterogeneous group of cells. For example, the number of distinct combining sites on lymphocyte receptors of an adult human far exceeds 10^9.

Two broad classes of lymphocytes are recognized: the B lymphocytes, which are the precursors of antibody-secreting cells, and the T lymphocytes. T lymphocytes play an important role in regulating antibody production by the B lymphocytes as a consequence of a direct, cognate interaction between these two classes of lymphocytes. This interaction is, however, highly specific in that both cells must have specificities for determinants borne by the same antigen.

An encounter between antigen and the host preimmune B cell repertoire (i.e. the B cell pool prior to contact with an antigen) results in the activation of that subset bearing appropriate surface immunoglobulin (sIg) receptors. The principal outcome of such a recognition is the induction of antigen-specific IgM antibodies. Subsequent developments include antibody isotype switch (usually predominantly to IgG) and B cell differentiation into either memory or antibody-secreting plasma cells. In T-dependent responses, progression of antigen-activated B cells through each of these stages is driven by antigen-specific activated T helper cells. B cells bind antigen through their sIg receptors, which is then followed by endocytosis, antigen processing and presentation of appropriate fragments in the context of MHC Class II molecules. Such antigen-presenting B cells then engage relevant T helper cells in a cognate interaction, one consequence of which is B cell proliferation and differentiation into antibody-secreting plasmacytes[2].

Problem of selective and hierarchical immunodominance

Unequal antibody responses to the spectrum of epitopes displayed by a multideterminant antigen have remained a long-standing paradox. The seemingly large preimmune B cell repertoire endows a given mammalian host with the potential to recognize a virtually limitless array of antigenic determinants. This along with the fact that B cells generally recognize protein antigens in their native form has led to the dogma that the entire accessible surface of a protein represents an antigenic continuum[3]. While this dogma is a logical derivative of our understanding of the mechanics of humoral (B cell) responses it, nevertheless, contrasts with experimental observations. With a variety of protein antigens[4,5], it has been the common finding that only a fraction of the B cell epitopes presented is actually successful at eliciting antibodies. While such 'selective' immunodominance clearly represents one aspect of the problem, there is yet another which is characterized by unequal levels of antibodies obtained against even those that are recognized in the humoral response. Thus the collective phenomenon of selective and hierarchical immunodominance implicates the involvement of parameters in addition to that of surface accessibility of the determinant alone in the induction and progression of humoral responses to polypeptide antigens.

The problem as stated above achieves practical significance in the context of design of new generation vaccines. With rapid advances in immunology it is increasingly more feasible to precisely identify neutralization epitopes, i.e. those epitopes capable of inducing neutralizing antibodies, on pathogen-derived proteins. This ability is also concurrently paving the way for the eventual development of 'minimalistic' vaccines that

incorporate only the relevant epitopes rather than the whole pathogen or its antigenic components. Towards this end a variety of modalities are being explored[6] that include, among others, incorporation of epitopes into live vector vehicles[7–10], chemical linking of epitopes[11,12], co-oligomerization of self-assembling peptides[13], and collinear assembly of polyvalent immunogens either by chemical synthesis[14,15] or by rDNA routes[16]. Regardless of the particular route one adopts, an important prerequisite for the eventual success of this strategy is that the included epitopes must retain their immunodominance in the new construct. It is from this standpoint that the resolution of the problem of selective and hierarchical immunodominance becomes particularly critical.

Selective immunodominance is immunologically regulated

To address some of these issues we initially expressed a designed, chemically synthesized and assembled gene coding for a hundred amino acid polypeptide, MEP-1 (ref. 16). This polypeptide included select determinants from the large protein of the surface antigens of Hepatitis B virus. A preliminary analysis indicated that MEP-1 was highly immunogenic in a variety of mouse strains and therefore held promise. However, the problem of selective and hierarchical immunodominance was also true of MEP-1. Of the three domains incorporated, only two were recognized by antibodies as revealed by peptide mapping[17]. Furthermore, of these two domains, antibodies against the amino-terminal epitope (epitope B1) predominated, accounting for over 90% of the total antibody population. On the other hand, those against the carboxy-terminal determinant (epitope B2) only constituted a minority[17]. Subsequent studies revealed that immunodominance of B1 was not a consequence of primary B cell recognition[18]. Indeed, in the first week following an immunization with MEP-1 it was the anti-B2 antibodies which predominated. However, subsequent maturation from this early primary response was accompanied by a rapid increase in anti-B1 titres, whereas the anti-B2 response was suppressed. This was found to correlate well with the ability of B cells directed against epitope B1 to productively interact with antigen-specific T helper cells relative to those against epitope B2 (ref. 18). It must be noted here that the ability of a given antigen-activated B cell to recruit help from antigen-specific T helper cells would represent the primary determinant of the extent to which this B cell would proliferate and differentiate into antibody-secreting cells. Thus the facility with which individual B cell populations activated against the various epitopes on a multideterminant antigen can access T cell help would directly correlate with the level of antibodies produced against each of those epitopes.

Further dissection of these initial results revealed an interesting paradox. Among the two initially induced polyclonal subpopulations, it was the B2-specific subset which had an affinity for antigen that was markedly higher than that against the B1. Given that the immunoglobulin (antibody) secreted by a given B cell is virtually identical to the sIg receptor on its surface, this would also imply that the B2-specific B cells would bind antigen with a higher affinity and thereby better present it to T helper cells relative to the antiB1 B cells[19]. However our observations were to the contrary. This puzzle was subsequently resolved with our finding that secreted immunoglobulins also have a critical role to play by competing with the corresponding B cells for binding to the epitope. Because of the soluble nature of secreted antibodies as opposed to the B cell surface bound receptor, they have faster on-rates for antigen binding – though affinities are identical. Thus when a given epitope induces large amounts of very high-affinity antibodies in the early primary response, it creates an equilibrium situation where the epitope on the free antigen molecules is completely saturated by the secreted antibodies (Figure 1). As a result, the antigen is now inaccessible for binding by the corresponding B cell. This renders it unable to recruit T cell help, ensuring its elimination from the response[18].

Affinity-mediated selection – the other side of the coin

These initial results clearly demonstrated that subsequent selection of B cells from the early antigen activated pool is a more complex process than hitherto anticipated. In order to delve further into this process we resorted to synthetic peptides as tools that are more amenable to both sequence and structural modifications. The parent peptide employed (peptide PS1CT3) was chimaeric peptide that included a known B cell epitope from the surface antigen of Hepatitis B virus (segment PS1) and

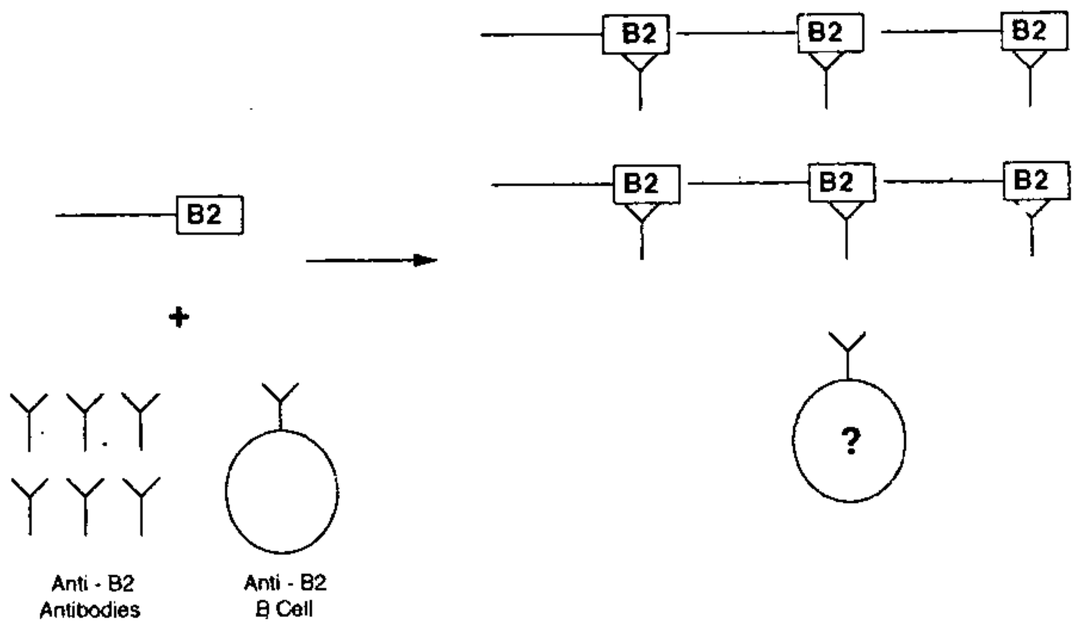

Figure 1. Secretion of epitope-specific high-affinity antibodies in the early primary humoral response down regulates further progression. Figure shows a model epitope (B2) on antigen which elicits large amounts of high-affinity antibodies in the early stages of a primary antibody response. This results in a three-way competition where the secreted antibody, by virtue of its higher on-rates, preferentially saturates the epitope. As a result, the antigen is inaccessible for B cell recognition. Further details are described in the text.

a known T cell epitope derived for the circumsporozoite protein of the malaria parasite, *Plasmodium falciparum* (segment CT3). Both epitopes were separated by a spacer of two glycine residues for reasons described earlier[20].

Immunization of mice with peptide PS1CT3 yielded an early primary IgM response that was heterogeneous with respect to fine-specificities recognized within the PS1 segment. However, subsequent progression of the humoral response saw a stringent selection for a restricted antibody specificity, exclusively directed against a tetrapeptide segment within PS1 of sequence DPAF (Figure 2). In parallel we also ascertained, from experiments in nude mice, that peptide PS1CT3 represented a T-dependent antigen during both the IgM and IgG stages of the response[21]. This peptide therefore provides a valid model for the study of B cell selection.

Our first set of experiments was to determine the quantum of T cell help required for the individual stages of primary B cell response to this peptide. While both the induction of IgM antibodies and B cell differentiation into a memory pool was readily achieved in the presence of limiting numbers of antigen-primed T cells, antibody class switch from IgM to IgG required at least an order of magnitude larger pool size of T cells. This finding allowed us to propose that induction and progression of a primary T-dependent humoral response was comprised of a single rate limiting step, in terms of T helper cell requirements, represented by the antibody isotype switch. From this we also postulated that this step would also constitute the principal 'filtering-out' point where only a fraction of the originally induced B cells are retained. This inference could subsequently be confirmed by our demonstration that selection for the 'DPAF'-specificity in the murine primary humoral response to peptide PS1CT3 also occurs concomitantly with class switch[22].

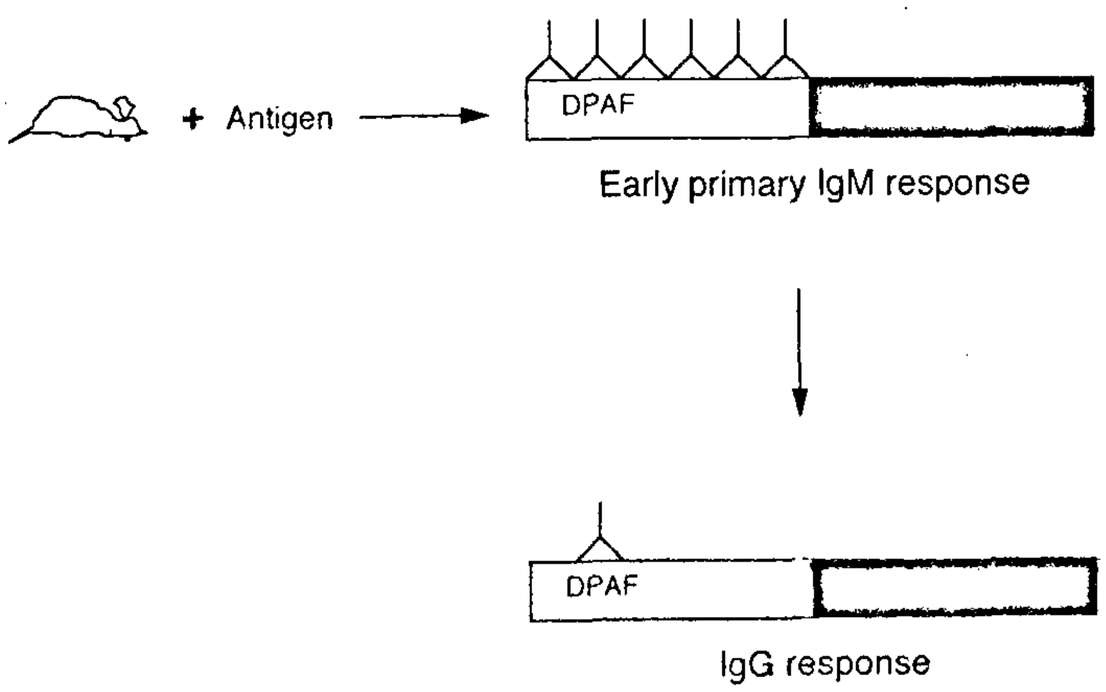

Figure 2. Maturation of a murine primary antibody response to peptide PS1CT3. Immunization of mice with peptide PS1CT3 initially induces IgM antibodies against the entire PS1 sequence (open box; sequence: HQLDPAFGANSTNPD). Maturation into an IgG response however, involves selection for antibodies only against a tetrapeptide segment, DPAF. The position of the DPAF segment within PS1 is indicated in the figure. The closed box represents the T epitope segment, CT3.

So what then are the immunological processes that guide exclusive selection for the anti-DPAF antibodies? It was this aspect that we next investigated. The circular dichroism spectrum of this peptide indicated a random distribution of conformations, suggesting that surface accessibility is unlikely to be a limiting factor. Indeed, dissection of the early primary IgM response into individual monoclonal antibodies confirmed this by revealing a composition that collectively recognized the sequence of the PS1 region. However, monoclonal antibodies derived from the late IgG stages of the response were all uniquely directed only against the DPAF sequence. Interestingly, though the IgG response was monospecific for DPAF it was nevertheless oligoclonal. Nucleotide sequencing of the antibody heavy chain variable region genes indicated at least four independent B cells contributing towards this response[21].

To examine the basis of DPAF selection, we synthesized a set of PS1CT3 analogues involving single amino acid substitutions for glycine within the DPAF segment. Of particular interest was our finding that immunization of mice with these analogues gave IgG antibody responses whose fine-specificities had now shifted to segments flanking the DPAF region. However, the affinity of these antibodies was markedly lower than that obtained against the DPAF sequence in PS1CT3. Collectively these and related studies seemed to suggest that selection for the DPAF specificity was the outcome of a competitive process where B cells directed against alternate domains were suppressed. Furthermore, based on affinities of secreted antibodies, it also seemed likely that positive selection was determined by the affinity of B cell sIg receptor for antigen. These speculations were subsequently ratified in mixing experiments where mice were immunized with an equimolar mixture of peptide PS1CT3 and one of its analogues, where antibody responses to the analogue were now completely suppressed[21].

Interestingly, our follow-up studies revealed that the driving force for competitive selection was the available pool size of antigen-specific T helper cells which, in the early stages of an immune response, is necessarily limiting. The affinity of the B cell sIg receptor for antigen merely served to define its ability – by way of antigen binding, processing and presentation – to recruit help from the limiting T cell pool and thereby denying it those with a lower affinity for antigen[21] (Figure 3).

While the results discussed above suggest that it is the limiting pool of antigen-specific T helper cells during an early stage primary antibody response that constitute the cut-off for the range of B cell affinities selected, our more recent experiments indicate that it is not an invariant barrier. Rather it represents a dynamic equilibrium between affinity of B cell for antigen and the actual pool size of antigen-specific T helper cells[22]. Thus for antigens which are more efficient at priming T cells,

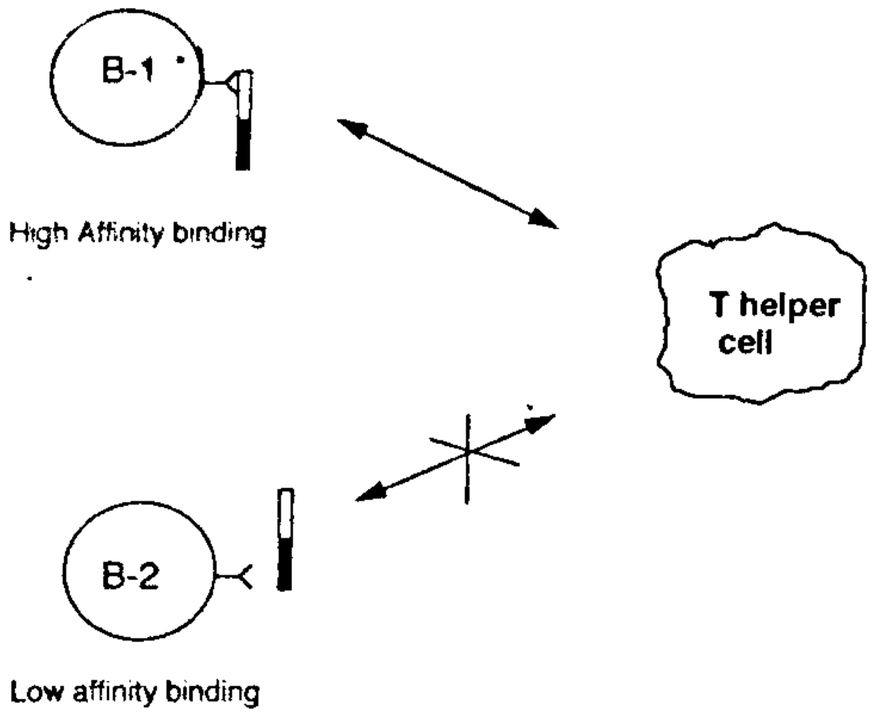

Figure 3. A limiting pool of T cell help enforces an affinity-driven competition between early activated antigen-specific B cells. The pool of antigen-specific T helper cells generated in the early stages of an immune response is insufficient to service the gamut of preimmune B cells that are activated upon initial antigen exposure. This enforces a competitive process where B cells with higher affinity for their corresponding epitope recruit help from this pool unidirectionally, leaving none available for B cells with lower affinities for their corresponding epitopes, which are then eliminated as a result.

even B cells with relatively lower affinities for antigen are selected. On the other hand, those antigens which prime T cells poorly will induce an IgG response that is far more restricted in terms of both fine-specificity and clonal diversity.

Concomitant with the above studies, we have also been examining parameters which modulate binding of antigen (through the epitope) to B cells. For this we have again used single amino acid substituted analogues of peptide PS1CT3, but with glycine substitutions performed outside of the DPAF segment though within the PS1 sequence. Such substitutions were found to alter binding properties of the resultant analogues but without affecting the fine specificity of recognition (i.e. binding through the DPAF segment was retained). These studies revealed that, in addition to affinity, the on-rate of antigen binding to receptor also influences the extent of B cell proliferation and subsequent antibody production (manuscript submitted). Thus, among a population of B cells with comparable affinities for their corresponding epitopes, those with higher epitope binding on-rates will proliferate preferentially, resulting in an antibody response that is biased in favour of such epitopes.

Summary

We believe that our investigations over the last three years have now provided us with at least a glimpse into the subtleties underlying T-dependent B cell responses. The early primary IgM response is indeed consistent with the dogma in that all accessible regions on the antigen surface are recognized[21,23]. It is the subsequent maturation from this phase where restriction is enforced. Positive selection of a particular B cell appears to be primarily determined by its ability to recruit the appropriate level of T cell help. This in turn is regulated by its affinity for epitope which indirectly defines its antigen-presenting efficacy to T helper cells. However, there is an upper threshold limit. Affinities above this threshold have a suppressive effect by ensuring elimination of the corresponding B cells as a result of competition with it for the same epitope. On the other hand, too low an affinity for epitope will also obviously not permit its recruitment by antigen. These results can therefore be summarized into an 'affinity window' hypothesis that sets upper and lower limits for affinity of preimmune B cell receptor for epitope in order to be fruitfully recruited by antigen into a humoral response. Within the limits of this window, affinity is expected to play a positive regulatory role. In addition to affinity, the extent of B cell proliferation is also modulated by the on-rate of antigen binding. This would be particularly true in situations where B cells of independent specificities have similar affinities for their corresponding epitopes. In such cases, on-rates may represent the primary determinant of which response dominates over the other. Such a hypothesis may serve towards explaining the basis of both selective and hierarchical dominance.

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