Antigens

Introductory article

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The term 'antigen' describes molecular species that are capable of specific recognition by one or more constituents of the acquired immune response. Collectively they encompass the entire range, from the simplest of chemical compounds to the most complex of macromolecules.

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Introduction

The adaptive immune system is an extremely efficient defence mechanism found in its most advanced form in higher vertebrates. It provides the means to generate rapid, highly specific and often protective responses against the myriad of pathogens that inhabit the world in which we live. For the immune system to mount a response against any microorganism it must first be able to recognize it. It is here that the adaptive immune system distinguishes itself from any other system in the body, being equipped with the ability to recognize a variety of molecular species that form the constituents of the microbe. This article will discuss the underlying principles of immune recognition, and the nature of its molecular targets. Such targets are commonly referred to as 'antigens'.

The term antigen represents a functional annotation that is generally used in the context of adaptive or acquired immunity. Substances or, more specifically, molecules that can be recognized by one or more constituents of an acquired immune response are referred to as antigens. Given that the immune system has evolved to recognize all molecular species foreign to the host, in principle antigens encompass a broad spectrum covering all classes of organic molecules, from the simplest of chemical compounds to the most complex of macromolecules.

The property of antigenicity is, however, operationally distinct from that of immunogenicity. While antigenicity refers to recognition by the effector components of an immune response, immunogenicity denotes the ability to elicit such a response. As a result, although all molecules which are immunogenic also possess the property of antigenicity, the reverse is not necessarily true. For example, many small molecules possess the property of antigenicity but are incapable, by themselves, of inducing a specific immune response. These are commonly referred to as haptens. Haptens are rendered immunogenic only upon chemical conjugation to larger proteins, which function as carriers. Thus, while antigenicity represents an intrinsic property of the molecule, immunogenicity characterizes a condition that is influenced by a number of additional factors involved in the total biological system.

Types of Antigens

As noted above, the adaptive immune system can recognize a wide variety of molecular species, ranging from a few hundred daltons in molecular mass to structurally complex macromolecules. Neither does the chemical nature of the entity pose a restriction, as targets for immune recognition can include small organic molecules such as dinitrophenol, simple and complex sugars, lipids, proteins and even nucleic acids. While all of these may be characterized as antigens there are, nevertheless, subtle distinctions specified by the constituents of the immune system.

The adaptive immune system is now generally accepted to be composed of two effector arms: humoral immunity, the arm derived from the antibody-producing B lymphocytes, and cell-mediated immunity, comprising immune-effector responses derived from T lymphocytes. Substances that are recognized by antibody (or B cells) are called 'B-cell antigens', whereas those recognized by T lymphocytes are known as 'T-cell antigens'. Recognition of antigen by T and B lymphocytes is performed by cell surface antigen receptors. These are associated in the lymphocyte membrane with signal-transducing proteins, which together serve to activate the lymphocyte in response to binding specific antigen. The antigen receptors of T and B lymphocytes are termed T-cell receptor (TCR) and B-cell receptor (BCR), respectively. After activation by antigen, B lymphocytes secrete a soluble form of their receptors (commonly termed antibody), the term BCR being normally reserved for the membrane-bound, signal-transducing form only.

Fundamental differences in the way B- and T-lymphocyte antigen receptors recognize an antigen determine which molecular features are recognized. B lymphocytes recognize antigens in soluble form and, therefore, are able to target molecular species of wide-ranging size, structural and chemical heterogeneity. Thus, for example, antibodies against a variety of small molecules such as phosphorylated amino acids, steroids, etc. have been successfully obtained, and it is equally feasible to generate antibodies against more complex chemical species, such as chemical polymers, branched sugars, oligomeric proteins and DNA. Furthermore, B-cell or antibody recognition is targeted

against the surface topology provided by the antigen and is therefore independent of the nature of the underlying structural units that contribute to it. As a result, humoral responses to protein antigens can include antibodies directed against linear segments of sequence presented in either an extended conformation or in any one of the numerous possible secondary structural configurations at the surface of the antigen. In addition, antibodies can be obtained that recognize surfaces generated by noncontiguous segments juxtaposed as a consequence of either tertiary or quaternary structural folding of the native protein (**Figure 1**).

A good example of the heterogeneous recognition potential of humoral immunity can be had from looking at the antibody specificities produced in response to infection by a pathogenic agent. A bacterial infection yields individual antibodies directed against a host of molecules that represent the chemical constituents of the organism. These include capsular polysaccharides, glycolipids, lipoproteins, low molecular weight constituents of the cell wall, membrane-bound and intracellular proteins, small polypeptides and, occasionally, lipids and nucleic acid.

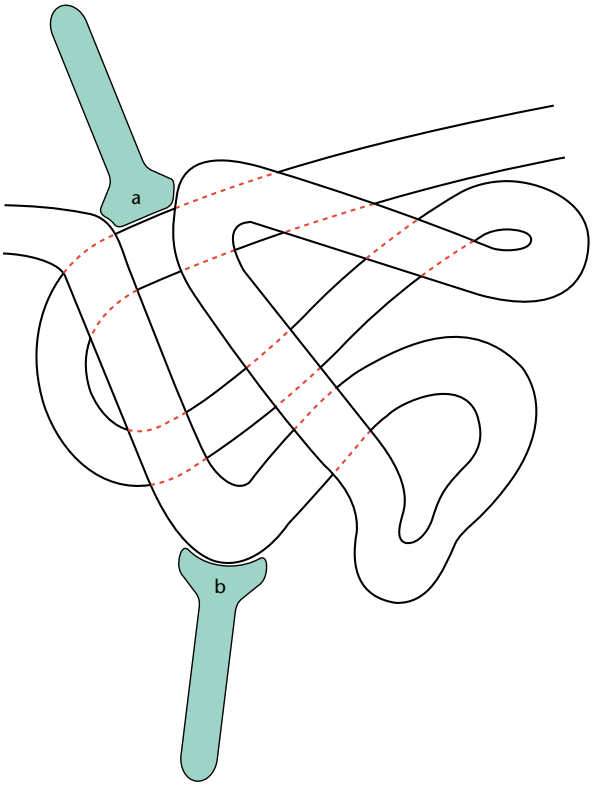

Figure 1 Antibody binding to a protein antigen. The figure shows a cartoon of a folded protein (black) with antibodies (red) bound to selected epitopes on it. Note that, while antibody a binds an epitope composed of 'noncontiguous' protein segments, antibody b recognizes a contiguous epitope.

In stark contrast to the broad canvas that B-cell antigens represent, recognition by T lymphocytes is principally restricted to polypeptide fragments that are usually derived from proteolytic degradation (also called antigen processing) of native proteins. Furthermore, unlike B lymphocytes which recognize soluble antigen, T-cell antigens are targeted only when associated with specific molecules on the surface of an appropriate cell. Thus T-cell antigenicity restricts itself to a single class of molecules, namely small peptides without dramatic structural variation and where the only true element of diversity permitted is at the level of the primary amino acid sequence.

Immune Recognition

The function of the adaptive immune system can be divided into two interrelated activities – recognition and response. The former defines the property of antigenicity, whereas an ability to invoke the latter describes the quality of immunogenicity. Regardless of qualitative distinctions in the recognition potential of B and T lymphocytes there is, nevertheless, a common feature relevant to recognition of larger, more complex antigens. Neither subset of immunocytes interact with, or recognize, the entire antigen molecule. Rather, lymphocytes individually recognize discrete sites on macromolecules. Such sites are called 'antigenic determinants' or 'epitopes'. Thus, antigens may also be defined as those molecules which possess within their structure recognition-competent domains for either T or B lymphocytes.

The hallmark of the adaptive immune system is not only the wide array of chemical entities that it can potentially recognize, but also the specificity of such individual recognition. Thus antibodies generated against a given protein, particularly in the later stages of the response, will not crossreact with another, even if they are closely related. Thus recognition of individual epitopes on an antigen by either B or T lymphocytes is of a highly specific nature. The basis for such exquisite specificity derives from the fact that immune recognition is dependent upon binding of antigen to antigen receptors on the surface of either B or T lymphocytes. If the antigen also happens to be immunogenic (e.g. protein or hapten conjugated to a carrier), then such a recognition results in activation of that lymphocyte, eventually leading to an effector immune response.

It is noteworthy that both the specificity of individual recognition and the wide range of antigens recognized stem from the requirement of receptor-mediated interactions. The antigen receptors on T and B lymphocytes are distinct from any other class of receptor in that the ligand-binding site displays a high degree of variability from one cell to another. Such variability derives from the fact that the antigen-combining site (or paratope) of both kinds of receptor is generated by rearrangement of multiple gene

segments where each segment is randomly selected from one of many members of individual gene families. Nondirected imprecision at the site of joining of the gene segments, as well as variable options of subunit pairing further add to the diversity of the antigen-combining sites generated. Whereas the principle of 'allelic exclusion' ensures that a given T or B lymphocyte will only bear identical receptors all expressing a common paratope, these are nevertheless distinct from those borne by its cohorts. As a result, while recognition by individual lymphocytes is extremely specific, the B- and T-lymphocyte pools collectively define a broad repertoire of antigen specificities. A fundamental distinction, however, remains between antigen receptors present on T lymphocytes and that borne on B lymphocytes. While the TCR always remains cell associated, B lymphocytes are empowered with the ability to secrete modified versions of their receptor as soluble antibody.

Antigen recognition by B lymphocytes

It has been noted earlier that BCRs recognize soluble antigen, which implies that antigens are generally recognized in the native form. This becomes particularly relevant for structurally complex macromolecules such as proteins and branched polysaccharides, where the topology of the molecular surface constitutes the epitopic repertoire available for B-cell (or antibody) recognition (**Figure 1**). Proteins, for example, are normally folded into a three-dimensional structure such that the hydrophobic domains are buried within the interior, whereas the hydrophilic domains remain exposed on the surface. In addition, secondary structural elements such as β turns also contribute to surface topology by protruding out from the protein surface, thereby facilitating binding by B cells bearing the appropriate receptor. Consequently, given the virtually limitless repertoire of B-cell specificities, it is generally believed that the entire accessible surface of a protein constitutes an antigenic continuum. This notion has spurred the generation of a variety of predictive algorithms that seek to identify surface-accessible domains on proteins whose primary amino acid sequence, but not the three-dimensional structure, is known, as potential approaches to localize B-cell epitopes on proteins. Identification has been attempted on the basis of anticipated biophysical properties such as local hydrophilicity, domain flexibility (or mobility), secondary structural motifs, etc. Thus, for example, any extended stretch of primary amino acid sequence which is composed predominantly of hydrophilic residues is expected to be present on the surface of a fully folded protein – thereby being available for recognition by B cells. Similarly, B-cell epitopes have also been predicted on flexible domains of proteins and segments with pronounced secondary structural motifs, such as β turns and either hydrophilic or amphipathic α helices. It must be mentioned, however, that such attempts yielded only limited success when predicted epitopes on a variety of protein antigens were compared with those experimentally determined by fine analysis of polyclonal antibodies produced against the same protein in an appropriate host. This further underscores the distinction between the potential antigenicity of a polypeptide segment and its functional immunogenicity.

Recognition of an antigen (or epitope) either by an antibody or its corresponding BCR follows the established norms of all protein–protein interactions. Recognition principally implies a binding interaction between the paratope of antibody (or BCR) and epitope, which is achieved only when two necessary preconditions are met: (1) There must be topological complementarity between the interacting paratope and epitope surfaces. (2) There must be a sufficient number of short range, noncovalent interactions (hydrogen bonding, Van der Waals interactions, salt-bridging, etc.) between the two contact surfaces, so as to cumulatively contribute favourably to the overall free energy of binding. In general, it has been found that the free energy of binding in an antigen–antibody interaction ranges between 18 and 25 $\mathrm{kcal\,mol^{-1}}$, with a contact surface area between 600 and 900 $\mathrm{Å^2}$.

In the literature, B-cell antigens are frequently described as either T-dependent or T-independent. This characterization is used to distinguish between antigens that induce a humoral response only in the presence of T-cell help (T-dependent), and those that do not require assistance from T-helper cells to elicit antibodies (T-independent). T-independent antigens have been further subclassified as type I if they are directly mitogenic for B cells and type II if they are nonmitogenic. Type I antigens frequently represent components of bacterial cell walls, the best known of which is lipopolysaccharide (LPS). Type II antigens, on the other hand, represent polymeric compounds presenting repeating units of the same antigenic determinant (e.g. polysaccharides). It is believed that binding of such antigens results, by virtue of their multivalency, in extensive crosslinking of the BCR, leading to cellular activation and proliferation without a requirement for T-cell help.

T-dependent antigens are molecules with either a mono- or limited valency of individual epitopes. Such antigens are unable to induce BCR crosslinking and, consequently, require assistance from T-helper cells to stimulate B-cell proliferation and differentiation. Most protein antigens fall under this category. The assignment of the terms T-dependent or T-independent, therefore, essentially define properties related to immunogenicity. It may perhaps be more appropriate then, to describe such molecules as T-dependent or T-independent B-cell immunogens.

Antigen recognition by T lymphocytes

Unlike B cells, the antigen receptor on T cells does not recognize native antigen, but only peptide fragments derived from the processing of larger proteins. Furthermore, recognition only occurs when the peptide fragment is presented on the surface of an appropriate antigen-presenting cell (APC), and in association with a member of the family of transmembrane proteins called the major histocompatibility complex (MHC) molecules. Thus, antigen recognition by T lymphocytes involves the formation of a trimolecular complex between the TCR, antigenic peptide and a self-MHC molecule. Another distinction between the two arms of adaptive immunity is that while B-cell (or antibody) recognition is solely directed against the antigenic determinant, T-cell recognition requires simultaneous contact between the TCR paratope and both the residues presented by the antigenic peptide and those provided by regions of the MHC molecule adjacent to the buried peptide. In other words, the overall structure bound by the TCR effectively represents a topological surface that is a composite of contributions from the nonself antigenic peptide and the self-MHC molecule (**Figure 2**).

Although the TCR alone is capable of binding peptide–MHC complexes the affinity for such interactions is low

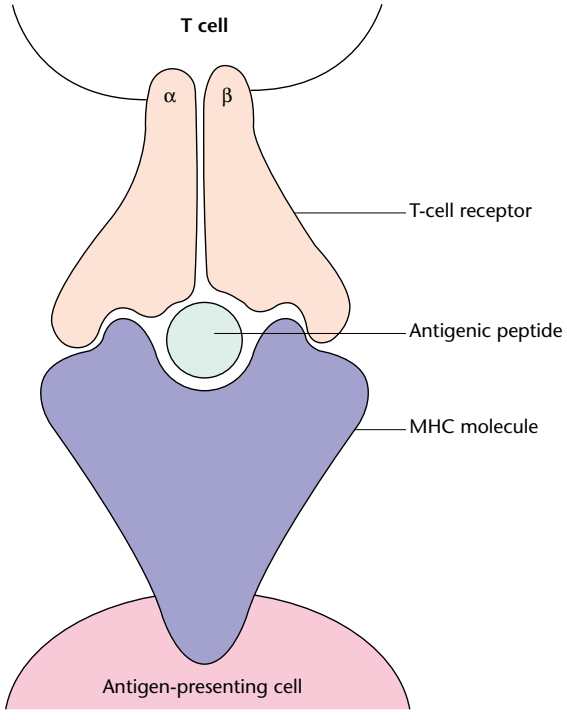

Figure 2 TCR recognition of a peptide–MHC complex, showing the interaction between an αβ TCR heterodimer and a peptide presented by an MHC molecule. Note that the TCR contact is with surfaces provided by both the antigenic peptide and neighbouring regions of the MHC molecule.

(K_d between 10^{-4} and 10^{-7} mol L^{-1}). As a result, binding of a T cell to an APC or target cell cannot depend on the affinity of this interaction alone. This is further compounded by the fact that, in normal circumstances, the frequency of both cells presenting a particular antigen and T lymphocytes with the appropriate receptor for binding to this peptide–MHC complex are low. Indeed a series of elegant studies have shown that antigen recognition by T lymphocytes does not constitute a direct receptor–ligand interaction, but represents a multistep process guided by assistance from accessory molecules. Adhesion molecules on the surface of both T lymphocytes and APCs act first to ensure a contact between the two cell types. Once cell-to-cell contact is made, the TCR then scans the APC membrane for peptide–MHC complexes that it can bind to. Binding then initiates a complex series of molecular events that eventually lead to proliferation and differentiation of the participant T lymphocyte into effector cells. In the event that the interacting APC does not express an appropriate peptide–MHC complex that can be recognized by the TCR, the cell-to-cell contact weakens and the T lymphocyte dissociates to continue its search for the right partner. It is clear, therefore, that antigen recognition by T lymphocytes represents a process that is far more complex and involved than that by B lymphocytes.

Depending on whether the antigenic peptide is presented in association with a self-MHC class I or class II molecule, it can serve as a target for recognition by either the CD8 + or CD4 + subset of T lymphocytes, respectively. Extracellular antigens or microbes that are taken up by APCs via receptor-mediated endocytosis, phagocytosis or pinocytosis are targeted to the lysosomes, where the protein components are degraded by lysosomal proteases. A select proportion of the fragments thus generated – those which have the requisite sequence – bind to the MHC class II molecule. Such peptide–MHC complexes are subsequently transported to the cell surface to await recognition by a CD4 + T lymphocyte bearing the appropriate TCR.

MHC class I molecules, on the other hand, present peptides derived largely from endogenously synthesized proteins. Endogenous antigens, such as those produced by a virus replicating within an infected cell, are degraded by the proteasome complex in the cytoplasm. Select peptide fragments are then transported to the endoplasmic reticulum where they bind to newly synthesized MHC class I molecules, before being transported to the cell surface for interaction with appropriate CD8 + T cells.

Crystal structure studies of peptides bound to MHC class I and class II molecules have revealed that the peptide usually is bound – in both cases – in an extended conformation with one face buried within the cleft of the MHC molecule, and the other exposed for TCR binding. Thus, for any peptide to possess T-cell antigenicity it must necessarily contain two distinct interaction sites. One site is required for binding to the MHC molecule, and the antigen residues involved in this interaction are called 'agretopic

residues'. The other site interacts with the TCR and the residues which comprise this site are known as 'epitopic residues'. While a given MHC allele can bind a variety of different peptides, the specificity of immune recognition is defined by the stringency imposed by the TCR. In this connection, the low affinity of TCR for the peptide–MHC complex serves as an important check since any variation in peptide sequence will result in an interaction that is of too low an affinity to be biologically relevant.

As in the case of B-cell recognition, attempts have also been made to predict – *a priori* – the locations of T-cell epitopes on protein antigens. While one such approach relied on the identification of specific motifs within the primary amino acid sequence, another predicted on the basis of segments capable of forming amphipathic alpha-helices in solution. Both of these approaches, however, have met with limited success – the reasons for which have become apparent since the elucidation of crystal structures of MHC–peptide complexes. As already alluded to earlier, these latter studies have revealed that the peptide is bound in an extended confirmation. A complicating factor in the prediction of T-cell epitopes is that it requires prediction of two distinct binding sites – one for MHC and the other for the TCR. This is further compounded by the diversity of MHC alleles, each with its own range of peptide specificities. Attempts are underway, in many laboratories, to delineate sequence motifs for binding to the various alleles of MHC class I and class II proteins.

In addition to the conventional T lymphocytes expressing a TCR composed of α and β subunits, there is also a small population of T cells with a receptor made up of γ and δ subunits. This subset of T lymphocytes appears to violate the established norms of T-cell recognition – namely, that all T cells are self-MHC restricted and recognize peptide antigens displayed in the cleft of the self-MHC molecule. Indeed $\gamma\delta$ T cells appear far more promiscuous in the type of antigens that they recognize and, in this respect, resemble B lymphocytes. For example, a $\gamma\delta$ T-cell clone has been derived that can bind directly to a herpes virus protein without requiring processing and presentation together with MHC. Again like B lymphocytes, antigen specificity of $\gamma\delta$ T cells does not appear to be restricted solely to polypeptide antigens. Activated $\gamma\delta$ T cells induced in response to a mycobacterial infection have been shown to bind some bacterial antigens that are protease resistant. One of these antigens has recently been identified to be isopentyl pyrophosphate. Thus, though many more details on this subpopulation remain to be elucidated, $\gamma\delta$ T cells appear to represent yet another (possibly more ancient) dimension to the cognitive armamentarium of the adaptive immune system.

It becomes obvious then, that the adaptive immune system is endowed with the enormous capability to recognize virtually any molecular species that it encounters. This ability, coupled with a 'learning' process that imparts a self versus nonself discriminatory capacity, enables it to mount a selective effector response against only those antigens that are foreign to the host. Nevertheless, immune responses against self antigens that are either altered in form (e.g. denatured proteins) or over-expressed (as occurs in some tumour cells) are also sometimes produced. While in general such responses are beneficial, constituting a surveillance machinery for cellular aberrations within the host, there are instances where a breakdown in self-tolerance leads to autoimmune responses with pathological consequences.

Antigenic Competition

Our understanding of the recognition repertoire of the adaptive immune system logically leads us to expect that virtually any antigen it encounters can be recognized and, subsequently, responded to. While this is undoubtedly true in principle, in practice, however, factors relating to immunogenicity often complicate the issue. For instance, it is not uncommon to find that immunization of a host with a complex mixture of diverse antigens – such as that represented by a microorganism – leads to an unequal immune response against the individual constituents of the mixture. This is largely dictated by variables in intrinsic immunogenicity between the antigenic components. Thus, for example, antibody responses to the T-independent antigen constituents would remain muted, primarily due to the absence of T-helper cell involvement – a factor critical for the amplification and differentiation of responder B cells. Even among the T-dependent antigens, the proportion of immune-effector responses directed against each will depend on their immunogenicity at the T-cell level, which can vary markedly from one antigen to another. As already discussed, T-helper cell antigenicity and immunogenicity requires proteolytic degradation of the target protein within the lysosome of APCs, and binding of the peptide fragments generated to MHC class II molecules. It can be envisaged, therefore, that T-cell immunogenicity, as defined by the ability to activate specific T cells and induce their proliferation, would then depend on the affinity of peptide binding to the MHC molecule because this would determine the number of MHC–peptide complexes available on the APC surface for TCR interaction. In addition to affinity, the concentration of peptide fragments generated within lysosomes will also determine the number of MHC–peptide complexes produced. This, in turn, will depend on the processing efficiency of the antigen, and the number of antigen molecules present in the original mixture (or microorganism). Finally, it must be remembered that a multitude of proteins are simultaneously being proteolysed in the lysosome. These include, apart from the antigen in question, other constituents of the antigen mixture, ligands endocytosed by unrelated receptors on the APC surface, and self proteins of membrane origin. As a

result, immunogenicity at the T-cell level is the outcome of an intensely competitive process in which antigenic peptides in the lysosomes compete not only among each other, but also with those derived from unrelated proteins – including self proteins – for binding to a limited number of available MHC class II molecules. It is the degree of success achieved in this process that will ultimately determine immunogenicity, not only at the level of T-helper cells, but also at the level of humoral immunity for T-dependent antigens.

It may be pertinent to emphasize here that neither antigen receptors nor the MHC molecules possess the inherent capacity to distinguish between epitopes derived from self and nonself antigens. Indeed, immune recognition (or binding) is an unprejudiced process governed by conventional physicochemical rules. Discrimination is enforced at the level of response, where 'self recognition' leads to tolerance either by deletion or inactivation of autoreactive lymphocytes. The discriminatory capacity is further enhanced by cellular components of the underlying innate immune system. In response to foreign antigens, particularly those derived from infectious agents, these cells produce a variety of chemical messengers which are now being commonly referred to as 'danger signals'. These molecules act on the lymphocytes, potentiating their ability to generate effector responses.

The phenomenon of 'selective' immunodominance is even more pronounced when the immunogenicities of individual epitopes on multideterminant antigens such as proteins are compared. The common finding here has been that not all B- and T-cell epitopes present on a protein antigen are equally immunogenic. While some remain cryptic, a 'hierarchy' of immunodominance is frequently observed against those determinants which display functional immunogenicity. Selective and hierarchical immunodominance for T-cell responses can easily be rationalized as the outcome of quantitative differences in loading of individual peptides onto MHC, resulting from the competitive processes described above. In such instances, competition would also include that among the various peptide fragments derived from the same antigen.

Selective and hierarchical immunodominance in T-dependent humoral responses has, however, proved more difficult to rationalize, particularly since B cells (or antibodies) directly bind their respective epitopes without taking recourse to accessory mechanisms. A variety of explanations has been put forward, of which the most prominent one proposes that the aetiology of selective recognition resides in the fact that antigen supplies become limiting soon after administration in the host. This is presumably due to proteolytic degradation. It is argued that a limiting antigen availability will restrict B-cell recognition to only those paratope–epitope fits that are of a high enough affinity, thereby restricting the spectrum of antibody specificities produced. More recent studies, however, suggest that it is not limiting antigen supply but rather a limiting frequency of antigen-primed T-helper cells that drives competitive selection, at least in a primary humoral response. These studies have demonstrated that while the early stage of a primary humoral response is indeed consistent with expectations in that all accessible domains of antigen are recognized, competitive selection is enforced during the subsequent stages of response maturation. Survival of an antigen-activated B cell continually depends on its ability to recruit T-cell help. Consequently, a limiting population of antigen-primed T-helper cells in an early primary response acts to enforce a competition between the numerous early activated B-cell clonotypes, or clonal subsets with distinct BCRs, for survival. The efficiency with which an antigen-activated B cell can recruit T-cell help is known to be directly related to the facility of antigen binding (either in terms of affinity or kinetics) by its BCR. This influences by defining the amount of antigen endocytosed by the BCR, subsequent to its processing and presentation of appropriate fragments as complexes with MHC for TCR recognition. As a result, those early activated B-cell clonotypes with favourable antigen-binding properties selectively engage the antigen-primed T-cell population, thereby denying it to the less competent clones, resulting in their elimination.

Antigen-binding characteristics of the BCR have also been shown to be critical in events downstream of the selection process by defining the quantum of T-cell help that a positively selected clonotype can recruit. This, in turn, will regulate the extent to which that particular cell can proliferate and eventually differentiate into antibody-secreting plasma cells. Collectively, these results provide a reasonable rationale for the observed phenomenon of selective and hierarchical immunodominance in humoral responses to T-dependent antigens.

In view of the enormous effort and energy that goes into equipping the adaptive immune system with a virtually unlimited recognition potential, why then does it restrict itself in its responses towards either multiple antigens or multiple determinants on a given antigen? The reason for this is not known. However, one may speculate that – in a situation where the total pool size must always remain constant – unrestricted proliferation of a given clonotype will always be at the expense of other, unrelated, cohorts. This could well lead to a 'dent' in the overall recognition repertoire. Yet another probable cause may stem from the need to minimize the possibility of generating autoimmune reactivities. Nondiscriminatory responses to a spectrum of determinants may increase the likelihood of generating effector responses that crossreact with one or more self antigens. Nevertheless, regardless of what the true explanation might be, it is abundantly clear that the adaptive immune system is virtually limitless in its potential but – at the same time – highly disciplined in its behaviour.

Further Reading

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