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Antigenicity and Immunogenicity of Viral Proteins

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Glossary

g0005 **B-cell epitope** Surface region of a native protein antigen that is specifically recognized by the binding sites of free and membrane-bound antibody molecules. The membrane-bound antibodies are the B-cell receptors that recognize the antigen during the immunization process.

g0010 **Cryptotope** Antigenic site or epitope hidden in polymerized proteins and virions because it is present on the surface subunits that become buried. Cryptotopes are antigenically active only after dissociation of protein aggregates and virions.

g0015 **Mimotope** A peptide possessing similar binding activity as that of a peptide epitope but showing little or no sequence similarity with it. Originally, mimotopes were defined as peptides able to bind an antibody but showing no sequence similarity with the protein antigen used to induce the antibody, usually because the antibody was directed as a discontinuous epitope.

g0020 **Neotope** Antigenic site or epitope specific for the quaternary structure of polymerized proteins or virions. Neotopes arise from the juxtaposition of residues from neighboring subunits or through conformational changes in the monomers resulting from intersubunit interactions.

g0025 **Paratope** Binding site of an antibody molecule that binds specifically to an epitope of the antigen. Paratopes are constituted of residues from six complementarity-determining regions (CDRs) located on the heavy and light chains of immunoglobulins. The CDRs vary greatly in sequence and in length in individual antibodies.

Virus Antigenicity

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p0005 The antigenic reactivity or antigenicity of viruses corresponds to their capacity to bind specifically to the functional binding sites of certain immunoglobulin molecules. Once such binding has been observed experimentally, the particular immunoglobulin becomes known as an antibody specific for the virus.

p0010 The antigenicity of nonenveloped viruses resides in the viral proteins that form the viral capsid, whereas the antigenicity of enveloped viruses resides mostly in the exposed proteins and glycoproteins that are anchored in the viral, lipid membrane. The oligosaccharide side chains of viral glycoproteins contribute significantly to the antigenic properties of enveloped viruses.

p0015 The antigenic sites or B-cell epitopes of viral proteins correspond to those parts of viral capsids and envelope proteins that are specifically recognized by the binding sites or paratopes of free and membrane-bound antibody molecules. Antibody molecules anchored in the outer membrane of B-cells correspond to the receptors of B-cells that recognize the antigen when it is administered to a vertebrate host during immunization. The B-cell receptors recognize the native tertiary and quaternary structure of viral proteins and the antibody molecules that are released when the B-cells have matured into plasmocytes, and also recognize the native conformation of the proteins.

p0020 During a natural viral infection or after experimental immunization, the immune system of the host may also encounter dissociated viral protein subunits and it will elicit antibodies specific for these components. In addition, during a viral infection, nonstructural viral proteins that are not incorporated into the virions will also induce the production of specific antibodies in the infected host. Diagnostic tests that detect antibodies to nonstructural viral proteins are useful for differentiating animals infected with, for instance, foot-and-mouth disease virus from vaccinated animals that possess only antibodies directed to the capsid proteins of the virus. In this case, the ability to differentiate vaccinated animals from infected animals by a suitable immunoassay is an important prerequisite for convincing trading partners that a cattle-exporting country is free of foot-and-mouth disease.

Viral Antigenic Sites

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p0025 In the absence of further qualification, the term epitope used in the present article refers to an antigenic site of a protein recognized by B-cells. Immune responses are also mediated by T-cells, that is, by lymphocytes that recognize protein antigens through T-cell receptors, after the antigen has been processed into peptide fragments. T-cell responses to viral antigens which involve the so-called T-cell epitopes will not be discussed here.

p0030 Because of the additional antigenic complexity that arises in viral proteins as a result of the quaternary structure of virions, it is useful to distinguish two categories of viral epitopes known as cryptotopes and neotopes. Cryptotopes are epitopes that are hidden in the intact, assembled capsid. They are located on the surfaces of viral

subunits that become buried when the subunits associate into capsids, and they are antigenically active only after dissociation or denaturation of virions. Neotopes are epitopes that are specific for the quaternary structure of virions and which are absent in the dissociated protein subunits. Neotopes arise either through conformational changes in the monomers induced by intersubunit interactions or result from the juxtaposition of residues from neighboring residues. These terms are useful when it is important to distinguish between the epitopes carried by different aggregation states of viral proteins. The availability of monoclonal antibodies has made it easy to identify neotopes and cryptotopes in many viruses whose antigenic structure has been analyzed in detail. There is evidence, for instance, that the trimeric form of the gp160 protein of human immunodeficiency virus (HIV) possesses neotopes that are not present in the monomeric form but are important for the induction of neutralizing antibodies.

p0035 Another important category of viral epitopes are the neutralization epitopes that are recognized by antibodies able to neutralize viral infectivity. Since it is the antibodies that bring about neutralization, it is appropriate to talk of neutralizing antibodies and not of neutralizing epitopes. The epitopes are best referred to as neutralization epitopes.

p0040 Infectivity neutralization depends on properties of the virus, the antibody, and the host cell. The ability of an antibody to interfere with the process of infection always takes place in a specific biological context and it cannot be described adequately only in terms of a binding reaction between virus and antibody. In many cases loss of infectivity occurs when the bound antibody molecules inhibit the ability of the virus to attach to certain receptors of the host cell. It can happen that the antibody prevents the virus from infecting one type of host cell but not another type.

p0045 Some picornaviruses such as the rhinoviruses occur in the form of as many as 100 different variants known as serotypes, each serotype being neutralized by its own antibodies but not by antibodies specific for other serotypes. In contrast, another picornavirus, that is, poliovirus, exists only as three serotypes. The stuctural basis for this difference in the number of neutralization serotypes between different picornaviruses has not yet been clarified.

s0015 Antibodies to Viral Antigens

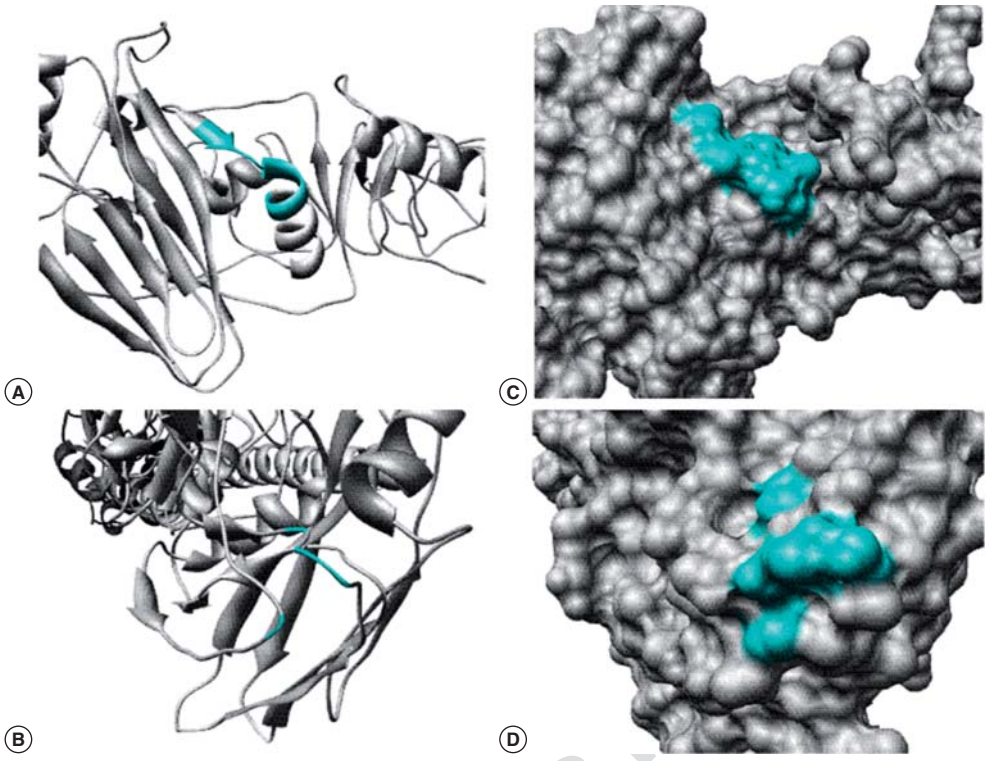
p0050 The capacity of antibodies to recognize viral epitopes resides in their functional binding sites or paratopes. The most common type of antibody is an immunoglobulin known as IgG which possesses two identical binding sites. These paratopes are constituted of three complementarity-determining regions (CDRs) located in a heavy chain and three CDRs in a light chain. The CDRs comprise a total of about 50 amino-acid residues and form six loops that vary greatly not only in sequence but also in length from one antibody to another. Each individual paratope is made up of atoms from not more than 15–20 CDR residues which make contact with a specific epitope. The so-called antibody footprint corresponds to an area on the surface of the protein antigen of about 800 $Å^2$. This means that as many as two-thirds of the CDR residues of an antibody molecule do not participate directly in the interaction with an individual epitope. These residues remain potentially capable of binding to other epitopes that may have little structural resemblance with the first epitope, a situation which gives rise to antibody multispecificity. The relation between an antibody and its antigen is thus never of an exclusive nature and antigenic cross-reactivity will be observed whenever it is looked for.

Continuous and Discontinuous Epitopes s0020

p0055 Protein epitopes are usually classified as either continuous or discontinuous depending on whether the amino acids included in the epitope are contiguous in the polypeptide chain or not (**Figure 1**). This terminology may Au3 give the impression that the elements of recognition operative in epitope–paratope interactions are individual amino acids, whereas it is in fact at the level of individual atoms that the recognition occurs. The distinction between continuous and discontinuous epitopes is not clear-cut since discontinuous epitopes often contain stretches of a few contiguous residues that may be able, on their own, to bind to antibodies directed to the cognate protein. As a result, such short stretches of residues may sometimes be given the status of continuous epitopes. On the other hand, continuous epitopes often contain a number of indifferent residues that are not implicated in the binding interaction and can be replaced by any of the other 19 amino acids without impairing antigenic activity. Such continuous epitopes could then be considered to be discontinuous.

p0060 Since discontinuous epitopes consist of surface residues brought together by the folding of the peptide chain, their antigenic reactivity obviously depends on the native conformation of the protein. When the protein is denatured, the residues from distant parts of the sequence that collectively made up the epitope are scattered and they will usually no longer be individually recognized by antibodies raised against the discontinuous epitope.

p0065 Discontinuous epitopes are often called conformational epitopes because of their dependence on the intact conformation of the native protein. However, this terminology may be confusing since it seems to imply that continuous epitopes, also called sequential epitopes, are



f0005 **Figure 1** Illustration of the difference between a continuous (a, b) and discontinuous epitope (c, d) in the hemagglutinin proteins of influenza virus. The residues that are part of epitopes are colored in blue ; the remaining residues are gray. (a) Ribbon representation of a continuous epitope. (b) Surface representation of the epitope shown in (a). (c) Ribbon representation of a discontinuous epitope. Residues distant in the sequence are brought close together by the folding. (d) Surface representation of the epitope in (c). From Greenbaum *et al*., 2007, with permission.

conformation independent. In reality the linear peptides that constitute continuous epitopes necessarily also have a conformation which, however, is mostly different from that of the corresponding region in the intact protein.

p0070 Most of our knowledge of the structure of discontinuous epitopes has been obtained from a small number of X-ray crystallographic studies of antibody–antigen complexes. It is important to realize that the structure of epitopes and paratopes seen in the complex may be different from the structure of the respective binding sites in the free antigen and antibody molecules, that is, before they have been altered by the mutual adaptation or induced fit that occurs during the binding interaction. For this reason, the structure of epitopes after complexation tends to be an unreliable guide for identifying the exact epitope structure that was recognized by the B-cell receptors during the immunization process. Crystallographic studies of antigen–antibody complexes have shown that the vast majority of protein epitopes are discontinuous and consist of residues from between two and five segments of the polypeptide chain of the antigen.

p0075 Most of our knowledge of protein antigenicity has not been obtained by X-ray crystallography but was derived from studies of the antigenic cross-reactivity between the intact protein and peptide fragments. In such studies, antibodies raised against the virus or isolated viral proteins are tested for their ability to react in various immunoassays with natural or synthetic 6–20-residue peptides derived from the protein sequence. Any linear peptide that is found to react in such an assay is labeled a continuous epitope of the protein. It is customary to test peptides of decreasing size and to give the status of epitope to the smallest peptide that retains a measurable level of antigenic reactivity. Usually this leads to the identification of continuous epitopes with a length of 5–8 residues, although the lower size limit tends to remain ill-defined. It is not unusual for certain di- or tripeptides to retain a significant binding capacity in particular types of solid-phase immunoassays.

p0080 On the other hand, increasing the length of peptides does not always lead to a higher level of cross-reactivity with antiprotein antibodies since longer peptides may adopt a conformation that is different from the one present in the intact protein. There is in fact no reason to assume that short peptides will have a unique conformation mimicking that of the corresponding region in the protein. Cross-reactivity of peptides with antiprotein antibodies is commonly observed because of antibody

multispecificity and of the induced fit and mutual adaptation capacity of the two partners.

p0085 Many investigators believe that the majority of so-called continuous epitopes described in the literature and listed in immunological databases actually correspond to unfolded regions of denatured protein molecules, that is, that they are not genuine epitopes of native proteins. They argue that there is usually little experimental evidence to show that short peptides are actually able to bind to antibodies specific for the native state of the cognate protein. It is indeed very difficult to demonstrate that the protein sample used in an immunoassay does not contain at least some denatured molecules that could be responsible for the observed binding reaction. Furthermore, claims made in the early 1980s that immunization with peptides always leads to a high frequency of induction of antibodies that recognize the native cognate protein are no longer considered valid. It is now accepted that such claims arose because the ability of antipeptide antibodies to recognize proteins was tested in solid-phase immunoassays in which the proteins had become denatured by adsorption to plastic.

s0025 Mimotopes

p0090 The multispecificity of antibody molecules is illustrated by the existence of so-called mimotopes. The term mimotope was coined in 1986 by Mario Geysen and was originally defined as a peptide that is able to bind to a particular antibody, but is unrelated in sequence to the protein antigen used to induce that antibody, usually because the antibody is directed to a discontinuous epitope. Currently, the term mimotope is applied to any epitope mimic, irrespective of whether the epitope being mimicked is continuous or discontinuous. It is indeed possible to mimic a continuous epitope with a cross-reactive mimotope peptide that shows little sequence similarity with the original epitope. This cross-reactivity of mimotopes is due to the fact that dissimilar amino acid residues may actually contain a sufficient number of identical atomic groups that will allow the peptide to interact with atoms of an antibody-combining site.

p0095 To qualify as a mimotope, a peptide should not only be able to bind a particular antibody but it should also be capable of eliciting antibodies that recognize the epitope being mimicked. This requirement stems from the fact that a single immunoglobulin always harbors a number of partly overlapping or nonoverlapping paratopes, each one capable of binding related or unrelated epitopes. When different subsites in the immunoglobulin-binding pocket partly overlap, binding to one epitope may also prevent a second unrelated epitope from being accommodated at a nearby location. Therefore, when a peptide is labeled a mimotope of epitope A because of its capacity either to bind to an anti-A antibody or to inhibit the binding of epitope A to the antibody, it cannot be excluded that the putative mimotope actually binds to a different subsite from the one that interacts with epitope A. This is why it is necessary to show that a peptide is also able to induce antibodies that cross-react with epitope A, thereby demonstrating that it really is a mimotope of epitope A.

p0100 Nowadays, mimotopes are often identified by testing combinatorial peptide libraries, obtained by chemical synthesis or phage display, for their capacity to bind monoclonal antibodies specific for viral proteins. It is also possible to screen a phage library by means of sera collected from individuals that recovered from a viral infection and have seroconverted. Mimotopes identified in this manner may find applications in peptide-based diagnostic assays. It is also believed that mimotopes could be used for developing peptide-based vaccines.

p0105 The antigenic reactivity of viral proteins discussed so far is based on chemical reactions between epitopes and paratopes and it can be described using parameters such as the structural and chemical complementarity of the two partners, electrostatic and hydrogen bond interactions between them, the kinetics and equilibrium affinity constants of the interaction, the discrimination potential of individual antibody molecules, etc. Such immunochemical investigations take the existence of antibodies for granted and do not ask questions about the biological origin, synthesis, or maturation of antibody molecules. The situation is different when it comes to investigations of the immunogenicity of viral proteins since this property cannot be analyzed outside of the biological context of an immunized host.

Immunogenicity s0030

p0110 Whereas antigenicity of proteins is a purely chemical property, their immunogenicity is a biological property that has a meaning only in the context of a particular host. Immunogenicity is the ability of a protein to give rise to an immune response in a competent host and it depends on extrinsic factors such as the host immunoglobulin repertoire, self tolerance, the production of cytokines, and various cellular and regulatory mechanisms definable only in a given biological context.

p0115 When a number of continuous epitopes of a protein have been identified, this knowledge does not provide information on which particular immunogenic structure, present in the antigen used for immunization, was recognized by B-cell receptors and initiated the production of antibodies. Our ignorance of the exact immunogenic stimulus is often referred to as the black box conundrum and this makes the study of immunogenicity very much an empirical endeavor.

p0120 Although any peptide identified as a continuous epitope will readily elicit antipeptide antibodies, it is only

rarely able to induce antibodies that also recognize the cognate, native protein antigen. In an immunoassay, an antibody raised against the native protein may be able to select one conformation in a peptide or it may induce a reactive conformation by an induced fit or mutual adaptation mechanism, the result in both cases being the occurrence of a cross-reaction between the peptide and the antiprotein antibody. On the other hand, when the same peptide meets a variety of B-cell receptors during the immunization process, it may not be able to bind preferentially to those rare paratopes in the receptors that, in addition to recognizing the peptide, also cross-react with the native protein. As a result most elicited antipeptide antibodies will only recognize the peptide and will not cross-react with the cognate protein.

p0125 As far as the immunogenicity of discontinuous epitopes is concerned, the situation is even more uncertain since the residues belonging to the epitope were identified by crystallographic analysis at the end of a process of mutual adaptation and conformational change in the two binding partners. The discontinuous epitope with its unique conformational features cannot be dissected out of the three-dimensional assembly of residues in the native protein and its immunogenic potential cannot therefore be studied independently of the rest of the protein antigen. As a result, the exact three-dimensional structure of the immunogen which was recognized by B-cell receptors and initiated the immune response cannot by known with certainty. Furthermore, reconstituting a discontinuous epitope in the form of a linear peptide that would include all the epitope residues from distant parts of the antigen sequence and would present them in the correct conformation appears to be an impossible task.

s0035 Implications for Vaccine Development

p0130 Our increasing knowledge of the structure of viral epitopes has given rise in some quarters to the expectation that it should be possible to develop peptide-based viral vaccines. However, the results so far have been disappointing. In spite of the hundreds of viral epitopes that have been identified by studying the antigenicity of peptide fragments of viral proteins, no commercial peptide vaccine has yet reached the marketplace.

p0135 Although most peptide fragments are immunogenic in the sense that they readily induce antibodies that react with the peptide immunogen, this type of immunogenicity is irrelevant for vaccination purposes. What is required is the induction of antibodies which, on the one hand, recognize the cognate, native viral antigen (so-called cross-reactive immunogenicity) and which in addition also neutralize the infectivity of the virus (so-called cross-protective immunogenicity). Unfortunately, very few of the continuous epitopes of viruses that have been described possess the required cross-reactive and cross-protective immunogenicity. Many attempts have been made to increase the conformational similarity between peptide and intact protein, for instance, by constraining the peptide conformation by cyclization but this has not resulted in peptides possessing adequate cross-protective immunogenicity. There is also some evidence that the intrinsic disorder in certain loop regions of viral proteins may be responsible for the finding that peptides corresponding to such disordered regions could potentially be better vaccine immunogens than peptides with a constrained conformation.

p0140 The difficulties that must be overcome to transform a continuous epitope into an effective vaccine immunogen are illustrated by the many studies of the peptide ELDKWAS corresponding to residues 662–668 of the gp41 protein of HIV-1. This peptide which is recognized by the anti-HIV-1 broadly cross-reactive neutralizing monoclonal antibody (Mab) 2F5 has for a long time been regarded as a promising vaccine candidate because it is located in a conserved region of gp41 necessary for envelope-mediated fusion of the virus.

p0145 The ELDKWAS peptide has been incorporated into a variety of immunogenic constructs in an attempt to have it elicit antibodies with the same neutralizing capacity as Mab 2F5. When additional gp41-derived flanking residues were added to the peptide or when its conformation was constrained, peptides were obtained which had a tenfold higher affinity for the 2F5 antibody than the unconstrained peptide. However, in spite of their improved antigenicity, the peptide constructs, when used as immunogens, were still unable to induce antibodies with detectable neutralizing capacity.

p0150 In an attempt to ascertain which structural elements close to the ELDKWAS residues in the gp41 immunogen may have influenced the induction of the neutralizing 2F5 antibody, the crystal structure of the 2F5 antibody in complex with various gp41 peptides was determined. The conformation of the bound peptides was found to differ significantly from the corresponding region in the gp41 protein, indicating that the putative ELDKWAS epitope was able to assume various conformations depending on the fusogenic state of gp41.However, it was not clear which conformation should be stabilized in the peptide constructs intended for vaccination.

p0155 It seems that the viral epitopes involved in the immunogenic stimulus may in fact be dynamic structures with variable conformations and it has been suggested that such epitopes should be referred to as transitional epitopes. Such a label would be an appropriate reminder that it is necessary to include the fourth dimension of time in the description of antigenic specificity.

p0160 The findings obtained with the ELDKWAS peptide suggest that these residues are part of a more complex

discontinuous epitope that elicited the neutralizing Mab 2F5. There is also evidence that the hydrophobic membrane environment close to the ELDKWAS sequence played a role in the induction of the neutralizing antibody 2F5.

p0165 Unfortunately, only some of the antibodies induced by viral antigens possess neutralizing activity and it is not known which structural features in the immunogen are responsible for the appearance of neutralizing rather than non-neutralizing antibodies. In recent years, attempts to find it out have relied mainly on the crystallographic analysis of viral proteins complexed with neutralizing monoclonal antibodies. Although such studies reveal the structure of static epitopes found in the complexes, they do not provide information on which transitional immunogenic epitopes are able to induce neutralizing antibodies. It seems likely that this type of information will only be obtained by systematic empirical trials in which numerous candidate immunogens are tested for their ability to induce protective immune responses.

See also: AIDS (00665); Immune response (00591); Neutralization of infectivity (00594); Vaccine strategies (00597).

Further Reading

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