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Multiplicity of Uses of Monoclonal Antibodies That Define Papillomavirus Linear Immunodominant Epitopes

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Abstract

During the last 10 yr, we have derived monoclonal antibodies from animals immunized with denatured bovine papillomaviruses type 1 major capsid (L1) protein, mapped their corresponding immunodominant epitopes to within a single amino acid (aa), and compared the reactivity of authentic L1 proteins to the predicted response by collinear analysis of the aa sequences of the same and other papillomaviruses (PVs). The data obtained from this approach has provided us with new insights into the sensitivity and specificity of the antibody response to viral proteins. We have included here some observations and conclusions that appear to be generic for the immune response, some of which might have applications for working with linear epitopes in other experimental systems.

Key Words

Immunodominant epitopes
Papillomavirus
Trimeric and pentameric peptides

Introduction

Viral serotyping has been used for many different purposes, including grouping of viruses into families *(1)*, identification and/or subtyping of new viral isolates, and detection of specific serologic responses to current (IgM response) or prior (IgG response) viral infections *(2)*. Seroepidemiological studies have provided invaluable information regarding the incidence and prevalence of specific viral infections (e.g., polio, rubella, and rubeola viruses, etc.) that have a significant impact on public health measures, frequently resulting in vaccine development *(3)*. Although viral serotyping can be accomplished by a variety of methods, availability and ease of rapid testing, as well as sensitivity and specificity of the tests are of paramount importance *(2)*. The use of viral or cell-associated conformational epitopes as antigens to test for reactivity with corresponding antibodies may be the most specific serological test available, since the antibodies react with epitopes related to biological functions that depend on conformational folding of viral proteins. For example, immunodominant, conformational bovine

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papillomavirus type-1 (BPV-1) major (L1) capsid epitopes elicit neutralizing antibodies that protect against formation of fibropapillomas in cattle. On the other hand, polyclonal antibodies (PAbs) and monoclonal antibodies (MAbs) produced against denatured L1 proteins are not protective. However, mapping these epitopes to the level of one amino acid (aa) by the Multipin Peptide Synthesis System has provided valuable insight into the characterization and use of these antibodies in experimental protocols.

Our interest in antibody recognition of immunodominant epitopes has evolved from MAb studies of linearized structural proteins of the BPV-1 L1 capsid proteins.We generated six MAbs, AU-1, AU-2, AU-3, AU-4, AU-5, and 1H8, whose minimal epitopes have been mapped using the predicted aa sequence encoded by the BPV-1 L1 gene. These studies were possible because recent advances in DNA technology and peptide chemistry *(4–9)* resulted in the ability to express PV proteins in bacteria and synthesize viral specific peptides in vitro *(9–15)*. These techniques introduced a new dimension of screening for PV gene products. This was important, because PV cannot be propagated in cell culture or transmitted easily to animal models, other than the xenograft system developed by Kreider et al. *(16)*.

Production of Highly Specific MAbs with Multiple Uses

PAbs and, especially MAbs, are highly useful for most (BPV-1) experimental protocols, especially those that include immunofluorescence (IF), immunocytochemistry (IC), immunoprecipitation (IP), enzyme-linked immunosorbent assay (ELISA), Western blotting, affinity chromatography, and epitope tagging. However, PAbs may yield a higher background of reactivity than desired. Also, MAbs frequently are poorly reactive, and the results obtained are equivocal. In our hands, there appear to be two major reasons for the latter. First, many MAbs are derived (because of time and cost) when the immune response is still maturing. These MAbs do not always appear to have the same high level of affinity and avidity as mature antibody populations that result from continual gene rearrangements and somatic mutations of antibody-producing cells. Second, and more importantly, MAbs should be screened by the same methods that reflect their intended use. For example. MAbs screened for positivity by ELISA may not be reactive with the same antigen by IF or IC, which makes it frustrating when the intended use is immunological identification of epitopes by the latter two techniques. Only about 1 in 70 candidate MAbs that recognize an epitope by one screening method can be expected to react with the same antigenic determinant using most methodologies. Thus, using standard methodologies with minimal modifications we derived MAbs that were highly cost effective.

Comparison of the Reactivity of Mab with Productive PV Infections with Homologous Minimal Epitopes Predicted by Collinear Alignment of the Major Capsid (L1) Proteins

AU-1 and AU-2 MAbs generated against sodium dodecyl sulfate disrupted BPV-1 capsids react with both BPV-1 and BPV-2 antigens in tissue sections by immunohistochemistry under conditions of very low dilution/high concentration. As reported previously *(17)* at 1:100 dilution AU-2 reacted with both PV types. However, at any dilutions greater than this, BPV-2 did not react, even though clean reactivity with no background was evident with BPV-1 at dilutions >1:2000. Comparisons of the L1 sequence of these two viruses (Tables 1 and 2) revealed that BPV-1 contained the FGAAN sequence that AU-2 recognized. BPV-2, however, had FGAAD, which accounted for the reactivity not found

Table 1. Comparison of immunohistochemical response to MAbs and sequence verification of the presence of the epitopes in the L1 genes of the various PVs by collinear analysis of L1 amino acid sequences

Virus	AU 1	1H8	AU-5
CRPV	LEDQYRYLQS	CEIG***FGA***MDHKT	VKS R_____ AY I
COPV	LDD**TYRY**INS	VDIG***FGA***MDFKA	VPKD T_____ YAT
HPV-2	LQD**TYRY**LQS	VETG***FGA***LDFATL	IPD E_____ LYIKS
HPV-6	LED**TYRY**VQS	VDTG***FGA***MNFAD	VPD T_____ LI IK
HPV-11	LED**TYRY**VQS	VDTG***FGA***MNFAD	VPD D_____ LLVK
HPV-16	LEDTYRFVT	VDTG***FGA***MDETTL	VPD D_____ LYIKG
HPV-18	LVDTYRFVQS	VDTGYGAMDFSTL	VPQ S_____ LY IKG
BPV-1	LED**TYRY**IES	MEIG***FGA***ANFKE[b]	APTT D_____ FY L
BPV-2	LED**TYRY**IES	MEIG***FGA***ADFKT[c]	APSK D_____ FY LKN
DPV	LEDIYRFIDS	MDIG***FGA***ANFKE[b]	LPPE A_____ YY LKN

[a]The correlation between actual reactivity and predicted reactivity was essentially 100% (underlined aa sequences are immunodominant epitopes defined by MAb). CRPV, Cottontail rabbit papillomavirus; COPV, canine oral papillomavirus; HPV, human papillomavirus; BPV, bovine papillomavirus; DPV, deer paplllomavirus.

[b]Reacts at high dilution with AU-2 (FGAAN).

[c]Reacts at low dilution only with AU-2 (FGAAD).

Table 2. Hierarchy of reactivity (under identical conditions) of MAbs 1H8 and AU-2 peptide sequences for MAbs IH8 and AU-2

1H8	AU-2
MEIG**FGA**	**FGAANF**
EIG**FGAA**	G**FGAAN**
MEIG**FGAA**	**FGAANFK**
MMEIG**FGA**	G**FGAANF**
IG**FGAA**	G**FGAANFK**
FGAANFK	IG**FGAANF**
EIG**FGAAN**	**FGAAN**
FGAAN	EIG**FGAAN**
G**FGAA**	IG**FGAAN**
EIG**FGA**	
FGAANF	
IG**FGAAN**	

in any other species of PV, except those from white-tailed and mule deer. These results indicate that *N*-(asparagine) is required to amplify the antigenicity of this sequence.

MAbs AU-1, AU-5, and 1H8 were reacted by IC with productive infections of 11 distinct (typed by molecular analysis of PV DNA) nonhuman and human PVs. Positive reactivities with authentic L1 proteins were compared to collinear analysis of the predicted aa sequences of the same PV to determine the extent of correlation between actual and predicted results (Table 2). The lack of reactivity of one MAb only did not correspond to the predicted sequence of a single human papillomaviruses (HPV) lesion. This was the only lesion in which the DNA was not typed, but predicted to be HPV-2 based on anatomic location in the patient, suggesting that it might in fact be HPV-1, which did not have the appropriate aa sequence. Thus, reactivity of highly specific MAbs with their corresponding immunodominant epitope can be predicted based on the presence of the critical aa residues of the epitope. This has allowed us to serotype productive PV infections using a multiplicity of MAbs that define different linear epitopes, each shared by subsets of PV L1 proteins.

Identification of a Trimeric Epitope Within a Pentameric Epitope: Functional Implications

We *(17)* recently mapped murine MAbs and rabbit PAbs generated against denatured BPV-1 L1 epitopes by overlapping hexameric peptides (using the Multipin Peptide Synthesis System) corresponding to 95% of the L1 protein. We identified both MAbs and PAbs that reacted with minimal epitopes that were composed of 3, 4, 5, and 6 sequential aa residues. One of the most antigenic sites was represented, in part, by the critical aa sequence FGAAN, a minimal epitope recognized by the MAb AU-2. MAb 1H8 recognized the amino acid sequence FGA, which is relatively highly conserved within many, but not all PV major capsid (L1) proteins (Tables 1 and 2). FGAAN is only present in the L1 protein of BPV-1 and DPV. From these results, it is apparent that 1H8 identifies an immunodominant trimeric epitope within the immunodominant pentameric epitope defined by AU-2 (**FGA**AN). The size of the minimal epitope appeared, in large part, to determine its specificity, and perhaps, molecular mimcry *(18)*.

Epitope Tagging Using Minimal Epitopes

One of the unexpected benefits of immunodominant epitope mapping has been the use of the corresponding MAbs for epitope tagging. AU-1 defines the critical aa sequence TYRY under all experimental conditions. When the BPV-1 L1 nucleotide sequences corresponding to TYRY and the natural flanking aa residues (DTYRYI) are cloned into a gene by polymerase chain reaction (PCR) (no kit is required), the gene product can be identified by its reactivity with MAb AU-1. This technique is particularly useful for localizing or identifying proteins expressed after transfection or infection of recombinant genes encoding, the tagged epitope *(19–21)*. The epitope tag has not interfered with the native function of the expressed polypeptide/protein. The flanking aa sequences that add significantly to the antigenicity of the critical amino acids were derived from a modified window net application of the Multipin Peptide Synthesis Sequence.

Naturally Occurring Antibodies May Recognize Minimal Epitopes

Minimal (contact) aa residues recognized by selected MAbs and PAbs sometimes appear to be recognized by low levels of naturally occurring antibodies. There are several possible explanations for this finding. If a protein released into the circulation from a disintegrating cell or microorganism retained its functional conformation, the consequences of its exposure to different cells and potential receptor sites might be deleterious. Therefore, it is logical to assume that an antibody response to denatured proteins processed by macrophages (and other antigen-presenting cells) would recognize many of the nonconformational epitopes in linearized polypeptides. On the other hand, small immunodominant peptides may induce a crossreactive antibody response against native proteins up to 6% of the time because of molecular mimicry *(18)*.

Recognition of Minimal Epitopes by Different Species

Although antibodies from different species frequently recognize the same antigenic site on PV L1 proteins, the corresponding minimal epitopes appear to differ, sometimes by as little as 1 aa. Therefore, it is difficult to block specific reactivities of antibodies from one species with those of another. However, it has been our experience that antibodies (PAbs and MAbs) from different species may recognize the same antigenic site up to 70% of time *(22)*.

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