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Mapping of viral epitopes with prokaryotic expression products

Brief Review

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Summary. Several systems are available for the expression of foreign gene sequences in *Escherichia coli*. We describe the use of prokaryotic expression products of viral gene fragments in order to identify the regions that specify the binding sites of antibodies. This approach is particulary successful if the antigenicity does not depend on the native protein, but only on the amino acid sequence, i.e., if the epitope is sequential. Combining prokaryotic expression with the use of synthetic peptides often permits a fast and accurate mapping of an epitope. The occurrence of immunodominant sequential epitopes on the surface of viruses seems to be a widespread phenomenon.

Introduction

As in most areas of biology and biochemistry, the study of viruses and their interactions with the immune system has been revolutionized by the recombinant-DNA technology. This review describes a new method for studying the antigenicity of viral proteins which involves the insertion of fragments from the gene coding for the protein into one of the many available expression vectors. By testing the antigenicity of the expression product, the protein segment involved in antibody binding can then be identified.

After the initial studies of Rüther et al. [98] with lysozyme, this approach led to the localization of several B-cell and T-cell epitopes. Recombinant antigens are most suitable for identifying the same category of epitopes that are detected by the use of synthetic peptides, namely the so-called linear or sequential epitopes. Expression products of viral genes are also useful for serodiagnosis and for studying virus neutralization.

Definitions and operational criteria

The antigenicity of proteins has been a controversial issue for many years [7, 10, 11, 129–131]. One source of confusion lies in the use of several terms that have overlapping meanings. Therefore, we will briefly define these terms as they are used in this review.

An *epitope* is the part of the antigen that is involved in the binding of a particular antibody. This definition implies than an epitope is specified by the antibody; and that an antigen may have as many epitopes as there are different antibodies recognizing the antigen [130]. Further, it should be noted that an epitope also depends on the method of localization. For instance, the use of peptides cross-reacting with the antigen may delineate an epitope that corresponds to part of the antigen-antibody interface observed by X-ray crystallography [131].

Not all residues within an epitope need to be essential for binding. A residue or a group of residues may be denoted as an *antigenic determinant* to indicate its measurable contribution to the binding of the antibody, rather than a passive presence in the epitope. Antigenic determinants can be defined by the analysis of escape mutants [84, 94, 137] or by the systematic replacement or deletion of residues [40, 73, 74].

Monoclonal antibodies that have identical, overlapping or adjacent epitopes will compete for binding to the antigen. We use the term *antigenic site* to denote the part of the antigen that is the target of a group of mutually competing antibodies. Although the whole surface of a protein may be potentially antigenic [10, 11], the number of antigenic sites seems to be limited.

Epitopes can be classified as *sequential* (or *linear*) or as *conformational* [106, 128]. Epitopes are sequential, if the antigenicity only depends on the primary structure of the protein antigen. Operationally, this is indicated if the antigenicity is retained after denaturation of the protein or if the epitopes can be mimicked by a peptide with the same sequence as the protein segment, but without any stable native-like conformation. Conversely, antibodies with conformational epitopes only bind to the antigen in its native conformation. The terms *conformation-dependent* or *conformation-independent* can be regarded as synonyms of conformational and sequential, respectively, 'conformation' referring to the *native* structure of the antigen.

Presumably, any epitope will have a defined conformation when bound to the antibody. Conceivably, conformational epitopes depend on the same intramolecular interactions that stabilize the structure of the antigen, while the conformation of a sequential epitope is mainly stabilized by intermolecular interaction with the antibody. This agrees with the notion that sequential epitopes correspond to those parts of the surface that are relatively mobile [121, 129, 136] or even disordered [2].

An independent criterion distinguishes between *continuous* and *discontinuous* epitopes [7]. In most cases, conformational epitopes will be discontinuous, i.e., formed by residues that are not contiguous in the amino acid sequence but are

brought together by the folding of the protein. However, continuous epitopes may also depend on the protein conformation [7]. Further, since replacement analysis [40, 73] has demonstrated that within an sequential epitope antigenic residues can be interspersed by non-antigenic ones, the distinction between continuous and discontinuous seems somewhat arbitrary [129]. Another reason for our preference of the original terms of Sela [106] is that a simple experiment—testing the effect of denaturation on antigenicity—decides on the classification.

Expression systems

In order to be expressed as a functional gene in a prokaryotic cell, a coding sequence must be flanked by a number of signals [16, 65, 101]: a promotor, a transcription termination site, a ribosome binding site, an ATG start codon and a stop codon. Except for the start and stop codons, these signals are specific for the host cell. Normally, all these signals are provided by the expression vector.

Three main factors determine the yield of an expression product:

The strength of the promotor

In most cases, a strong promotor, such as P_{tac} or λP_L, is preferred for a high level of gene expression. Further, to prevent continuous accumulation of the expression products leading to growth retardation or cell death, an inducible promotor is needed.

Factors governing the initiation of protein synthesis

These factors are the distance between the ribosome-binding site and the start codon, local mRNA secondary structure and other features of the sequence [104]. The problem of optimizing the initiation rate can be circumvented by fusing the foreign sequence to a well-expressed bacterial gene downstream of the bacterial start codon, leading to the synthesis of a hybrid protein.

The level of proteolytic degradation

This is probably the most unpredictable and critical factor. Another advantage of fusing the sequence to a bacterial gene is that hybrid proteins are often relatively stable, particularly when the accumulated expression product precipitates inside the cell [65, 69, 115].

Many applications require posttranslational processing and correct folding of the polypeptide [12]. If eukaryotic processing such as phosphorylation or glycosylation is necessary for antigenic activity, prokaryotic expression systems are of no use at all. Intracellular precipitation competes with correct folding of the protein and necessitates the use of elaborate in vitro renaturation procedures [76].

Table 1. *E. coli* expression vectors

Vector	Carrier protein or fusion gene[a]	Insertion site[a]	Promotor[a]	Restriction enzyme sites for insertion[b]	Reference
Phage λ vectors					
λgt11	*lacZ*	#1007-1008 #1007-1008	P_{lac} P_{lac}	*Eco*RI	[142]
λgt22	*lacZ*	#1007-1008	P_{lac}	*Eco*RI, *Sal*I, *Xba*I, *Not*I[c]	[47]
λJK2	*lacZ*	N-terminal	P_{lac}	several	[109]
λJK4	*lacZ*	C-terminal	P_{lac}	several	[109]
LacZ fusion plasmids					
pEX	*cro-lacI-lacZ*	C-terminal	λP_R	as pUC8[d, e]	[116]
pEX11-13	*cro-lacI-lacZ*	C-terminal	λP_R	as pUC8 + *Kpn*I, *Spe*I, *Sph*I[d]	[61 a]
pUEX[g]	*cro-lacI-lacZ*	C-terminal	λP_R	as pEX[d, e]	[13]
pEX627	*cro-lacI-lacZ* #1-181	C-terminal	λP_R	as pEX2	[117]
pEX3407	*cro-lacI-lacZ* #1-407	C-terminal	λP_R	*Bam*HI, *Sal*I, *Pst*I	[57]
pHK413	*cro-lacI-lacZ*	between	P_{lacUV5}	*Bgl*II, *Hin*dIII,	[112]
pHK414	*cro-lacI-lacZ*	*cro* and	P_{lacUV5}	*Sma*I, and	[112]
pTC413	*cro-lacI-lacZ*	*lacI*	P_{lac}	*Bam*HI	[55]
pMR[h]	*cI* (#1-157)-*lacI-lacZ*	between *cI* and *lacI*	P_{lac}	*Hin*dIII, *Bam*HI, *Sma*I, *Bam*HI[d]	[45]
pORF[h]	*ompF* (#1-37)-*lacZ*	between *ompF* and *lacZ*	P_{omp}	*Sal*I, *Bam*HI, *Sma*I, *Bam*HI or *Bgl*II, *Bam*HI, *Sma*I, *Bam*HI[d]	[134, 135]
pUI[i]	*lacZ*	N-terminal	P_{lac}	*Stu*I, *Dra*I, or *Sma*I (blunt)	[120]
pUK	*lacZ*	#5-6	P_{lac}	*Pst*I, *Bam*HI, *Eco*RI	[59]

pUR	*lacZ*	C-terminal	P_{lac}	*Bam*HI, *Sal*I, *Xba*I, *Hin*dIII or *Bam*HI, *Sal*I, *Pst*I, *Hin*dIII[d]	[99]
pWS50	(c*II* #1-12)-*lacZ*	between *cII* and *lacZ*	λP_L	*Nru*I	[110]
pWR590	*lacZ* #1-590	C-terminal	P_{lac}	several	[64]
Plasmids with fusion to other sequences					
pATH	*trpE* (336 codons)	C-terminal	P_{trp}	several	[139]
pCG150	*malE* (#28-392)-*lacZα*[k]	between *malE* and *lacZα*	P_{lac}	several	[29]
pCG806	*malE* (#1-392)-*lacZα*[k]		P_{lac}	several	[29]
pEx30	MS2 polymerase #1-99	C-terminal	λP_L	*Eco*RI, *Cla*I, *Hin*dIII, *Pst*I, *Xba*I, *Hin*dIII[d]	[119]
pEx31	MS2 polymerase #1-99	C-terminal	λP_L	*Eco*RI, *Bam*HI, *Pst*I, *Bgl*II, *Xba*I, *Hin*dIII[d]	[119]
pFS2.2	*E. coli* enterotoxin subunit B	C-terminal	P_{tac}	*Sau*I, *Bam*HI, *Eco*RV	[103]
pHE6	λ N-protein #1-33	C-terminal	λP_L	*Sma*I, *Bam*HI, *Sal*I, *Pst*I, *Hin*dIII	[75]
pIN-I	outer	#3-4	P_{lpp}	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[32]
pIN-II[l]	membrane	#3-4	$P_{lpp} + P_{lac}$	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[32]
pINIII-A[m]	pro-	#3-4	$P_{lpp} + P_{lac}$	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[32]
pINIII-B[m]	lipo-	#20-21	$P_{lpp} + P_{lac}$	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[52]
pINIII-C[m]	protein	#27-28	$P_{lpp} + P_{lac}$	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[32]
pIN-III-OmpA[l]	signal peptide of OmpA protein	C-terminal	$P_{lpp} + P_{lac}$	*Eco*RI, *Hin*dIII, *Bam*HI[d]	[32]
pJL6	λ *c*II #1-13	C-terminal	λP_L	*Cla*I or *Hin*dIII	[63]
pJLA16	λ *c*II #1-13	C-terminal	λP_L	*Cla*I or *Hin*dIII	[63]
pNGS	artificial #1-15[n]	C-terminal	P_{lac}	as pUC8 or pUC18	[118]
pRIT2[l]	staphylococcal	C-terminal	λP_R	*Eco*RI, *Sma*I, *Bam*HI	[81]
pRIT5[l, o]	protein A[k]	C-terminal	P_{spa}	*Sal*I, *Pst*I, and *Hin*dIII	[1]
pRX	*trpE* #1-18	C-terminal	P_{trp}	several[d]	[91]

Table 1 continued

Vector	*Carrier protein or fusion gene*[a]	Insertion site[a]	Promotor[a]	Restriction enzyme sites for insertion[b]	Reference
Plasmids allowing cleavage of hybrid protein					
pCG806fX[p]	as pCG806	as pCG806	P_{lac}	several	[68]
pEAP8[q]	penicillinase signal sequence	C-terminal	from penicillinase gene	*Hin*dIII	[54]
pGEX[k, r]	gluthathione S-transferase[s]	C-terminal	P_{tac}	*Bam*HI, *Sma*I, *Eco*RI	[113]
pJC264[t]	*cheY*	C-terminal	P_{lac}	*Pst*I, *Eco*RI	[38]
pJG200[u]	pro-α-2 collagen (60 residues)-*lacZ* #9-end	N-terminal	λP_R	*Bam*HI	[39]
pLcII-(nic-0)[p]	*c*II #1-31	C-terminal	λP_L	*Bam*HI, *Xba*I, *Sal*I, *Pst*I, *Hin*dIII	[79]
Plasmids without gene fusion					
pANK, pANH, pPL2	—		λP_L	*Kpn*I, *Hpa*I, or *Bam*HI	[107]
pKC30[v]	—		λP_L	*Bam*HI	[93]
pAS	—		λP_L	*Bam*HI, *Asp*718, *Sal*I, *Nco*I, *Nde*I, *Sma*I, *Xma*I, *Nru*I, *Hpa*I, or *Eco*RV	[108]
pOTS[w]	—		λP_L	*Bam*HI	[26]
pRC23[v]	—		λP_L	*Eco*RI	[23]
pEV-vrf	—		λP_L	*Eco*RI, *Bam*HI, *Cla*I[d]	[23]
pRDB8 pRDB9[x]	—		T4 gene *32*	several	[33]

ptac-12	—	P_{tac}	*Pvu*II	[4]
pKK233-2	—	P_{trc}	*Nco*I, *Pst*I, *Hin*dIII	[5]
pXmnATG	—	P_{tac}	*Xmn*I	[48]

[a] # Amino acid residues or codons. Abbreviations in italics indicate genes from which fragments have been used either as part of the fusion gene or (preceded by '*P*') as inducible promotor. *c*I: phage λ repressor gene, *c*II: phage λ regulator gene, *cro*: phage λ antirepressor gene, *galK*: *E. coli* galactokinase gene, *lacI*: *E. coli lac* repressor gene, *lacZ*: *E. coli* β-galactosidase gene, *malE*: *E. coli* maltose binding protein, *ompF*: *E. coli* outer membrane porin gene, *tac, trc*: *trp-lacZ* hybrid promotor, *trp*: *E. coli* anthranilate synthetase

[b] From 5′ to 3′

[c] λgt22 contains no *Sac*I site (Promega Notes 18, 1989), as originally reported [47]

[d] Available for expression in all three reading frames

[e] *Eco*RI site only present in one of the reading frames

[g] Essentially identical to pEX, but containing the c1857 gene coding for the temperature-sensitive repressor

[h] Synthesis of β-galactoside only if the insertion restores the reading frame

[i] Broad-host-range vectors

[k] Allows affinity purification of the hybrid protein

[l] Secretion of expression product

[m] Plasmid carrying *lac*I coding for the lac repressor

[n] Containing an Asn_6 sequence to stabilize the expression product

[o] Shuttle vector for secretion in periplasmatic space in *E. coli* and for extracellular secretion in gram-positive hosts

[p] The sequence Ile-Glu-Gly-Arg at the C-terminus of the carrier protein allows specific cleavage by the blood coagulation protease Factor X

[q] Excretion vector; signal sequence cleaved off after transport of product through inner membrane; outer membrane made permeable by the expression of the *kil* gene on the plasmid

[r] Expression products of pGEX-2T and pGEX-3X contain C-terminal of the carrier protein the recognition sequences of thrombin, and factor Xa, respectively, allowing specific cleavage of the hybrid protein

[s] Expression products reported to be both soluble and stable

[t] A methionine C-terminal of the cheY protein allows specific cleavage of the hybrid protein by CNBr

[u] The collagen sequence allows specific cleavage by collagenase

[v] No start codon provided by the vector

[w] Derivative of pAS1 containing the λ t_0 terminator

[x] Proteolytic degradation inhibited by infection with phage T4

Other expression vectors are described in [25] and [89]

A number of prokaryotic expression vectors are listed in Table 1. Indicated are the bacterial gene used to generate a hybrid protein (setting the compromise between solubility and degradation), the promotor and the unique restriction sites available for inserting the foreign sequence. The first expression system used to map viral antigenic determinants was the phage λ Charon 16 [82]. This phage is comparable to λ gt11 [142], one of the most popular expression vectors. In both phage systems, the foreign gene fragment is inserted in the *Eco*RI site near the 3′ end of the *lacZ* gene. After adding the synthetic inducer isopropyl-β-D-thiogalactopyranoside, the foreign sequence is expressed as part of a β-galactosidase hybrid protein. Direct immunoscreening allows the selection of recombinant plaques synthesizing an antigenic sequence. The main advantages of phage λ systems are the high efficiency of transfection, the possibility of screening plaques at a high density and the availability of worked-out, reliable protocols. The main disadvantage of λgt11 is the availability of only an *Eco*RI site for insertion; this has been eliminated in a new variant λ gt22 [47].

Like λgt11, several of the plasmid expression systems have been devised originally for the construction of cDNA expression libraries. Other systems have been constructed to investigate the products of open reading frames (pORF, pMR) or to produce native-like proteins. An advantage of plasmids is that the procedures for plating out, growing and DNA isolation are very simple. Furthermore, the new technique of electroporation allows efficiencies of transformation that are at least comparable to those of the packaging and transfection with λ DNA.

The most popular system for epitope mapping is the pEX system [116]. These plasmids contain the strong λ P_R promotor, regulated by a temperature-sensitive repressor, and a polylinker region at the end of a *cro-lacZ* fusion gene, available in the three different readings frames. During the development of this vector [115], it was found that insertions at the 3′ end of the fusion gene gave more stable expression products than insertions at the 5′ end. The pEX expression products are quite insoluble, ensuring that the product of virtually any foreign sequence is protected effectively against degradation. Further, lysis in SDS and transfer to nitrocellulose filters allow a direct immunoscreening of colonies.

To increase the versatility of the pEX system, the plasmids pEX11, pEX12, and pEX13 were constructed by incorporating a polylinker with 7 different sites [61a]. Incorporation in the plasmid of the cI857 gene coding for the temperature-sensitive P_R repressor yielded the pUEX plasmids, which can be propagated in normal host strains [13].

Mapping strategies

A prerequisite for epitope mapping via heterologous gene expression is the availability of recombinant DNA clones containing the relevant coding information. Different strategies have been used to generate subgenomic fragments

which, after insertion in a expression vector, direct the synthesis of an antigenic expression product.

Results can be obtained rapidly by using restriction enzymes, but the accuracy of localization in this case obviously depends on the presence of suitable cleavage sites. More accurate localization may be obtained by constructing a library of small DNase I fragments and selecting epitope-producing clones by immunoscreening of colonies [31, 25, 58, 66, 72, 82].

A third approach is the construction of a series of deletion clones with *Bal*31 [30, 100, 125], exonuclease III [17, 52] or restriction enzymes [41, 132]. However, epitope delineation by progressive deletions from only one side may lead to erroneous interpretations. Since the antigenicity of the expression product is destroyed as soon as one essential antigenic determinant is deleted, it is this determinant that is mapped and not the complete epitope [17, 41, 125].

Finally, a delineation with a resolution of a single amino acid residue can be obtained by expressing synthetic oligonucleotides [20, 61].

Viral epitopes synthesized in *E. coli*

Table 2 compiles the use of recombinant antigens for the mapping of viral epitopes.

With only a few exceptions [17, 55, 80] fusion proteins were solubilized in buffers containing SDS and a reducing agent. Subsequently, the products are fractionated by gel electrophoresis, transferred to nitrocellulose and incubated with antibodies. However, this procedure is only suitable for antibodies that are capable of recognizing the viral protein after Western blotting [9, 17, 20, 31, 36, 55, 61, 66, 67, 111, 123]. Conversely, negative results have been reported with monoclonal antibodies that recognize denaturation-sensitive epitopes [17, 36, 41, 61, 66]. An interesting exception is the conformational site IV on the G2 protein of Rift Valley fever virus [55], which could be localized within 20-residues by immunoprecipitation of an expression product.

Are epitopes synthesized in E. coli always sequential?

To what extent is an expression product inside the *E. coli* cell or immobilized on a blotting membrane able to fold to a native-like structure? In most cases the antigenicity appears to depend only on a small subsequence that can be flanked by any bacterial or viral sequence. In such cases formation to a stable native conformation is not likely and the epitope is evidently sequential. Indeed, several epitopes could be delineated further by testing synthetic peptides [44, 52, 56, 58, 61, 67, 82, 88, 100, 132, 141]. So, we may consider a prokaryotic expression product as antigenically equivalent to denatured protein. This does not exclude a local native-like structure, but only in the afore-mentioned case [55], this was substantiated by the negative effect of denaturation on antigenicity.

Table 2. Epitope mapping via expression in *E. coli*

Virus	Protein	Vector	Mapping strategy	Antibody[a]	Antigenic sequence(s)[b]	Reference
Herpesviruses						
Human cytomegalo-virus	gp58	pEX	restriction enzymes	NMAb, MAb	548-*614*-645[c]	[124]
Herpes simplex virus 1	gD	pEX	restriction enzymes	NMAb	1-55[d]	[56]
Herpes simplex virus 1	gD	pEX	DNase library	human	288-327, 355-369[d]	[58]
Epstein-Barr virus type A type B	EBNA 2 EBNA 2	pLCII pLCII	restriction enzymes	human, type specific	378-435 390-454	[95]
Hepadnaviruses						
Hepatitis B virus	HBcAg[e]	pEx31b	*Bal*31 3′ deletions, restriction enzymes	human, anti-(HBe + HBc) MAbs, anti-HBe2 MAbs, anti-HBc MAbs, other HBc sites	74-*83-89* 2-*79-138*, 2-*138-140*[f] 74-83, *(2-73)-(83-89)* 2-63, 2-*64-89*	[100]
Adenoviruses						
Adenovirus 2	E1A protein	pKRS101[g]	restriction-enzyme deletions	MAbs, rabbit	23-120[h]	[123]
Adenovirus 2	fibre polypeptide	pUC	restriction enzymes	MAb, NMabs, three sites	383-583	[133]
Papovaviruses						
Human papilloma virus 6b	L1 ORF product	pATH	restriction enzymes Exo III deletions[i]	human rabbit	420-433 197-222	[52]

Human papilloma virus 1	E4 gene product	pEX	*Bal*31 3′ deletions	MAbs	15-28, 15-*35-37*, 15-*61-63*, 15-*77-82*	[30]
SV40	T-antigen	pUR	restriction enzymes	NMAbs	446-708	[77]
Reoviridae						
Reovirus 3	σ1	pUC13	restriction-enzyme deletions	NMAbs	5-*125-226*-368 *(125-225)-(226-368)* *226-368*-455	[80]
Coronaviruses						
Mouse hepatitis virus	M	pEX	*Bal*31 5′ deletions	rabbit, immuno-dominant site[k]	217-227 *213-217*-227	[122]
Mouse hepatitis virus	S	pEX	restriction enzymes	NMAb, site A	839-862[l]	[67]
Infectious bronchitis virus	S	pEX	restriction enzymes, DNase library, oligonucleotides	rabbit, mouse chicken chicken MAb, site G MAb, site G MAb, site G NMAb, site G NMAb, site G	549-*570-582*[m] 549-566 *(553-555)-(565-567)* 549-*570-582* *555*-556-561[l] 555-*565-567*[l] *(553)-(554)*-563 *(549-554)-(567-569)-(570-574)*	[61, 66]
Transmissible gastroenteritis virus, feline infectious peritonitis virus	S	pEX	restriction enzymes	MAb, site B NMAb, site C MAb, site D NMAb COR5	*1-325*-378 *1-325*-378 326-*379-529*-558 607-*629-651*-660	[36, 88]
Paramyxoviruses						
Measles virus	fusion protein	pEX	*Bal*31 3′ deletions	MAbs	1-*17-306* 1-*330-513*[l]	[132]

Table 2 continued

Virus	*Protein*	*Vector*	*Mapping strategy*	*Antibody*[a]	Antigenic sequence(s)[b]	Reference
Measles virus	nucleoprotein	pRIT2	restriction enzymes, Exo III 3′ deletions[i]	MAbs, site I MAbs, site II MAbs, site III	23-*122-150* 457-475 457-*519-525*	[17]
Sendai virus	nucleoprotein	pRC23	5′ deletions with restriction enzymes	MAbs	1-*456-517* 1-*426-455* 1-*290-425* 1-*290-295*	[41]
				rabbit	1-*290-425*	
Newcastle disease virus	HN	pUC19	restriction enzymes oligonucleotides	NMAb	346-353	[20]
Rhabdoviruses						
Infectious hematopoietic necrosis virus	G	pATH3	*Sau*3AI	rabbit	335-450	[42]
Bunyaviridae						
Rift Valley fever virus	G2	pTC413	*Bal*31 deletions, restriction enzymes	NMAb, site I NMAb, site II MAb, site III NMAb, site IV	*(105-121)-(127-138)* 229-362 362-375 127-146[n]	[55]
Retroviruses						
Feline leukemia virus	gp70	λ Charon 16	DNase library	NMAb cI.25	213-226	[82]
Human immunodeficiency virus I	gp120	λ gt11	DNase library	MAbs, site a MAbs, site b[o] MAbs, site c MAb, site d MAb, site e	187-276, 210-274 393-408, 403-457 387-449, 409-499 21-85 34-55	[31]

Human immunodeficiency virus I	gp120	pOTS	restriction enzymes	chimpansee, immunodominant site	288-467[l]	[44]
Human immunodeficiency virus	gp41	λgt11	DNase library	human MAb	83-93	[9]
Human immunodeficiency virus I	gp41	pATH	restriction enzymes	human, immunodominant site	76-127[p]	[139]
Human immunodeficiency virus	p24	pUC	restriction enzymes	MAb	1-*177-244*	[60]
Picornaviruses						
Poliovirus 1	VP1	pBR322	restriction-enzymes and *Bal*31 deletions	NMAb, site 1	1-*96-104*[q]	[125, 141]
Human rhinovirus 2	VP2	pEx34[r]	*Bal*31 deletions	NMAb	1-*153-164*	[111]

[a] Species names denote polyclonal antisera
[b] Numbers amino acid or codon numbers. In italics, sequences of residues that have been shown to contain antigenic determinants
[c] The reported epitopes boundary 608-625 is only based on an assumed epitope length of 6 and 9 residues
[d] More accurate localizations by testing synthetic peptides
[e] HBc, HBe1, and HBe2 denote three antigenic sites on the core antigen HBcAg or on its antigenic variant HBeAg, both products of the C gene. HBc has been mapped with monoclonal antibodies, but is also the most immunodominant site recognized by human polyclonal antisera
[f] Evidence cited that residues 2-77 contain essential determinants of site HBe2
[g] pBR322 derivative with a *BglII* cloning site near the 3′ end of the *trp* gene
[h] Localized more accurately by testing antibody binding of protein fragments or adenovirus-SV40 protein fragments
[i] Exo III, exonuclease III
[k] Affinity purification via adsorption to the expression products yielded two fractions that recognized the two different epitopes
[l] Localized more accurately by PEPSCAN peptide analysis
[m] Residue numbering according to [61]
[n] Epitope sensitive to denaturation by boiling in SDS and dithiothreitol and localized by immunoprecipitation
[o] MAbs capable of blocking the interaction with the cell receptor CD4
[p] Same epitope localized with peptides [43, 105]
[q] Epitope localization confirmed by analysing escape mutants [84]
[r] Similar to pEx30 and pEx31

As mentioned before, a sequential epitope may represent a component of the complete epitope as would be observed by X-ray crystallography. This is exemplified by two discontinuous epitopes of foot-and-mouth disease virus [87]. A similar situation may exist for site IV or D of transmissible gastroenteritis virus [88].

T-cell epitopes

All epitopes discussed so far are the targets of the soluble immunoglobulins, which are relevant for the humoral immune response. The cellular response is mediated by the T-cell receptor of T-lymphocytes. According to the current consensus, T-cell antigens are processed inside the antigen-presenting cells. This generates antigen fragments, which are bound on the cell surface by class-I (for cytotoxic T-cells) or class-II (for helper T cells) major histocompatibility antigens [for a review, see 24]. As a consequence of this process, T-cell epitopes are inherently sequential and can be mimicked by peptides [96, 97] or λgt11 expression products [62]. In two recent reports, pEX expression products have been used to localize T-cell epitopes of a viral protein, the F protein of measles virus [27] or the E2 protein of Semliki Forest virus [114].

Comparison with other methods of epitope mapping

The suggestion that the antigenicity of bacterial expression products is almost exclusively limited to sequential epitopes implies that the same epitopes can also be mimicked by synthetic peptides. However, expression of gene fragments in *E. coli* should be considered as an approach complementary to the use of synthetic peptides, rather than as an alternative [61, 66]. Expression products can localize an antigenic sequence within 20 to 100 residues, depending on the available restriction sites and the mapping strategy. Within such a region, peptides can then be used for an exact localization. The combination of expression in pEX and PEPSCAN peptide synthesis has been applied successfully to measles virus [132], different coronaviruses [61, 66, 88] and to a T-cell epitope of *Mycobacterium tuberculosis* [126, 127].

A number of epitopes delineated by using expression products could not be detected by PEPSCAN analysis [61, 88]. This is most likely explained by the length of these epitopes: 11 and more than 17 residues for two epitopes of infectious bronchitis virus [61] and more than 21 residues for an epitope of feline infectious peritonitis virus [88].

Recently [87], the use of combinations of peptides to delineate discontinuous epitopes of foot-and-mouth disease virus was reported. This method, if generally applicable, would be a useful alternative to the analysis of MAb-resistant or non-binding mutants, which in principle only gives information about antigenic determinants.

A few reports [21, 34, 51, 70, 77, 123] describe the use of eukaryotic expression for epitope mapping. Three epitopes on the gD protein of herpes

simplex virus I were sensitive to reduction and alkylation, but not to 0.1% SDS [21], suggesting that these epitopes were partially conformational. However, other epitopes localized by eukaryotic expression could also be mimicked by prokaryotic products [77, 123] or peptides [28, 51]. It seems unlikely, therefore, that eukaryotic expression of gene fragments is a general method to localize conformational epitopes.

Serological studies

Polyclonal antisera are likely to contain antibodies which, by their specificity for linear epitopes, recognize prokaryotic expression products. This then allows the use of such products for serodiagnosis. The sera of AIDS patients appeared to recognize the bacterial expression products of *env* [18, 19, 22, 50, 53, 139], *pol* [83], or *gag* [140] fragments from human immunodeficiency virus (HIV). In addition, differences between individual sera could be defined by expression of the HIF *tat* gene [3] or fragments of the *env* [139] or *gag* [140] genes. Similar studies have been carried out with human T-cell leukemia virus-I [53, 86, 102]. A fusion protein containing the sequence coded by the BMRF1 open reading frame of Epstein-Barr virus was considered as serodiagnostic indicator protein [92].

Immunogenicity of expression products

Antisera raised against bacterial expression products that cross-react with the native antigen will have, like anti-peptide sera, a predetermined specificity. Studying the neutralization of viral infection by such antisera could be of relevance for vaccine development. Despite negative results with canine parvovirus [112], infectious bursal disease virus [8] and bovine rotavirus [37], there are several reports about expression products that did induce in vitro neutralizing sera. Examples are the gp120 sequence from HIV [90], the VP7 sequence from simian rotavirus [6], the VP7c sequence from bovine rotavirus [71], the major antigenic site on VP1 from foot-and-mouth-disease virus [14, 15, 138], the VP1 regions 52-302 and 24-129 from poliovirus I [49], the VP2 sequence from infectious pancreatic necrosis virus [64], and the N-terminal gD sequence from herpex simplex virus I [56, 58]. More spectacular is the induction of protective immunity. This was observed with a recombinant immunogen containing the core-antigen sequence of hepatitis B virus in one of two chimpanzees [78], with the G sequence from hematopoietic necrosis virus in fish [42] and with E2 sequences from Semliki Forest virus in mice [46]. In the latter case, the sequences eliciting partial or complete protection were localized within residues 114-149 and 216-288, respectively. Remarkably, no in vitro neutralization was observed.

A view on viral antigenicity

The available information on the location of sequential epitopes allows a few generalization to be made.

The distinction between antigenic sites, recognized by a group of mutually competing MAbs, and the epitopes of individual MAbs has now be substantiated [31, 61, 66, 88, 100]. The number of these sites found on a viral protein is usually limited. Often, one of the sites is immunodominant and is recognized by the majority of polyclonal antisera and/or monoclonal antibodies [43, 44, 61, 66, 84, 85, 94, 100, 105, 140]. All these sites appear to be sequential. Therefore, the preference of the immune system for certain sites may be explained by the location of regions that by their segmental mobility can conform to the paratopes of the antibody [136]. This does not exclude, however, the presence of conformational sites on other parts of the accessible surface. So, the concept of an antigenic structure, specifying a limited number of antigenic sites [7], can be reconciled partially with the notion that the whole surface of the protein is potentially antigenic [10, 11].

By their location, viral surface proteins are likely to be involved in molecular recognition processes and to interact with the host immune system. Conceivably, flexible regions on the surface are a typical feature of this category of proteins.

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