The Type-specific Epitopes of the Epstein–Barr Virus Nuclear Antigen 2 Are Near the Carboxy Terminus of the Protein

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SUMMARY

The Epstein–Barr virus nuclear antigen 2 (EBNA 2) shows serotype variation and two serologically distinct groups of viruses have been identified. These correspond to the two hybridization groups of viruses (A and B) that are distinguished by a highly substituted nucleic acid sequence in the middle of the open reading frame of the EBNA 2 gene. An epitope survey of the EBNA 2-coding region was carried out using a new prokaryotic expression vector tailored to express DNA fragments from the M13 sequencing libraries of the B95-8 (type A) and Jijoye (type B) prototype virus strains. Short overlapping stretches of EBNA 2 sequence were expressed as fusion proteins and used in Western blotting with human sera that contained serotype-specific antibodies. The type A-specific epitope was located between residues 378 and 435 of the B95-8 EBNA 2 polypeptide and the type B-specific epitope mapped between residues 390 and 454, at the carboxy terminus of the Jijoye polypeptide chain. All of the type-specific anti-EBNA 2 sera tested reacted with fusion proteins containing one or other of these epitopes. Despite the direct correlation between the hybridization and serological phenotypes, the type-specific epitopes appear to lie in the relatively conserved carboxyl-terminal region of EBNA 2. There was no indication that the residues of the non-homologous region contributed to the formation of antibody-combining sites.

INTRODUCTION

The immortalization of human lymphocytes in vitro by Epstein–Barr virus (EBV) has been used to form a model of the processes of transformation that may result in the virus-associated tumours Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) (de Thé, 1982). In both immortalized lymphocytes and tumour cells the viral genome is present as an autonomously replicating episome (Pope et al., 1968; Kaschka-Dierich et al., 1976; Lindahl et al., 1976; Raab-Traub & Flynn, 1986; Brown et al., 1988) that may express up to six nuclear proteins (EBNA 1, 2, 3A, 3B, 3C, 4) and one membrane protein (LMP) (Summers et al., 1982; Bodescot et al., 1984; Dambaugh et al., 1984; Addlinder et al., 1985; Dillner et al., 1985a, b; Hennessy et al., 1985; Hennessy & Kieff, 1985; Rowe et al., 1985, 1987; Petti et al., 1988; Ricksten et al., 1988). These products are encoded by latent genes which represent a small subset of the genes carried within the viral genome (Baer et al., 1984). Recent evidence indicates that a second potential membrane protein may be encoded by a highly spliced gene that is formed by fusion of the viral terminal repeats shortly after infection and is also expressed in latently infected cells (Laux et al., 1988).

Some, and possibly all, of these genes are expected to have roles in the immortalization process. Expression of LMP in RAT-1 cells can lead to morphological transformation and a tumorigenic phenotype (Wang et al., 1985). In addition, spontaneous deletion events in long-term cultured BL cell lines have produced non-immortalizing mutants of EBV that lack the EBNA 2 open reading frame and the 3' unique part of the EBNA 4 (also called LP or EBNA 5)
coding sequence (Miller et al., 1974; Rabson, 1982; Bodescot et al., 1984). Although the effects of EBNA 4 expression on lymphocytes remain unclear, a number of significant biological effects have been attributed to EBNA 2 expression. In particular the induction of CD23 activation antigen on the surface of infected lymphocytes appears to correlate with EBNA 2 expression (Wang et al., 1987). The gene for EBNA 2 contains within its open reading frame a region of nucleotide sequence diversity that has been used to classify viruses into two groups (type A and type B). The EBNA 2 protein differs between type A and B viruses; type A protein is 488 amino acids long and has an $M_r$ of 52K whereas the B protein is 455 residues long and has an $M_r$ of 49K. Variations in the frequency of transformation and the phenotype of immortalized lymphocytes have been attributed to virus types (Rickinson et al., 1987). Epidemiological studies have shown that there is a geographical localization of virus types; the A type is found primarily in Europe and North America and the B type is predominantly of African origin (Zimber et al., 1986).

EBNA 2 has been implicated as a target for both cell-mediated and humoral immune responses in infected individuals. Although uncloned virus-specific cytotoxic T cells from individuals infected with type A virus lyse type A- or type B-infected cells equally well (Wallace et al., 1987), clonal lines of T cells have shown some virus type-specific interactions with targets (Moss et al., 1988). Serologically, 8% of normal control individuals have anti-EBNA 2 antibody at titres of greater than 1:10. The proportion of antibody-positive individuals is as high as 40% in NPC and EBV-associated BL patients (Seigneurin et al., 1987). Human sera that recognize EBNA 2 on Western immunoblots appear to do so in a type-specific manner (Dambaugh et al., 1984: Rowe et al., 1985). So far the EBNA 2 reactivity of human sera on a number of lymphocyte cell lines directly parallels the A or B type nucleic acid sequence homology of the resident viral genome. It might be suggested that this correlation indicates an underlying causal relationship in which the region of sequence diversity directly alters the epitope recognized by human sera.

An epitope map would be required to confirm this speculation and to identify the amino acid sequences that are involved in forming type-specific epitopes. The most common approaches to determining amino acid sequences of epitopes involve either sequence analyses of neutralizing antibody-resistant viruses or screenings of purified proteolytic fragments or collections of synthetic overlapping peptides (for review see Benjamin et al., 1984; Berofsky, 1985). Recently Mehra et al. (1986) have used a lambda gtl 1-based fusion protein expression system to define epitopes on the 65K protein antigen of Mycobacterium leprae. We have taken a similar approach using a new prokaryotic expression vector to synthesize small fusion products which express short stretches of overlapping EBNA 2 amino acid sequence. The reactivity of human anti-EBNA 2 sera with these fusion fragments allows a tentative identification of sequences in the carboxy-terminal region of the protein as encoding type-specific and type-common epitopes.

**METHODS**

**Materials.** Restriction enzymes, DNA polymerase I (Klenow fragment), mung bean nuclease and T4 DNA ligase were obtained from New England Biolabs. Plasmid pLclII and phage M13mp11 FX were gifts of K. Nagai (MRC, Cambridge, U.K.). The M13 library of EBV clones was obtained from B. Barrell (MRC, Cambridge). The wild-type lambda lysogen EQ192 was obtained from J. Karn (MRC, Cambridge) and the temperature-sensitive CI lambda lysogen N4830 was purchased from Pharmacia. Radioactive nucleotides and 125I-labelled Staphylococcus aureus Protein A were supplied by Amersham.

**Expression vector construction.** A 28 bp oligomer (5'CGACATCTGGAATTCTGAGTGAGTGAGG3') and a complementary 26 bp oligomer (5'CCTCAGTCATCGAGTGGAGGTGG3') were synthesized. This sequence contains a BglII site, an EcoRI site, and an all-phase chain termination signal. Digestion of the annealed oligonucleotides with mung bean nuclease or filling in the 5' overlap with Klenow polymerase produced oligomers of various lengths. The hybridized oligomers were cloned into the Stnl site of the phage M13mp11 FX (Nagai & Thogerson, 1984) to produce three clones varying by one nucleotide (mFEX0, -1 or -2). The oligomers together with the Factor X (FX) recognition sequence in mp11 FX were then excised from the phage replicative form as BamHI fragments and cloned into the BamHI site of plasmid pLclII (Nagai & Thogerson, 1984) to create a series of three vectors, pLEX0, -1 and -2 (Fig. 1). Before this cloning step the sole EcoRI site in pLclII was destroyed by digesting the DNA with EcoRI, filling in the overhanging 5' ends and religating the plasmid.
**Location of EBNA 2 epitopes**

![Diagram of the pLEX expression plasmid](image)

Fig. 1. Structure of the pLEX expression plasmid. The pL promoter of lambda, upstream from the CI/nut repressor-binding sequence, drives the inducible expression of the first 31 amino acids of the CII open reading frame. A synthetic oligonucleotide encoding the FX recognition sequence, a variable number of guanine residues, BglII and EcoRI restriction sites, and an all-phase chain terminator were inserted into the BamHI site in the CII open reading frame. The BglII–EcoRI site was used to insert BamHI–EcoRI fragments from the EBV M13 library.

**Cloning and expression of EBV DNA.** Selected M13 clones from an EBV sequence library contained short stretches of EBV sequence (300 to 500 bases) cloned at the Smal site of M13mp8. EBV insert lengths were determined by primer extension radiolabelling of the DNA followed by excision with BamHI and EcoRI, and electrophoresis in 5% sequencing gels. Where necessary the nucleotide sequence across the M13 BamHI site into the EBV DNA was determined by the dideoxynucleotide chain termination method (Sanger et al., 1977). Clones with EBNA 2 open reading frames in a 5′ BamHI and 3′ EcoRI sense orientation were excised and the fragments were purified after agarose gel electrophoresis on 1% gels with Tris-borate-EDTA buffer in a minigel apparatus (Raven Instruments).

The bacterial expression plasmids (pLEX0, -1 and -2) were digested with BglII and EcoRI and then treated with alkaline phosphatase as previously described (Maniatis et al., 1982). Purified BamHI–EcoRI EBV fragments from the M13 library were ligated into the appropriate pLEX expression vector which maintained the continuity of the CII and EBV open reading frames. Ligation mixtures were used to transform the lambda lysogen EQ192. Plasmids with inserts (pEX) were transferred to the expression strain N4830. Overnight cultures of plasmid-harbouring N4830 cells grown at 30 °C were diluted 1:100 and grown to an O.D. of 0.7 in 2 x TYE medium (Difco). An equal volume of medium preheated to 65 °C was added and cultures were incubated at 42 °C for an additional 3 h. Induction was monitored by labelling 1 ml aliquots of culture with 50 μCi of [35S]methionine for 5 min or by Western blotting with an anti-peptide serum directed against the amino-terminal amino acid sequence of the CII gene product.

**Sera.** The human anti-EBNA 2 sera used in this study were collected from normal individuals, chronic mononucleosis (CM) patients and BL patients. Type specificity was analysed by Western immunoblotting against a panel of three cell lines: BIAB, an EBV-negative BL line; K50-7, an EBV type A-positive cord blood lymphoblastoid transformant; IARC-BL16, an EBV type B-positive BL line.

**SDS-PAGE and Western blotting.** The lymphocytes were solubilized in sample buffer (0-625 M-Tris–HCl pH 6.8, 2% SDS, 0.1% 2-mercaptoethanol, 10% glycerol, 0.02 mM-bromophenol blue) and proteins were separated by the discontinuous gel electrophoresis technique (Laemmli, 1970). Gels, 1.5 mm thick and 180 mm long, consisted of a 10% resolving gel (acrylamide:bis-acrylamide, 30:0.8) and a 5% stacking gel. Generally 20 μl of sample representing 2 × 10^6 to 3 × 10^6 cells was electrophoresed at 60 V for 12 h and blotted onto nitrocellulose by procedures which have been described previously (Burnette, 1981; Fischer et al., 1984; Berofsky, 1985). After...
blotting, the non-specific antibody-binding sites on the filter were blocked with dilute skimmed milk (Johnson et al., 1984). The immunoreagents, when bound to the nitrocellulose filters, were detected with $^{125}$I-labelled staphylococcal Protein A. Mr determinations were made using prestained high and low Mr standards (Bio-Rad), run on the same gels.

RESULTS

Sequence comparison of type A and type B EBNA 2

Previously, a sequence analysis of the EBNA 2 open reading frames of EBV isolates B95-8 (type A) and AG876 (type B) had revealed a region of divergence near the middle of the encoded proteins (Dambaugh et al., 1984). Sequencing of the EBNA 2 gene of a second type B isolate, Jijoye, was recently completed (B. Barrell, personal communication) and surprisingly it showed complete identity with AG876. Over the entire 1464 bases of the open reading frame not a single base substitution had occurred, in marked contrast to the high degree of substitution between the type A and type B viruses. Fig. 2 shows the homology plot for a comparison of the B95-8 and Jijoye amino acid sequence by the Dayhoff proportionality algorithm method (Staden, 1982). The plot shows two 'bubbles' along the diagonal homology line. The first (nearest the amino terminus) is due to the presence of the Pro repeat region which is 38 amino acids long in B85-8 but only 13 amino acids long in Jijoye. Spurious homology appears both vertically and horizontally with respect to this region and is due to the high Pro content of the EBNA 2 polypeptide. The other bubble is due to the degenerate Arg-Gly repeat which is approximately 14 amino acids long in both strains of virus. A region of 130 residues in the middle of both proteins shows maximum divergence and almost completely different amino acid sequences. Some tendency does seem to exist, however, towards conservation of amino acid composition, as the sequences of both proteins are rich in Pro residues (Fig. 2b). A linear plot of the occurrence of consecutive Pro residues reveals the limits of the divergent sequence but provides no indication of an evolutionary relationship between the sequences of the two proteins. This is the region from which the nucleotide sequence probes that distinguish type A from type B EBNA 2 are derived, and might be considered the best candidate for harbouring epitopes for the type-specific responses of human sera against EBNA 2. Of the 31 virus isolates we have examined (21 type A and 10 type B) there has been a direct correlation between nucleic acid hybridization and serological phenotypes (data not shown).

Fusion fragments of the EBNA 2 open reading frame

The main morphological features of the EBNA 2 proteins revealed by sequence analysis are shown on a linear map in Fig. 3. Initially, M13 clones were selected from the sequence libraries of B95-8 and Jijoye such that the entire open reading frame of each gene was covered in five overlapping segments for B95-8 and four overlapping segments for Jijoye. Table 1 lists the sequence of amino acids contained within each of the clones shown above and below the linear map (Fig. 3). Amino acids 110 to 115 of the B95-8 sequence have not been covered by the fusion clones. This is the region in the vicinity of the BamHI site between BamH1 Y and BamH1 H DNA fragments and, because of the strategy employed in the sequencing project, clones spanning this region do not occur in the B95-8 library. However, the homologous region of the Jijoye strain of EBV is present in the amino-terminal clone pEX13. Each of the fusion proteins contains an amino-terminal leader sequence consisting of the first 31 amino acids of the lambda CII gene product and a linker of nine or 10 amino acids (depending upon the expression vector used) that includes the tetrapeptide recognition sequence for the blood coagulation protease FX. The carboxy termini of fusion proteins expressing internal EBNA 2 fragments have three to six amino acids attached depending upon which of the stop codons in the chain termination sequence is being used. Note that the B95-8 amino-terminal clone pEX10 contains 16 additional amino acids encoded upstream of the initiator methionine and that the Jijoye amino-terminal clone pEX13 encodes eight upstream amino acids. For both fusion proteins (CX10 and CX13) these amino acids are derived from upstream in-phase EBV sequences that were present in the M13 fragments selected from the library. This selection was for the closest available
translational start that includes the amino-terminal sequences of the EBNA 2 protein translated from the in vivo initiator methionine. The constructs were made in the wild-type lambda lysogen EQ192 and plasmids with the correct structures were transferred to the expression strain N4830 (Gottesman et al., 1980).

Western blotting using human serum as probe

A panel of N4830 clones containing the five plasmids pEX10, -11, -21, -12 and -3 representing the amino- to carboxy-terminal fragments of type A EBNA 2, along with the four clones containing plasmids pEX13, -14, -19 and -20 representing the amino- to carboxy-terminal spanning fragments of type B EBNA 2 were grown, induced, and prepared for PAGE (see
Fig. 3. Overlapping fragments from the EBNA 2 region of the viral genome. The linear maps of EBNA 2 types A and B are presented above and below the scale which is divided into units of 25 amino acid residues. BamHI and EcoRI fragments used in this study are shown above and below the linear maps with the clone number designations. Shaded boxes indicate areas of Pro repeats, unshaded boxes show non-homologous sequences and hatched boxes are areas of Arg-Gly repeats.

Table 1. Structure of fusion proteins for epitope survey

<table>
<thead>
<tr>
<th>EBNA 2 type</th>
<th>Expression plasmid</th>
<th>Vector</th>
<th>EBNA 2 amino acid sequence</th>
<th>Additional amino acids</th>
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<tr>
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<td>pEX10</td>
<td>pLEX1</td>
<td>-16-109</td>
<td>41, 6</td>
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<tr>
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<td>pLEX2</td>
<td>116-219</td>
<td>42, 5</td>
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<td>186-299</td>
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</tr>
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<td>pLEX1</td>
<td>-8-154</td>
<td>41, 6</td>
</tr>
<tr>
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<td>pLEX1</td>
<td>126-197</td>
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<td>272-351</td>
<td>41, 3</td>
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<td>pLEX1</td>
<td>393-455</td>
<td>41, 0</td>
</tr>
</tbody>
</table>

Methods). Aliquots of extracts were run on 14% gels, immunoblotted, and probed with human sera at dilutions at which the sera showed type-specific reactivity with lymphocyte-derived EBNA 2. Fig. 4(a) and (b) show results of screening the pEX clones with two human sera (sera 1 and 2) which show type A specificity. At the predetermined dilutions, both sera reacted strongly with the CX3 fusion protein expressing the carboxy-terminal 110 amino acids of type A EBNA 2. No reactivity of these sera was seen with the CX11, CX21 or CX12 fusion proteins containing...
Fig. 4. Reactivity of human anti-EBNA 2 sera with fusion proteins. Extracts of induced bacteria containing the pEX expression constructs were run on 14% SDS-polyacrylamide gels. The lanes corresponding to five constructs, pEX10, -11, -21, -12 and -3 (lanes 2 to 6, respectively) with type A EBNA 2 sequences and four constructs, pEX13, -14, -19 and -20 (lanes 7 to 10, respectively) with type B EBNA 2 sequences are arranged to represent the amino to carboxy sequence of the EBNA 2 protein. Western blots were probed with type A or B EBNA 2-specific sera at dilutions that gave type-specific reactions with lymphocyte-derived EBNA 2. (a) Serum 1 (1/500) and (b) serum 2 (1/500) are type A EBNA 2-specific. (c) Serum 7 (1/1000) and (d) serum 9 (1/500) are type B EBNA 2-specific. Lanes 1 show the response to induced bacteria not containing an expression vector.

sequences from the region of greatest diversity between EBNA 2 of types A and B. The reactivity with CX3 did appear to be type-specific as there was no reaction with the CX20 fusion protein containing the carboxy-terminal 177 amino acids of type B EBNA 2. At lower dilutions these sera did show weak reactions with CX4, CX19, CX20 and CX21 (Fig. 3 and Table 2).

When an anti-EBNA 2 type B-specific human serum (serum 7 in Table 2) was reacted with the same set of clones (Fig. 4c) the strongest reaction was with the CX20 protein although significant cross-reactivity with CX3, presenting the homologous region of EBNA 2 type A, was observed. This pattern of reactivity directly paralleled the higher cross-reactivity of EBNA 2 type B-specific sera against whole type A or type B protein; this appears to be a characteristic of these sera and is not seen for anti-EBNA 2 type A-specific sera. The serum (serum 9 in Table 2) used to probe the blot in Fig. 4(d) shows an equal affinity for CX3 and CX20 and also reacts equally well with the type A or B EBNA 2 proteins in lymphocytes. All of the anti-EBNA 2 human sera that we have tested react with either CX3 or CX20, or both. We have not encountered an EBNA 2-positive serum that does not react with these proteins and nor have we
found an EBNA 2-negative serum that does react. These observations indicate that a major type-specific epitope recognized by human sera is located near the carboxy terminus of EBNA 2.

**Fine mapping of type-specific epitopes**

We have mapped type-specific epitopes to within the 110 carboxy-terminal amino acids of EBNA 2 type A and the 177 carboxy-terminal amino acids of EBNA 2 type B. To map these epitopes more closely, additional M13 clones which contained less of the carboxy terminus of EBNA 2 were selected from the sequence library. Expression clones pEX22 containing the last 62 amino acids of EBNA 2 type A, pEX4 containing the last 61 amino acids of EBNA 2 type B and pEX15 containing 79 amino acids overlapping the amino-terminal portion of pEX20 were made. Aliquots of induced bacteria were run on polyacrylamide gels and probed with human EBNA 2 sera. Fig. 5(a) and (b) show Western blots probed with sera 1 and 2; the type-specific reaction with CX3-containing extracts detected previously is seen but no cross-reactions or reactivity with the shorter carboxy-terminal CX22 fragment. These results suggest that the type A-specific epitope lies at least 60 residues upstream of the carboxy terminus of EBNA 2.

The reactivity of EBNA 2 type B-specific sera with the same set of extracts was more complex. For example, in addition to the binding of antibodies to CX20 and CX3 the sera also contained antibodies which reacted with CX4, the carboxy-terminal 61 amino acids of type B EBNA 2 (Fig. 5c). This reactivity was type-specific and no reactivity was detected with CX22. The one serum (serum 9) which we have found to be more universally EBNA 2-reactive and not strictly type B-specific did not react with CX4 (Fig. 5d). These results may be interpreted to mean that CX20 contains two epitopes for antisera raised against type B EBNA 2, one which is cross-reactive and is also present in CX3 (but not CX22) and another which is type B-specific and is recognized in CX20 and CX4.

A list of the sera which have been characterized for reactivity with the fusion fragments is presented in Table 2. Six type A and four type B EBNA 2-specific sera are shown. Although the number of type A-specific sera we have analysed exceeds 12 the pattern appears to be invariant. Two of the four type B-specific sera (7 and 8) were obtained from other laboratories and the remainder were selected by screening a panel of BL sera originating from central Africa (A. Levinson, personal communication). Some of the type A EBNA 2-specific sera showed a slight reactivity at low dilutions with fusion proteins CX12, CX21, CX19, CX20 and CX15, suggesting that an additional minor cross-reacting epitope may be recognized by some anti-type A sera.

### Table 2. Summary of human anti-EBNA 2 serum reactivities*

<table>
<thead>
<tr>
<th>Serum</th>
<th>EBNA 2 type</th>
<th>Source†</th>
<th>EBNA 2 type A fusion protein</th>
<th>EBNA type B fusion protein</th>
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<td>4</td>
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<td>CM</td>
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<td>BL</td>
<td>+ + + + + +</td>
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</tr>
</tbody>
</table>

* Serum reactivities are described by the intensity of bands on Western blots: + + +, strongest reactivity; + +, readily detectable; +, weak but detected.

† SC, seroconverted patient; CM, chronic mononucleosis; BL, Burkitt's lymphoma.
Fig. 5. Carboxy-terminal localization of EBNA 2 epitopes. pEX clones 22 and 4 (lanes 2 and 5, respectively) which contain the carboxy-terminal 60 amino acids of type A and B EBNA 2, respectively, and pEX clone 15 (lane 4) containing sequences around and including the EBNA 2 type B Arg-Gly repeat. Lane 1 shows the type A EBNA 2 clone 3 and lane 3 shows the type B EBNA 2 clone 20. Parts (a) and (b) were probed with the EBNA 2 type A-specific sera and (c) and (d) were probed with the EBNA 2 type B-specific sera. These are the same sera used to probe the blots shown in Fig. 4.

DISCUSSION

Nucleic acid sequence probes and human sera have been used to distinguish two types of EBV (A and B) based on variations within the EBNA 2 gene. The hybridization data on EBV isolates have identified a geographically restricted prevalence of EBV type B to central Africa and New Guinea (Zimber *et al.*, 1986). Sequencing of the type A and B EBNA 2 genes has revealed a region of sequence diversity that encodes the middle 120 amino acids of the EBNA 2 open reading frame (Zimber *et al.*, 1986). Because the anti-EBNA 2 antibodies in human sera detect
either type A or type B EBNA 2 protein in lymphoblastoid cell lines, the sera have also been classified as A- or B-specific and it has been reasonable to assume a direct relationship exists between the sequence variation and antibody response.

The epitopes we have mapped by using Western blotting of fusion proteins (containing fragments of EBNA 2 polypeptide sequence) lie near the carboxy terminus of the intact protein. The differential reactivity of the anti-EBNA 2 type A-specific sera with the various fusion proteins suggests that there is a type-specific epitope between residues 378 and 435 of the EBNA 2 type A sequence (Fig. 6). This sequence of 57 amino acids contains 17 substitutions between
the type A and type B proteins. Although most of these changes are conservative, there is a region between residues 407 and 423 where the sequence is divergent with the type A protein containing bulkier and more hydrophilic side chains. This is characterized by a trough in the type A but not the type B hydrophobicity plot (Fig. 6a) and represents a unique hydrophilic region that may be contributing to the conformation of the type A-specific epitope. The corresponding region of the type B sequence between residues 355 and 405 also appears to contain an epitope which the cross-reacting anti-EBNA 2 type B sera recognize. This epitope might lie within the conserved hydrophilic sequences that flank the 374 to 390 hydrophobic peak on the EBNA 2 type B plot (Fig. 6a). The type-specific epitope of anti-type B sera appears to be contained in sequences downstream of residue 390 in the carboxy-terminal 65 amino acids of the type B protein. There are 18 substitutions between the type A and B sequences in this region but the conservative nature of these changes is reflected in the almost identical hydrophobicity plots of the two proteins (Fig. 6b). Further mapping with peptides will be necessary in order to define the epitope more precisely. When Western blots of the bacterial fusion proteins were probed with EBNA 2 type A-specific sera at low dilutions, fusion proteins CX12, CX15, CX19, CX20 and CX21 occasionally gave weak reactions. This suggests that some type A-specific sera recognize secondary cross-reacting epitopes. This is consistent with a previously reported reactivity of human sera with a fusion protein expressing residues 144 to 299 of the EBNA 2 type A open reading frame (Hennessy & Kieff, 1985). If this reactivity is due to a single epitope, it would appear to map between residues 275 and 300 of the type A EBNA 2 sequence which is just on the carboxy-terminal side of the non-homologous region. Despite the direct correlation between hybridization and serological phenomena there was no indication from our mapping studies that human antisera contained type-specific antibodies which recognized the amino acid sequences of the 120 residues encoded by the non-homologous region. It is not possible to rule out the existence of epitopes that rely on conformational differences or post-translational modifications that are only found in lymphocyte-derived EBNA 2. However, the type-specific and cross-reactive antibodies in human sera were first described as Western blot phenomena and absorption of antisera with CX3- or CX20-containing bacterial extracts blocks type-specific Western blot detection of EBNA 2. Indeed, the type-specific and cross-reactive epitopes we have mapped can account for most of the serological responses observed with the intact protein.

That no epitopes could be conclusively mapped to the non-homologous region was unexpected and there are several possible explanations for this. Potentially antigenic sites on protein molecules appear to be present over most of the accessible surface and buried residues do not normally participate directly in the formation of epitopes. Thus the non-homologous region would be unlikely to provoke an antibody response if it were concealed within the EBNA 2 protein. Alternatively, the dominant specificities expressed by type-specific sera may be subjected to immunoregulatory influences that skew the secondary response toward epitopes in the domain represented by the carboxy terminus of the protein even though more significant topological diversity occurs elsewhere on the surface of the protein.

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REFERENCES


Location of EBNA 2 epitopes


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