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Regulation of JC Virus Expression in B Lymphocytes

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The etiologic agent of progressive multifocal leukoencephalopathy, a subacute demyelinating disease of the central nervous system, is the human polyomavirus JC virus (JCV), which causes a lytic infection of myelin-producing oligodendrocytes. In infected individuals the JCV genome can be detected in brain tissue and B lymphocytes isolated from the blood, bone marrow, or lymph nodes. Using mobility shift assays and a radiolabeled oligonucleotide from the JCV promoter-enhancer region (JCV bp 130 to 160), referred to as domain B, we were able to detect specific bands of the same mobility in nuclear extracts from human fetal glial cells, U-251 glioma cells, different B-cell lines, and in vitro-activated tonsillar B lymphocytes but not from T cells. In addition, a specific shift was detected when using nuclear extracts from freshly isolated tonsillar or lymph node B cells from five AIDS patients, two of whom later developed progressive multifocal leukoencephalopathy. Somewhat surprisingly, the above gel shift was partially inhibited by unlabeled oligonucleotides containing a κE2-binding site. UV cross-linking of the protein-DNA complex from either B cells or glial cells and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed the presence of a 46-kDa band. Transient transfection of a reporter plasmid constructed by fusing a trimer of the domain B sequence to a minimal promoter revealed activity in B lymphocytes and glial cells but not in T cells. Mutational analysis of this region demonstrated that the core TGGC repeat was essential for enhancer activity. Thus, a similar protein in B lymphocytes and glial cells may account for the preferential replication of JCV in these two cell types.

Seroepidemiological surveys show that infection with the human polyomavirus JC virus (JCV) is very common and usually occurs in young individuals (29). Despite the high seroprevalence of exposure, progressive multifocal leukoencephalopathy (PML), a JCV-mediated demyelinating disease of the central nervous system, is rare (30, 31). In 1958, the occurrence of PML was found to be associated with underlying immune suppression (7). Further support for a role of immunosuppression in the pathogenesis of PML has been provided by the sharp increase in PML with the emergence of AIDS (9, 15). On the basis of these observations, it has been suggested that JCV can induce a latent infection which may be activated upon immune suppression (10).

Since JCV is not present in the central nervous system of normal individuals, the virus was presumed to reside in cells outside of the nervous system, where it remained dormant until reactivated during periods of immunosuppression. Upon reactivation, the cells harboring JCV could cross the blood-brain barrier and infect glial cells (22). To identify cells which might harbor the virus outside of the central nervous system, various tissues from patients with PML have been examined for the JCV genome by using in situ hybridization and immunohistochemistry techniques. These studies detected JCV in B lymphocytes from the bone marrow, spleen, and brain (16, 21). Recently, JCV DNA has been detected in the peripheral blood lymphocytes of 89% of tested patients with PML (38). It was also demonstrated that JCV-infected B cells are localized in demyelinating areas of brains of patients with PML (21). Thus, it has been proposed that hematogenous spread of the virus to the brain occurs via infected B lymphocytes (22).

Transfection studies with viral DNA and various cell types suggest that the tropism of JCV for B lymphocytes and oligodendroglial cells is dependent predominantly on intracellular factors controlling early viral transcription and replication rather than selective adsorption of JCV to a specific receptor (14). Most of the studies of the regulation of JCV transcription have focused on glial cells. A 98-bp repeat sequence in JCV has been shown to have enhancer-promoter activity in glial cells but not in HeLa cells (4, 21). This 98-bp region has been subdivided into three domains (A, B, and C). Although a common set of DNA-binding proteins present in HeLa and glial cells interact with domains A and C, a distinct set in each cell type interacts with domain B (4). Further characterization of domain B-binding proteins by UV cross-linking identified a 45-kDa glial cell-specific nuclear protein (18). This binding protein was affinity purified from glial cell nuclear extracts and shown to stimulate transcription of the JCV promoter in vitro when added to HeLa nuclear extracts (1). A recombinant cDNA clone was isolated from a brain expression library by using domain B as a ligand which contained an open reading frame that extended the entire length of the cDNA (17). The predicted protein from the translated open reading frame was termed GF_1 and would be expected to have a molecular mass of at least 40 kDa. The relationship between GF_1 and the previously identified glial cell-specific 45-kDa protein was not determined. Cotransfection experiments with a GF_1 expression vector and a JCV promoter reporter plasmid suggested that GF_1 stimulates JCV promoter transcription (17). However, a murine homolog of GF_1 has recently been isolated, and the predicted protein is 933 amino acids, much larger than the domain B-binding protein (24).

Other studies have shown that domain B is also bound by members of the *jun* family (5) and nuclear factor 1 (NF-1) or a protein related to NF-1 (4). However, there is no direct evidence that NF-1 or *jun* is present in gel shifts when using the domain B probe. In addition, NF-1 is larger than the 45-kDa glial cell nuclear protein identified by UV cross-linking. In this study we have found a JCV domain B-binding protein which is

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present in nuclear extracts prepared from human B lymphocytes and has a similar molecular mass to that of the previously reported 45-kDa glial cell-specific protein. We also noted that this protein is induced during B-cell activation and is present constitutively in B cells isolated from the tonsils or lymph nodes of patients with human immunodeficiency virus (HIV) infection. Finally, we have shown that domain B, when fused to a minimal promoter, is functional in B lymphocytes but not in T lymphocytes and that a TGGC repeat which is present in domain B is necessary for activity.

MATERIALS AND METHODS

Cells and cell lines. BJA-B (Epstein-Barr virus-negative B-cell lymphoma) cells were kindly provided by E. Oates, University of Miami, Miami, Fla. HS-Sultan (immunoglobulin G kappa myeloma) and Jurkat (T-cell leukemia) lines were obtained from the American Type Culture Collection, Rockville, Md., and U251 glioma cells were a gift from L. Vitkovic, National Institute of Aging. B cells from tonsils and lymph nodes were obtained by standard procedures (13). The purity of the B-cell preparation was routinely greater than 95% by fluorescence-activated cell sorter analysis for expression of the B-cell-specific marker CD19. All but U251 cells were cultured in RPMI 1640–10% fetal calf serum supplemented with antibiotics. U251 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. B cells were polyclonally activated with 0.01% formalin-inactivated *Staphylococcus aureus* Cowan 1 (Bethesda Research Laboratories, Gaithersburg, Md.) in the presence of 100 U of recombinant interleukin-2 (rIL-2) (Cetus Corp., Emeryville, Calif.) per ml.

Nuclear protein extraction and mobility shift assay. Nuclear protein extracts were prepared as previously described (28). The protein concentration in each nuclear extract was quantitated by the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). The binding reactions for the electrophoretic mobility shift assays (EMSA) were performed in a total volume of 25 μl for 30 min at room temperature with 10 μg of nuclear protein, 17 μl of binding buffer (Stratagene, La Jolla, Calif.), and 50 to 100 pg of double-stranded oligonucleotide probes which had been labeled with ^{32}P. In some reactions a 50-fold molar excess of unlabeled probe was used for competition. Samples were run on a 5% polyacrylamide gel at 120 V in a cold room as specified by the manufacturer (Stratagene). Gels were autoradiographed for 4 h at −70°C. All oligonucleotides were synthesized with a 381 A DNA synthesizer (Applied Biosystems, Foster City, Calif.). The nucleotide sequences of the competitor oligonucleotides were obtained from published sequences (see figure legends for exact sequences).

Transfection and CAT assay. Trimers of JCV 130-160, domain B, and mutations of this sequence were subcloned into the *Sal*I site of a c-*fos* promoter containing chloramphenicol acetyltransferase (CAT) plasmid (Δ71 CAT, kindly provided by M. Lenardo, National Institute of Allergy and Infectious Diseases). The sequences are as follows.

Domain B: 5′GAGCTCATGCTTGGCTGGCAGCCATCCCTTC
Domain B-M1: 5′GAGCTCATGCTAGACAGACAGCCATCCCTTC
Domain B-M2: 5′GAGCTCATGCTTGGCTGACAGCCATCCCTTC

The cells were transfected with 5 μg of each CAT construct by using 10% DEAE-dextran solution followed by a 30-min treatment of the cells with 0.1 mM chloroquine. Cells were harvested after 48 h in culture and lysed by three cycles of freeze-thaw. The protein concentration was normalized by the Bio-Rad protein assay and CAT activity was determined by a liquid scintillation assay as previously described (27). RSV CAT (kindly provided by M. Norcross, National Institute for Dental Research) was used as a control plasmid for the Jurkat cell line, and cytomegalovirus CAT (kindly provided by A. Dayton, National Institute of Allergy and Infectious Diseases) was used for the other cell lines. Results are given as the mean percentage of CAT conversion from at least three independent transfections.

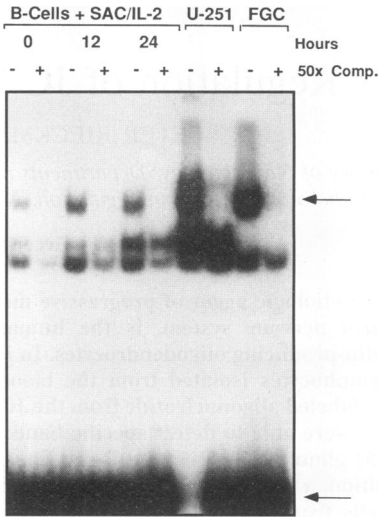

FIG. 1. EMSA with JCV 130–160 (domain B) and nuclear extracts from activated tonsillar B cells, U251 glioma cells, and fetal glial cells (FGC). B cells were activated with *S. aureus* Cowan 1 or IL-2 for the indicated time. Each reaction was inhibited with a 50-fold molar excess of an unlabeled domain B probe (lanes +). The upper arrow indicates the specific band shift. The arrow at the bottom points to the unbound probe. Nonspecific bands are present in some of the nuclear extracts.

UV cross-linking. The domain B oligonucleotides used for UV cross-linking were synthesized by using 5-bromo-2′-deoxyuridine phosphoramidites (Pharmacia). A 100-ng sample of the double-stranded oligonucleotides was end labeled to a specific activity of greater than 5×10^8 cpm/μg with Klenow fragment. Nuclear protein (20 μg) and 10^6 cpm of the oligonucleotide probe were incubated under the same conditions as described for the EMSA for 20 min followed by separation of the DNA-protein complex on an 8% polyacrylamide gel. The EMSA gel was exposed to UV-A (Fotodyne transilluminator; wavelength, 300 nM) for 20 min. The specific shifted band was identified by autoradiography, excised, and size fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 10% polyacrylamide). The gel was fixed in 10% methanol–10% acetic acid, dried, and autoradiographed overnight.

RESULTS

Nuclear factors from B cells bind to the 98-bp enhancer region from JCV. Nuclear extracts were prepared from human tonsillar B cells and tonsillar B cells which had been stimulated with *S. aureus* Cowan 1 and rIL-2 for 12 or 24 h. Similar amounts of nuclear extract protein were incubated with a domain B probe prior to fractionation on a nondenaturing polyacrylamide gel. A low level of a nuclear factor present in nuclear extracts from purified tonsillar B cells bound to domain B, whereas a much higher level was found in nuclear extracts prepared from in vitro-activated tonsillar B cells (Fig.

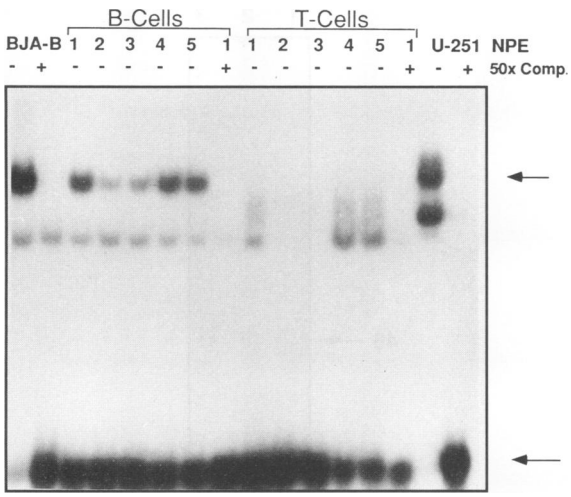

FIG. 2. EMSA with a domain B probe and nuclear extracts from B and T cells freshly isolated from tonsils (patients 1 to 3) or lymph nodes (patients 4 and 5) of HIV-infected individuals, BJA-B cells, and U251 cells. Specific band shift was inhibited with a 50-fold molar excess of unlabeled domain B probe (lanes +). NPE, nuclear protein extracts.

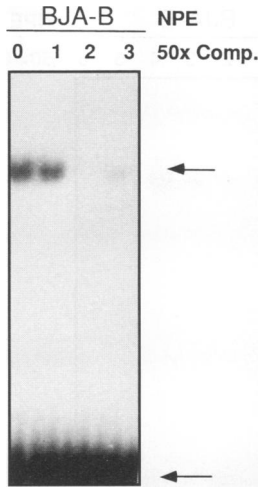

FIG. 3. Competition of domain B binding to nuclear protein extracts (NPE) from BJA-B cells with mutated oligonucleotides. A 50-fold molar excess of oligonucleotides was used to block the interaction. Lanes: 0, without competition; 1, competition with domain B M1; 2, competition with domain B; 3, competition with domain B M2. The upper arrow indicates the specific band shift, and the lower arrow denotes unbound oligonucleotides.

1). The protein-DNA complex was inhibited by a 50-fold molar excess of the same unlabeled oligonucleotide. The protein-DNA complex migrated at a similar rate to a complex obtained by using nuclear extracts prepared from U251 glioma cells or human fetal glial cells (Fig. 1). Of note, nuclear extracts from both the U251 cells and fetal glial cells contained a higher level of this binding factor than did extracts from either resting or activated B cells. A nonspecific band was present in most of the nuclear extracts which failed to compete with unlabeled probe. The presence of this nonspecific band was dependent on the cell type and the method used to prepare the nuclear extracts.

Using freshly isolated tonsillar B and T cells from HIV-infected individuals, we were able to detect a nuclear protein which bound to domain B present in nuclear extracts from B cells but not in extracts from T cells (Fig. 2). Of interest, three of the patients (patients 1, 4, and 5) had relatively high levels of the domain B-binding protein. These increases may reflect the high level of polyclonal B-cell activation which frequently accompanies HIV infection (2, 3, 19, 32). In addition, a similar protein-DNA complex was detected with nuclear extracts from BJA-B cells, a cell line derived from a patient with a B-cell lymphoma. Two (patients 4 and 5) of the three HIV-infected patients who had high levels of domain B-binding proteins subsequently developed PML.

Fine mapping of the binding of the domain B nuclear binding protein by competition studies. A TGGC tetranucleotide repeat within domain B is important for the glial cell nuclear factor binding to this region (18). In addition, a recent study has suggested that this repeat is important in binding of a protein present in nuclear extracts prepared from two different B-cell lines which bound to a 188-bp probe containing one of the 98-bp repeats of the JCV promoter (8). To analyze the role of this repeat in the binding of the nuclear protein from BJA-B cells to domain B, we synthesized oligonucleotides with altered nucleotides in the repeat regions and attempted to inhibit the binding of the domain B-binding protein (Fig. 3). If nucleotides in the tetranucleotide repeats were altered from the wild type, the resulting probe was incapable of inhibiting the binding to the labeled wild-type probe (Fig. 3, lane 1). In contrast, if only the second TGGC was altered, the probe still effectively inhibited the binding to the labeled wild-type probe (lane 3). This result with nuclear extracts from BJA-B cells is consistent with the findings in glial cells (data not shown).

To further characterize the interaction of domain B of the 98-bp repeat with B-cell nuclear extracts, we tried to inhibit the binding reaction with oligonucleotides containing the consensus sequence for other gene regulatory elements (NF-κB, Oct-2, κE2, AP-1, or an NF-1-binding site). A 50-fold molar excess of NF-κB or Oct-2 failed to inhibit the binding of the domain B-binding protein to domain B (Fig. 4, lanes 3 and 4), a similar excess of AP-1 and NF-1 inhibited binding only slightly (lanes 1 and 2); however, a 50-fold molar excess of oligonucleotides containing a κE2-binding site from the kappa intron enhancer inhibited the interaction (lane 6). Alignment of the κE2 site, the JCV promoter-enhancer between bp 130 and 160, and the NF-1-binding site is shown in Fig. 5. Thus, although domain B matches the NF-1 consensus binding site, this site only poorly inhibits the binding of the domain B-binding protein. Moreover, although domain B does not contain a consensus κE2 binding site, it does inhibit the binding.

JCV domain B-binding factors present in B and glial cell nuclear extracts are of the same size. A UV cross-linking technique was used to determine the molecular masses of the domain B-binding factors in B cells and to compare them with those of factors in glial cells. Nuclear extracts from *S. aureus* Cowan 1- or IL-2-activated B cells, from B cells obtained from an HIV-infected patient, and from fetal glial cells were used for a mobility shift assay with a ^{32}P-labeled DNA probe in which 5-bromo-2′-deoxyuridine was substituted for several of the thymidines in domain B. The DNA-protein complex from each of the cell types migrated at the same rate (Fig. 6; approximately 46 kDa), which is close to the reported molecular mass for the glial cell-specific nuclear protein (18). The DNA is likely to contribute several kilodaltons to the protein on SDS-PAGE.

Transfection of a domain B-CAT construct into various cell lines. Having demonstrated the binding of nuclear extracts

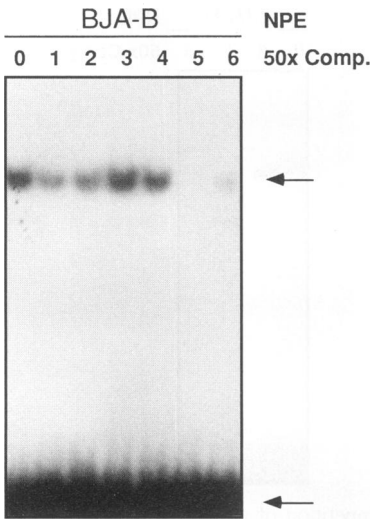

FIG. 4. Competition of domain B binding to nuclear protein extracts (NPE) from BJA-B cells with oligonucleotides representing binding sites for gene regulatory sequences. A 50-fold molar excess of each oligonucleotide was used to block the interaction. Lanes: 0, no competition; 1, NF-1 (Ad) (26); 2, AP-1 (5); 3, NF-κB (12); 4, Oct-2 (35); 5, JCV 130–160; 6, κE2 (25). The upper arrow indicates the specific band shift, and the lower arrow indicates unbound oligonucleotides.

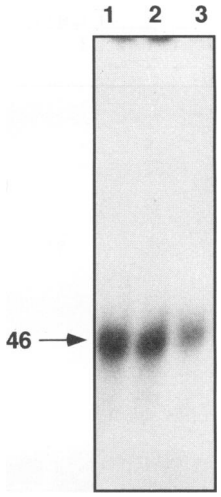

FIG. 6. Identification of domain B-binding proteins by UV cross-linking. Specific bands from EMSA performed with 5-bromo-2′-deoxyuridine containing oligonucleotides were excised, UV cross-linked, and separated on an SDS–10% polyacrylamide gel. Lanes: 1, BJA-B cells; 2, fetal glial cells; 3, B cells from HIV-infected patient 5. The molecular mass of the protein-DNA complex is ca. 46 kDa.

from various B-cell sources to domain B and analyzed the interaction, we used trimers of domain B of the JCV promoter-enhancer and the corresponding mutations domain B-M1 and domain B-M2 subcloned in a c-*fos*-containing reporter plasmid to functionally characterize these sequences in transfection experiments with BJA-B lymphoma cells, HS-Sultan cells, U251 glioma cells, and Jurkat cells. The results obtained corroborate the gel shift data. A strong induction of the domain–B-CAT construct as indicated by the rate of CAT conversion was observed in the two B-cell lines and the glioma cell line, whereas no activity was detected in Jurkat T cells. In addition, the mutation of both TGGC repeats, domain B-M1, which abolished binding to nuclear extracts (Fig. 3), also decreased CAT activity to baseline levels in the transfection assays. The second mutation, domain B-M2, leaving the first TGGC intact, almost restored CAT activity in the three cell lines but again had no effect on CAT induction in Jurkat cells (Fig. 7). Taken together, these results indicate that the interaction of the domain B-binding protein is of functional significance and implicates it as being important in the expression of JCV in B lymphocytes.

DISCUSSION

In this study we have shown that the central portion of the 98-bp repeat of the JCV promoter-enhancer can confer B-cell-specific expression on a minimal promoter and as such is a target for a transcription factor(s) expressed in B lymphocytes. Three copies of this sequence element fused to the c-*fos* promoter linked to the CAT gene was very active in B cells but not in T cells on transient transfection. Mutation of the TGGC repeats present in this region resulted in loss of activity, whereas mutation of only the second repeat did not. Identification of an approximately 40- to 45-kDa protein present in B-cell nuclear extracts which bound to the central portion of the JCV promoter-enhancer and whose detection was dependent on the TGGC repeats suggested that this protein has a role in the transcription of the CAT construct and perhaps JCV.

JCV domain B:	5'	GAGCTCATGCT**TGGC**TGGCAGCCATCCCTTC
NF1 consensus:	5'	NTTGGCNNNNNGCCAAN
κE2 site:	5'	AAGGCAGGTGGCCCAAGCT

FIG. 5. Alignment of the nucleotide sequences from JCV domain B, NF-1, and the κE2 site. The underlined nucleotides show homologous nucleotides within the region of the NF-1 consensus sequence. The bold nucleotides represent homologies to the κE2-binding site.

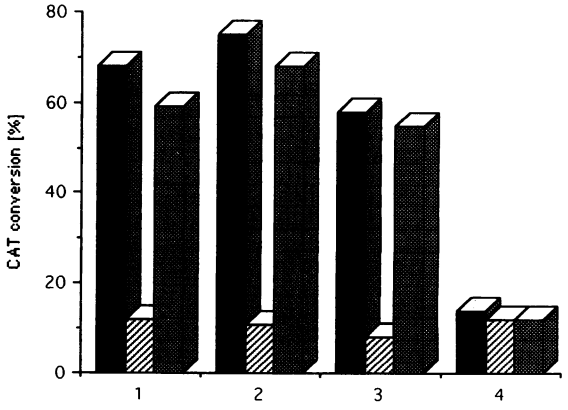

FIG. 7. Functional analysis of domain B enhancer activity. Trimers of domain B, domain B-M1, and domain B-M2 were subcloned into a c-*fos*-containing reporter plasmid, Δ71, and transfected. Results are expressed as a percentage of CAT conversion and are the mean of three independent transfections. The following cell lines were transfected: 1, BJA-B; 2, HS-Sultan; 3, U251; 4, Jurkat. The solid bars represent domain B-CAT, striped bars represent domain B-M1-CAT, and stippled bars represent domain B-M2-CAT.

Although a number of DNA-binding proteins in glial and HeLa cells have been identified to interact with the regulatory region of JCV, much interest has focused on the role of DNA-binding proteins thought to be unique to glial cells. The center region in the 98-bp repeat of the JCV promoter-enhancer, which has been termed domain B (4), has received much attention and is the area that we chose to focus upon in B cells. Two different avenues of investigation have led to disparate conclusions concerning the nature of the DNA-binding proteins in glial cells which interact with this region in the promoter. On the basis of comparison with known DNA target sequences, a role for *jun* and NF-1 has been sought (4, 8, 21, 37). Both recombinant *jun* protein and purified NF-1 protein have been shown to bind in vitro to overlapping sequence elements present in the domain B region (5). Furthermore, DNA footprints with purified NF-1 or *jun* mimics the footprints found with glial cell nuclear extracts, suggesting that these factors in glial cell nuclear extracts account for the DNA-binding activity noted in them (5). *cis* sequences in several promoters of neural specific genes have been identified which share these overlapping binding sites for NF-1 and *jun* (5, 24). Although this evidence is compelling, there is no direct evidence that NF-1, an NF-1-related protein, and *jun* actually are components of the gel shifts noted with glial cell nuclear extracts or that they are important in the regulation of JCV in vivo. Another difficulty in ascribing a role for NF-1 or *jun* in the regulation of JCV gene expression is that both proteins are ubiquitously expressed, making it difficult for them to account for glial cell-specific expression of JCV (6, 21, 26).

Using a more direct approach of affinity purification with domain B-containing oligonucleotides, we isolated a 45-kDa protein from glial cell nuclear extracts. This molecular mass matches the molecular mass of the domain B-binding protein we identified by UV cross-linking by using domain B and B lymphocyte nuclear extracts. The molecular mass of the GF_1 protein identified by expression cDNA cloning from a glial cell library with a domain B probe was unknown since the nucleotide sequence of a full-length cDNA was not reported (17). To our knowledge the relationship between GF_1, the glial 45-kDa protein identified by affinity purification, and the protein we identified by UV cross-linking with B-cell nuclear extracts is not known. There is evidence that GF_1 is present in spleen cells since small amounts of GF_1 mRNA was detected in mouse spleen RNA by a primer extension assay. The cell types expressing GF_1 in the spleen were not further evaluated (17). Recently, a murine cDNA encoding a protein which specifically binds 5′-phosphorylated G-rich single-stranded DNA was isolated by using a sequence motif from the immunoglobulin Sμ region (24). The predicted protein of 993 amino acid termed Sμbp-2 was found to be highly homologous to the GF_1 protein. Indeed, GF_1 corresponds to the polypeptide between residues 490 and 864 of Sμbp-2 and is probably the human equivalent of Sμbp-2. Further studies revealed that Sμbp-2 mRNA is ubiquitously expressed in murine cell lines and tissues but can be enhanced in spleen cells stimulated with lipopolysaccharide and IL-4. Analysis of the full-length protein suggests that Sμbp-2 is a member of the helicase superfamily, proteins known to be involved in DNA replication, recombination, and repair. No evidence was found to suggest that GF_1/Sμbp-2 is a sequence-specific transcriptional factor. Northern (RNA) blot analysis of RNA prepared from human B- and T-cell lines with a GF_1 polymerase chain reaction (PCR) product revealed higher levels of GF_1/Sμbp-2 in the T-cell lines than in the B-cell lines (40). Thus, it seems unlikely that GF_1/Sμbp-2 is the glial cell and B-cell factor identified by gel shift and UV cross-linking experiments. Further studies are needed to characterize the domain B-binding protein detected in gel shift assays with nuclear extracts from glial and B cells.

On initial inspection, our observation that oligonucleotides containing a κE2 site inhibit the binding of the B-cell protein to a domain B probe further complicates matters. E boxes, of which κE2 is a member, were first identified as a set of homologous motifs present in the immunoglobulin heavy chain gene enhancer which bound lymphoid specific proteins (11, 34). E boxes are found in all immunoglobulin enhancers including the kappa intronic enhancer, in which the κE2 site is important for kappa chain gene transcription (20). Molecular cloning of cDNAs for E motif-binding proteins revealed a new family of transcription factors, the helix-loop-helix (HLH) proteins (25). The κE2-binding proteins include E12, E47, and ITF-1, which are derived from alternative splicing of the same gene. The molecular masses of the κE2-binding proteins are larger than observed on UV cross-linking with the domain B probe and B-cell nuclear extracts; the consensus binding site for E12/E47 is not present in the domain B sequence. A binding-site selection method involving PCR and an initially random nucleotide sequence identified a consensus binding site for E47 and E12 homodimers as (G/A)CAG(G/C/A)TG (35). Although this site is not present in domain B, one of the clones isolated during the binding-site selection did not contain the sequence CANNNNTG, which is present in domain B. Nevertheless, this site is unlikely to bind E12/E47 since the immediately 5′ nucleotide is a T, which was never observed in an E12/E47-binding site (36). We think that it is unlikely that the domain B-binding protein in B-cell nuclear extracts is E12/E47, but, rather, we believe that the domain B-binding protein in B lymphocytes is capable of binding to the κE2 site. The 3′ portion of κE2 site does contain a TGGC, which appears to be important in binding the domain B-binding protein. This is the likely explanation for why the κE2 site inhibited the binding.

The B-cell domain B-binding protein was noted to be inducible in tonsillar B cells and to be constitutively present in B cells isolated from patients with AIDS. This suggests that the domain B-binding protein is a product of an activation gene in B cells. Its high constitutive level in B cells from AIDS patients is also consistent with its being an activation gene since there is both virus-specific and polyclonal B-cell activation in B cells from AIDS patients (2, 3, 19). Assuming that the domain B-binding protein is important in JCV transcription, the concomitant B-cell activation and deficiency of T-cell immunity may provide a good environment for JCV activation and replication in B cells. Some of these B cells probably cross the blood-brain barrier (33, 39), and they may provide a vehicle for infection of glial cells.

As has so often been the case, the study of viral gene transcription has led to important insights in the understanding not only of the regulation of viral gene expression but also of normal cellular physiology. The recognition of the tropism of JCV for B lymphocytes and glial cells will probably result in further insights into gene regulation in both these cell types.

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