Characterization of the EBV/C3d Receptor on the Human Jurkat T Cell Line: Evidence for a Novel Transcript

Suman K. Sinha,* Scott C. Todd,* Joseph A. Hedrick,* Catherine L. Speiser,* John D. Lambris,* Constantine D. Tsoukas**

*Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182; **Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, CA 92037; and *Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

ABSTRACT. EBV is a human herpes virus that causes mononucleosis and is associated with various tumors. EBV infects cells via the CR2 that was previously thought to be expressed only on the surface of B cells and certain epithelial cells. Recent findings in our laboratory and those of others, however, have shown that the EBV receptor is also present on T cells. Our study shows that Jurkat human T cells have a molecule that reacts with both anti-CR2 antibodies and the third component of complement, C3. Furthermore, the data indicate that this molecule binds EBV detected by incubation with biotin-conjugated virus and streptavidin phycoerythrin. Viral binding is specific, as it is inhibited by nonconjugated virus, with anti-CR2 antibodies, and with an antibody reactive with the glycoprotein (gp350) that EBV uses to bind CR2. In addition, EBV variably infects Jurkat cells as demonstrated by the presence of transcripts of Epstein Barr nuclear Ag (EBNA-1) using the polymerase chain reaction. Immunoprecipitation experiments with anti-CR2 antibodies and SDS-PAGE analysis reveal a protein with an apparent molecular mass of 155 kDa which is higher than the one seen in B cells. The size of this molecule is reduced to 119 kDa upon endoglycosidase F treatment. Northern blot analysis of Jurkat poly(A)+ RNA shows a transcript of 4.7 kb upon probing with the B cell CR2 cDNA. This size is consistent with that of B cell CR2 mRNA. Two cDNA clones were identified upon screening of a Jurkat cell cDNA library with the B cell CR2 cDNA. One of the clones possesses an identical nucleotide sequence to the one corresponding to B cell CR2, whereas the other represents a differentially spliced transcript which lacks the exon 8b of B cell CR2. Analysis of Jurkat and Raji mRNA by PCR demonstrated the presence of this novel splice variant in both cell lines. Journal of Immunology, 1993, 150: 5311.

EBV is the causative agent of infectious mononucleosis and is related to Burkitt's lymphoma, nasopharyngeal carcinoma (1), and B cell lymphomas in immunodeficiency cases (2). EBV has been also shown to be associated with cancers of T cells wherein the T cell lymphomas are positive for the EBV genome (3–9). The virus has been shown to target lymphocytes through CR2/CD21 (10–12), a molecule that is reactive with the C3d fragment of the third component of complement (13–15).

The elucidation of the genomic organization of B cell CR2 has revealed a gene that is organized in multiple, related exons, 15 or 16 of which code for the extracellular segment of the receptor (16). The extracellular portion of CR2 is arranged in tandem SCR3 with additional exons

---

Received for publication February 23, 1993. Accepted for publication March 22, 1993.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Grants GM 39518, GM 45765 from the National Institutes of Health, NSF Grant DCB 90-18731 from NSF, and by Grant R92-SDS-044 from the California Universitywide AIDS Research Program. SKS received support through a Scripps XI Scientific Research Society Scholarship. This is publication 7546-MEM from the Scripps Research Institute.

2 Address correspondence and reprint requests to Dr. Constantine D. Tsoukas, Ph.D., Department of Biology, San Diego State University, San Diego, CA 92182-0057.

3 Abbreviations used in this paper: SCR, Short Consensus Repeat; PE, Phycoerythrin; PCR, Polymerase Chain Reaction. BioEBV, biotin-conjugated EBV, EBNA-1 Epstein-Barr nuclear Ag.
coding for the transmembrane and cytoplasmic regions of the receptor, respectively (16). The SCR of CR2 are homologous to those described in CR1 and other C3 and C4 binding proteins thus, rendering all these genes members of a gene superfamily, the complement control protein gene superfamily (17–19).

Expression of CR2 or CR2-like molecules has been also detected on pharyngeal (20) and cervical epithelia (21), follicular dendritic cells of the tonsils and lymph nodes (22), with increasing evidence indicating its presence on cells of the T lineage (23–28). Several lines of evidence support the presence of CR2 on human T lymphocytes. Early studies by Menezes et al. (23) have demonstrated that EBV binds to the lymphoblastoid T cell line Molt 4. Finger et al. (24) have purified the CR2 protein from the human leukemic T cell line HPB-ALL and have provided limited NH₂-terminal sequencing. More recently, Delibrias et al. (28) demonstrated that HPB-ALL cells coexpress CR2 and CR1 and that a portion of these two C3 receptors are associated in the form of CR1/CR2 complexes in the cell membrane. Studies from our own laboratory have shown that human thymocytes react with anti-CR2 mAb and that the antibodies can be used to immunoprecipitate a molecule of the expected size for CR2 (25). In a more recent report, we have shown that EBV binds to a phenotypically immature subpopulation of human thymocytes and infects these cells as detected by the expression of EBNA-1 (26).

This binding and infection occurs through CR2 or a CR2-like receptor (26). Other studies have also shown the presence of CR2 on normal human peripheral blood T lymphocytes by immunofluorescence analysis with anti-CR2 antibodies and by immunoprecipitation (27).

In our investigation, we have extended our studies on the physicochemical and molecular characteristics of CR2 on T cells using the human Jurkat T cell line as a model system. Immunofluorescence analysis and flow cytometry with anti-CR2 mAb and C3, immunoprecipitation and SDS-PAGE with anti-CR2 antibodies, and Northern blotting analysis with a B cell CR2 cDNA demonstrate the presence of CR2 in Jurkat cells. We have also used biotin-conjugated EBV and streptavidin-phycocerythrin to show that EBV binds to Jurkat cells via CR2. The CR2 molecule on Jurkat seems to be functional as it allows EBV-infection demonstrated by the presence of EBNA-1. Finally, we have identified two Jurkat cDNA clones that represent the 15 SCR form of B cell CR2 and a novel, differentially spliced variant, respectively. We speculate that the latter clone which lacks exons 8b and terminates in exon 9, may represent a truncated form of CR2.

Materials and Methods

Cells and antibodies

The B cell lines Raji and BY, the T cell lines HPB-ALL and Jurkat, and the EBV-transformed marmoset leukocyte line B95-8 were all cultured in RPMI 1640 medium (Cellgro, Washington, DC) supplemented with FCS (10%) (HyClone, Logan, UT), penicillin (100 U/ml), and streptomycin (100 µg/ml) (GIBCO, Grand Island, NY). The cells were adjusted to a concentration of 2 × 10⁶/ml of the above medium every 3 days, and maintained at 37°C in a humidified atmosphere of 5% CO₂–95% air.

Anti-CR2 mAb OKD7 and B2 were purchased as purified Ig from Ortho Diagnostic Systems (Raritan, NJ) and Coulter Immunology (Hialeah, FL), respectively. Anti-CR2 mAb 6F7 containing culture supernatants were generously provided by M. Dierich (Innsbruck, Austria). The anti-CR2 mAb HB5 (HB-135) and the anti-gp350 mAb 72A1 (HB-168) were produced by hybridomas purchased from the American Type Culture Collection (Rockville, MD).

Cytosfluorometric analysis

It was performed as previously described (29). One × 10⁶ cells were stained with either the mAb followed by fluorescein-conjugated goat anti-mouse IgG (Jackson Immunoresearch, West Grove, PA) or with biotin-conjugated EBV followed by streptavidin-conjugated phycocerythrin (Biomedia Corp. Foster City, CA). The purification of EBV and its conjugation to biotin have been described previously (26). Binding of C3 was assessed with purified fluorescein-conjugated C3 (0.2 mg/ml) that had been aggregated with gluteraldehyde (30, 31).

Radiolabeling, immunoprecipitation, and gel electrophoresis

It was performed as previously described (29). Briefly, lysates from 10 × 10⁶ Raji, HPB-ALL, or Jurkat cells that had been labeled with Na¹²⁵I (Amersham Corp., Arlington Heights, IL) were reacted with anti-CR2 mAb HB5 or isotype (IgG2a) control antibody RPC-5 (Organon Teknika Corp., Westchester, PA), complexes were formed by incubating with rabbit anti-mouse Ig (Jackson Immunoresearch) and precipitated with Pansorbin (Calbiochem, La Jolla, CA). The presence of N-linked high-mannose complex oligosaccharides was assessed by treatment of the immune complexes with 1 U of endoglycosidase F for 2 h at 37°C. The immunoprecipitated material was analyzed on 7.5% SDS-PAGE under reducing conditions. Autoradiography was performed by exposing the dried gels to XAR-5 film (Kodak) at −70°C with intensifying screen for 10 to 14 days.

Northern blotting

RNA was isolated according to the procedure described by Sambrook et al. (32), and poly(A)⁺-enriched RNA was obtained chromatographically using an oligo(dT) cellulose column (Pharmacia, Fine Chemicals, Piscataway, NJ).
Northern blotting analysis was performed as described previously (33). The filters were probed with a previously described 32P-labeled B cell CR2 cDNA (34) that was provided to us by Dr. Glen Nemero (Scripps Research Institute, La Jolla, CA), and washed twice (10 min each wash) with 0.2× SSC, 0.1% SDS at 42°C. The filters were exposed to XAR-5 film (Kodak) for 4 to 5 days at −70°C with an intensifying screen.

PCR-facilitated amplification of mRNA

This method was used to assess the presence of EBNA-1 mRNA, and to detect the expression of CR2 transcripts. For EBNA-1 detection, Jurkat cells were cultured at 1 × 10⁶ cells/ml with EBV-containing B95-8 culture supernatants (1/4 dilution) for 7 days at 37°C. The RNA was isolated, treated or not treated with DNase 1, and then subjected to reverse transcription with oligo dT and Moloney murine leukemia virus reverse transcriptase, followed by PCR analysis as described previously (33). The oligonucleotide primers used to amplify EBNA-1 were 5’-TAGAGGACGTGAAAAGGC-3’ (extends downstream) and 5’-ACATGGACTCCTTAGC-3’ (extends upstream). These primers yield a product of 611 bp that was detected by Southern blot analysis and probing with a 32P-labeled primer that hybridizes to a portion of the amplified EBNA-1 product, and has the sequence 5’-GAGACGA-CTCAATGTTGAAGACGACATGTTGGAATAGCAA-GGGAGTTC-3’. Detection of CR2 transcripts was done as above with the exception that no DNase 1 treatment was performed and CR2 specific primers were used instead. Reverse transcription was carried out with a CR2-specific primer along with random hexamer primers (500 pmol each). The specific primer hybridizes across the junction of exons 12a and 12b and has the following sequence: 5’-AAGCTGGA-CCCATCT-3’. PCR analysis of the so generated cDNA was carried out using primers that flank exon 8b. The sequences of the primers are 5’-AATGCAAAGTGCCAGGCTG-TGAAG-3’ (hybridizes across the junction of exons 7 and 8a and extends downstream) and 5’-ACACTGGAATGTC-ACAGTGTCATT-3’ (hybridizes to exon 9, 10 and extends upstream). The PCR product yielded by these primers is either 420 or 497 bp depending on whether or not SCR 8b is present. After Southern transfer, the blot was probed with either an exon 8b-specific oligonucleotide with the sequence 5’-TGCTCAGATGTGCAAGGCAATCTCGT- GGTTTATTGAGA-3’ or with an exon 9, 10-specific probe with the sequence 5’-TCTAAGGAACTCCCGGTTGCTGTG-3’.

In all of the experiments, controls containing all reagents except the RNA were carried out to monitor for contaminants. Positive controls for reverse transcription were also included using identical conditions with the exception that primers that amplify the stimulatory GTP-binding protein GoS were used. These primers are 5’-GACTGCGCATC- CCTCGT-3’ (extends downstream), 5’-ACTGAAAGTG- AGAGGAAC-3’ (extends upstream).

Library construction, screening, and DNA sequencing

mRNA that was isolated from Jurkat cells, as described above, was used to construct a cDNA library in the λ-ZAP vector by following the manufacturer’s protocol (cat. no. 200400, Stratagene, San Diego, CA). The library represents approximately 2.6 × 10⁸ recombinants. It was screened with a previously described 32P-labeled B cell CR2 cDNA (34). Screening was performed on duplicate nitrocellulose filters as described (35) under low stringency hybridization (2× SS C, 0.1% SDS at 42°C). Positive phage plaques were replated several times until uniform hybridization was obtained. Two clones were selected and excised in pBluescript using the helper phage system (Stratagene), and analyzed by restriction endonuclease digestion. Both DNA strands of these clones were sequenced using a commercial sequencing kit (Sequenase, United States Biochemical Corporation, Cleveland, OH).

Results

Jurkat cells express CR2

Four anti-CR2 mAb that detect distinct epitopes across the molecule were used to compare the phenotypic profile of Jurkat cells to JY, a CR2−, EBV transformed lymphoblastoid B cell line (Fig. 1, left set of panels). In contrast to JY cells, which react equally well to all antibodies, Jurkat displays differential reactivity with the antibodies, reacting the best with mAb HB5. Furthermore, Jurkat cells bind much less aggregated C3 when compared to the binding on B cells (Fig. 1, left set of panels).

Jurkat cells bind EBV

Reactivity of Jurkat cells with EBV was determined by treating them with BioEBV, followed by streptavidin-phycocerythrin and analysis in the flow cytometer. It is apparent that BioEBV binds to Jurkat T cells (78% positive) and JY B cells (88% positive) at about equal proportions (Fig. 1, right set of panels). Preincubation of the virus with mAb 72A1, an antibody that is known to block binding of EBV to B cell CR2 (36) caused 100% inhibition of virus-binding to Jurkat (Fig. 1, right set of panels). Moreover, pretreatment of the cells with anti-CR2 mAb also inhibits EBV binding (Fig. 1, right set of panels). Antibody OK7 caused 94 and 100% inhibition of EBV binding to JY and Jurkat cells, respectively, whereas antibody HB5, in the presence of goat anti-mouse Ig caused 72% and 64% inhibition of virus binding to JY and Jurkat, respectively. The reason for the most efficient blocking with antibody OK7 is due to the fact that this antibody reacts with an epitope that is located in SCR 1 and 2, the same site where EBV
binds. Antibody HB5 binds to an epitope at SCR 3 and 4 (37, 38). Finally, preincubation of cells with nonconjugated EBV caused 100 and 94% inhibition of BioEBV binding to Jurkat and JY, respectively (Fig. 1, right set of panels). The above data, collectively, demonstrate that EBV binding to Jurkat cells occurs specifically via CR2 and it is not an artifact of biotinylation.

**EBV variably infects Jurkat cells**

To analyze the ability of EBV to infect Jurkat cells, we incubated virus-containing B95-8 cell culture supernatants with Jurkat for a period of 7 days and the expression of EBNA-1, a viral Ag important in the maintenance of the viral episome (39), was assessed by PCR. To this end, cellular RNA was isolated, as described in Materials and Methods, treated with DNAase 1 to eliminate carried-over viral DNA, and subsequently subjected to reverse transcriptase treatment. The resulting cDNA were analyzed by PCR for the detection of EBNA-1 using specific oligonucleotide primers (see Materials and Methods). The expected 611-bp band for EBNA-1 is detected in cells that had been exposed to the virus (Fig. 2, lane 3). This band is not seen in identical samples that receive DNAase 1, but not reverse transcriptase treatment (Fig. 2, lane 2) thus, indicating that it represents mRNA and not contaminating, carried-over EBV-DNA. The latter can be seen when neither DNAase 1 nor reverse transcriptase treatment of the samples is performed (Fig. 2, lane 1). Jurkat cells that have not been incubated with EBV do not express EBNA-1 (not shown). Lane 4 (Fig. 2), identical to lane 2 but without template, is a control to indicate that the reagents are free of contaminants. Although the above data demonstrate clearly that EBV can infect Jurkat cells, the infection was only variably observed. Of eight experiments performed similar to the one described above, two displayed EBNA-1 transcripts whereas the rest were negative.

**Molecular analysis of Jurkat CR2**

To characterize Jurkat CR2 and compare it to the B cell CR2, we studied its relative molecular size by SDS-PAGE. Cells from established T (Jurkat, HPR-ALL) and B (JY, Raji) cell lines were radiolabeled with $^{125}$I. Cell lysates were reacted with anti-CR2 mAb HB5 or an isotype (IgG2a) antibody control, and the immunoprecipitates were analyzed by SDS-PAGE. A reproducible difference in relative molecular sizes was seen between Jurkat CR2 and that of both R cell lines and HPR T cells (Fig. 3, A and B). The apparent molecular size of Jurkat CR2 is 155 kDa whereas
that of the B cells and HPB is 145 kDa. Treatment of the immunoprecipitates with endoglycosidase F reduced both the Jurkat and B cell CR2 to about 119 kDa (Fig. 3B) indicating that at least part of the size difference is due to N-linked, high-mannose, complex oligosaccharide modifications.

We also analyzed and compared the Jurkat CR2 mRNA to that of B cells. Northern blot analysis of RNA from Jurkat and Raji cells with a B cell-CR2 cDNA, under high hybridization stringency, reveals the presence of predominant bands at 4.7 kb (Fig. 4). Thus, no apparent differences in mRNA transcript size is observed.

Isolation and characterization of Jurkat CR2 cDNA clones

Purified mRNA from Jurkat cells was used to construct a cDNA library. On screening with a B cell CR2 cDNA, under low stringency hybridization, two positive clones were identified and studied further. One of the clones, pBCJ5d (2.3 kb in size), was analyzed by restriction endonuclease digestion and by sequencing of both DNA strands, and the results compared to those from three other sequences of B cell CR2 cDNA (16, 34, 40). The results (not shown) indicated that this clone is incomplete as it terminates in exon 7. The sequencing data revealed a sequence identical to the one reported by Moore et al. (34) with the exception that exon 11 is spliced out. This splicing event has been previously reported by Fujisaku et al. (16) and Weis et al. (40). PCR amplification of Jurkat mRNA was used to obtain sequencing information for the rest of this clone. Jurkat mRNA was subjected to reverse transcription as described in Materials and Methods with the difference that a 15-base oligonucleotide that spans the junction of exons 12a and 12b, instead of oligo-dT, was used. The so obtained product was amplified by PCR (as described in Materials and Methods) using primers that hybridize to exons 1 and 9,10, respectively. The derived 1575 bp product was subcloned with the TA cloning system following the manufacturers recommendation (Invitrogen, San Diego, CA). Sequencing analysis revealed a sequence identical to those previously reported (16, 34, 40).

Analysis of the second Jurkat CR2 cDNA clone, pBCJ2d, showed that it is identical to pBCJ5d above, but it represents a splice variant, as it lacks exon 8b (Fig. 5A). The elimination of exon 8b and subsequent joining of exons 8a and 9,10 introduces a frameshift mutation that results in a change of the amino acids encoded by exon 9,10 and the creation of a stop codon (Fig. 5A). These changes allow for the prediction of a truncated CR2 protein with 32 novel amino acids at its carboxy-terminus (Fig. 5A). Confirmation of the DNA sequence of this splice variant over the exon-8a/9,10 junction was performed by using two independent primers on the positive strand and one primer on the negative strand (Fig. 5B).

With this evidence of a novel CR2 splice variant in T
cells, we wanted to assess its presence in cells of the B lineage. Poly(A)^+ RNA from both Jurkat and Raji cells was subjected to reverse transcription and amplification by PCR using specific oligonucleotide primers flanking exon 8b (see Materials and Methods). The PCR products were resolved by agarose gel electrophoresis, and analyzed by Southern blotting and probing with radiolabeled oligonucleotides that specifically hybridize to either exon 8b or exon 9,10. The expected signals, of approximately 420 and 497 bp, respectively, are revealed in both Jurkat and Raji cells when blots are probed with the oligomer that hybridizes to exon 9,10 (Fig. 6A). In contrast, only the 497 bp band is seen when blots are probed with the exon 8b-specific oligonucleotide (Fig. 6A). These data indicate that both exon 8b-containing and exon 8b-lacking transcripts are encoded in both Jurkat and Raji cells. The specificity of the probing oligomers is demonstrated in Figure 6B where the B cell CR2 (AE41) (34) and pBC12d cDNAs are used as templates for PCR amplification and probed with either exon 8b or exon 9,10 specific oligonucleotides. As expected the exon 9,10-specific probe reveals only the 420 bp signal in the pBC12d cDNA whereas the exon 8b probe does not hybridize at all.

Discussion

In our investigation, we have extended the characterization of CR2 on T lymphocytes by using the human Jurkat T cell line as a model system. We have demonstrated a variable reactivity of Jurkat cells with anti-CR2 mAb, characterized the anti-CR2 reactive protein on Jurkat by immunoprecipitation and SDS-PAGE, and showed that EBV can bind and infect, albeit variably, Jurkat cells. EBV-binding to Jurkat is specific as demonstrated by its inhibition with anti-CR2 and anti-EBV antibodies. Northern blot analysis of Jurkat RNA reveals a message of 4.7 kb, consistent with the size of the B cell CR2 mRNA (34, 40). Analysis of cDNA clones from a Jurkat library uncovered the existence of one clone, pBC13d, whose sequence is identical to the one previously reported by Moore et al. (34) with the exception that exon 11 is spliced out. Another isolated cDNA clone, pBC12d, seems to represent a novel splice variant of CR2 as it lacks exon 8b. The deletion of exon 8b in this clone causes a frameshift mutation that predicts a truncated form of CR2 that carries a novel 32 amino acid carboxyl-terminus. Furthermore, we found that this exon 8b-lacking CR2 variant is also expressed in cells of the B lineage. The CR2 gene is found in the q32 band of chromosome
1 (41) in a cluster of genes belonging to a family known as the regulators of complement activation (42, 43). This family, among others, includes the genes that code for CR1 and 2, membrane cofactor protein, and factor H. Besides sharing various functional features some of these genes use alternative splicing to produce multiple transcriptional forms (44–49). In certain cases alternative splicing may be used to create both membrane-associated, as well as soluble forms of the coded proteins. Caras et al. (44) have proposed that an alternatively spliced form of decay accelerating factor, where a splicing event causes a shift in the reading frame near the carboxyl-terminus, may encode for a soluble form of the protein. Hourcade et al. (46) have described an alternatively processed transcript of CR1 that predicts the coding of a secreted form of this C3b/C4b receptor.

In view of the above, it remains plausible that the novel splice variant of CR2, pBC2d, we have described in this report, may represent a soluble form of this receptor. In fact, there exists evidence supporting the contention for a soluble form of CR2 in normal human serum (50) at levels comparable to those of soluble CR1 (51). Moreover, it has been previously shown that soluble CR2, or CR2-like, molecules can be isolated from the spent culture media of Raji B lymphoblastoid cells, and that its molecular mass is approximately 72 kDa (52, 53). However, it is not yet clear if such a protein represents a proteolytic cleavage product of CR2 or whether it results from alternative splicing of CR2 mRNA, as has been described above. It is therefore possible that the pBC2d cDNA could produce a protein with a predicted molecular mass of about 67 kDa, which upon glycosylation could conceivably represent the 72-kDa form of CR2 previously reported in serum and in the supernatants of Raji B cell cultures (50, 52, 53).

In comparison to B cells, Jurkat cells display differential reactivity with monoclonal anti-CR2 antibodies and with C3. One possibility that could explain this could be differences in the primary structure of the two CR2 molecules whereas another possibility, not exclusive of the former, could be differences in posttranslational modifications, such as those involving N-linked, high-mannose, complex oligosaccharide modifications. The lack of differences in nucleic acid sequences between B cell and Jurkat CR2 cDNA does not support the former explanation. However, the data in Figure 3, indicating differences in apparent molecular sizes before, but not after Endo-F treatment, support the possibility that posttranslational modification of Jurkat CR2 is different than that in B cells which may very well explain the differential reactivity.

In contrast to their differential reactivity with anti-CR2 antibodies and C3, Jurkat cells are able to bind EBV at a level comparable to that of B cells. It has been shown that the sites on CR2 to which C3, EBV and mAb OKB7 and HB5 bind are distinct (37, 38, 54, 55). Both EBV and antibody OKB7 reactive epitopes require SCR 1 and 2 for their binding whereas antibody HB5 needs SCR 3 and 4 (37, 38). The binding of EBV and OKB7 does not rely on specific amino acid sequences, but is dependent on distinct receptor conformation involving discontinuous regions of the CR2 primary structure (54, 55).

The infection of Jurkat cells by EBV was assessed by the transcriptional activation of EBNA-1, a protein important for the maintenance of the viral genome (39). Even though clear evidence of infection was obtained, it was variable, as only two out of eight experiments displayed transcription of EBNA-1. Despite the variability, however, other evidence from both our own laboratory and others corroborates infectability of T cells by EBV. Expression of EBNA-1 in thymocytes cultured with EBV was demonstrated by Western blotting analysis (26), whereas EBNA-1 transcripts, using PCR as described here with Jurkat, have been detected in HSB-2 T cells on exposure to EBV-containing supernatants (33). The HTLV 1 harboring T cell line MT-2 expresses high levels of CR2 and produces the EBV-coded Ag EBNA-1 and LMP after viral infection (56). The variability in EBNA-1 expression, we see with Jurkat cells, may reflect individual differences among the various cell targets. For example, variations in expression of cellular transcription factors among different cell types that
can interact synergistically with EBV-coded transactivators and determine the viral state have been previously described (57). Whether such events can explain variability in infecting Jurkat cells in our experiments will require further investigation that may lead to important insights in EBV-host interactions.

The presence of CR2 or related molecules on T cells may have implications on the significance of these receptors in both physiologic and pathologic conditions. Although the physiologic significance of CR2 on T cells is not yet elucidated, our recent reports on the presence of such molecules on immature human thymocytes (25, 26) and the suspected role of CR2 on B lymphocytes (42) suggest involvement in differentiation and/or activation. Anti-CR2 antibody reactivity and EBV binding is present in CD1+ (25, 26), CD4+CD8+ double positive (our unpublished observations) thymocytes, and expression of CR2 is lacking during the stem cell and pre-B cell stages, but it starts appearing at the time surface IgM is also detected (58, 59).

The presence of CR2 or related receptors on thymocytes (25, 26) and in normal peripheral blood T lymphocytes (27, 60), as well as the ability of EBV to infect cells of the T lineage (present report) (26, 33, 56) may have significant implications for EBV pathology. Of particular importance in this regard are the following reports that describe cases of patients with EBV-genome positive T cell lymphomas (3–9, 61). Kikuta et al. (3) reported the case of a patient who, after chronic EBV infection, developed a Kawasaki-like disease with the interesting characteristic of circulating T cells that carried EBV genome. Jones et al. (4) described three different cases of patients with T cell lymphomas that carried the EBV genome who, similar to Kikuta’s observations, had primary EBV infections before the development of the lymphoma. Harabuchi et al. (5) reported EBV-positive nasal T cell lymphomas in five patients with lethal midline granulomas (5), and Ho et al. (7) described 11 cases of primary nasal lymphomas, 9 of which had a T cell phenotype and were positive for EBV-DNA. Finally, Richel et al. (8) and Su et al. (9) have described CD8+ T cell lymphomas and aggressive peripheral T cell lymphomas respectively, all of which were positive for EBV-DNA. In view of these clinical reports and the evidence of EBV receptors in T cells, the frequency of EBV+ T cell lymphomas should be addressed.

Opsonization of HIV-1 particles with complement enhances infection of the human MT2 T cell line under low doses of viral infectivity (62). This phenomenon is CD4 independent and can be inhibited by anti-CR2 antibodies (62). Thus, notwithstanding the fact that the CD4 glycoprotein is the primary HIV-1 receptor, the above finding suggests that CR2 may play a critical role in HIV-mediated pathogenesis by facilitating infection in patients with low viral titers.

In conclusion, we have presented evidence that confirms and further extends the finding of EBV/C3d receptors on cells of the T lineage. This report constitutes the first demonstration of a molecular CR2 cDNA clone in T cells. Our information, the clinical data of T cell lymphomas bearing the EBV-genome, and the possible involvement of CR2 receptors in facilitating infection of T cells by HIV, emphasize the need for further study on the role of CR2 in T cell normal physiology and immunopathology.

Acknowledgments

The authors thank Dr. M. Dierich of the Innsbruck Institute of Hygiene, Innsbruck, Austria for the 6F7 mAb, Drs. M. Moore and G. Nemerow for the CR2-cDNA, Sabine Escobar for assisting with DNA sequencing, Gary Sumnicht and Richard Barnet of San Diego State University for oligonucleotide synthesis, Steve Barlow of San Diego State University for assistance with the photography, and Dennis Young of the University of California Cancer Center for flow cytometry. We also thank Dr. Kathleen McGuire of San Diego State University for invaluable suggestions.

References


receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein Barr Virus receptor map to 1q32. J. Immunol. 138:312.


