Inhibition of Epstein-Barr Virus Infection In Vitro and In Vivo by Soluble CR2 (CD21) Containing Two Short Consensus Repeats

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The extracellular domain of CR2, the Epstein-Barr virus (EBV)/C3d receptor of B lymphocytes, contains 15 or 16 tandemly arranged short consensus repeat elements (SCR). Recombinant CR2 proteins containing SCR 1 and 2 fused to *Staphylococcus aureus* protein A (PA-CR2) and to murine complement factor H SCR 20 (CR2FH) were expressed in *Escherichia coli* and in insect cells, respectively. These recombinant CR2 molecules retained functional activity as indicated by their ability to bind to C3dg in an enzyme-linked immunosorbent assay and to inhibit EBV gp350/220 binding to B cells. PA-CR2 and CR2FH were as efficient in blocking EBV gp350/220 binding as the full-length CR2 extracellular domain, indicating that the first two SCR of CR2 contain the majority of the ligand binding activity of the receptor. PA-CR2 and CR2FH inhibited EBV-induced B-cell proliferation in vitro and blocked the development of EBV-induced lymphoproliferative disease in severe combined immunodeficient mice reconstituted with human lymphocytes. These studies indicate that soluble forms of truncated CR2 proteins may have potential therapeutic value in the treatment of EBV-induced lymphoproliferative disorders in humans that involve viral replication.

Epstein-Barr virus (EBV), a human herpesvirus which is the agent of infectious mononucleosis (11), is carried in a latent state in B lymphocytes in the majority of adult individuals. Although latent infection with EBV usually does not result in disease, EBV nonetheless possesses oncogenic potential and has been implicated as a cofactor in nasopharyngeal carcinoma (27) and African Burkitt's lymphoma (6). EBV-induced B-cell lymphoproliferative disease is also associated with immunosuppressed patients with AIDS (34) or in individuals receiving organ transplants (5). Recently, an animal model of EBV-induced lymphoproliferative disease using mice with severe combined immunodeficiency (SCID) has been developed (3, 18). SCID mice transplanted with peripheral blood lymphocytes (PBL) from EBV-seropositive donors develop EBV-containing tumors spontaneously, while mice reconstituted with PBL from EBV-seronegative donors succumb to fatal lymphoproliferative disease following injection of exogenous EBV (3).

EBV is one of the few human herpesviruses for which the earliest events in attachment and entry have been elucidated on a molecular level. EBV binding to B cells is mediated by the gp350/220 envelope glycoprotein (21, 30) which binds to the cellular complement C3dg receptor designated CR2 (CD21) (7, 8, 24). A 9-amino-acid sequence in the EBV gp350/220 envelope glycoprotein (EDPGFFNVE) which is very similar to a region in C3dg (EDPGKQLYNVE) (14) appears to play a dominant role in ligand binding to CR2 (20). CR2, the EBV/C3dg receptor, is composed of an extracellular domain of 16 (16) or 15 (32) short consensus repeat elements (SCR) of 60 to 75 amino acids that is followed by a predicted transmembrane domain and a short cytoplasmic tail. The entire 16-SCR extracellular domain of CR2 has been expressed as a soluble protein in insect cells, allowing a detailed analysis of its physiochemical and ligand binding properties as well as certain of its structural features (17). This soluble protein blocks EBV infection in vitro (23). Transient expression of recombinant CR2 on the surface of non-B cells allows EBV to infect these cells (1). The EBV gp350/220 and C3dg binding site(s) has been colocalized to the first two amino-terminal SCR of CR2 by expression of truncated and chimeric forms of CR2 and CR1 on the surface of non-B cells (15).

In the studies presented here, we examined whether soluble CR2 containing SCR-1 and SCR-2, expressed as a chimeric or fusion protein, retains EBV and C3dg binding activity. In addition, the ability of soluble CR2 to block EBV-induced lymphoproliferation in vitro and in vivo was analyzed.

MATERIALS AND METHODS

Cells, viruses, and CR2 ligands. The transforming strain of EBV was produced in the B95-8 cell line as previously described (19). B95-8 and Raji B-cell lines were cultured in Dulbecco modified Eagle medium containing 10% fetal bovine serum, 25 µg of gentamicin (MA Bioproducts) per ml, 20 mM glutamine, and 0.2 U of amphotericin B (Fungizone) (GIBCO) per ml. Insect cells (*Spodoptera frugiperda* clone 9 [Sf9]), were cultured in EX-Cell 400 insect medium (J. R. Scientific, Woodland, Calif.) containing 1% fetal bovine serum. The C3dg complement fragment was isolated from human plasma by glutaraldehyde cross-linking as previously described (31). Soluble recombinant gp350/220 was isolated from culture supernatants derived from GH3 Δ19 rat pituitary cells expressing soluble recombinant gp350/220 (33) as previously described (30).

CR2 expression vector constructs. Expression and purification of the complete CR2 extracellular domain produced in insect cells [CR2(1-16)] has been previously reported (23). A bacterial expression vector containing the first two SCR of CR2 fused to *Staphylococcus aureus* protein A (PA) was

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constructed as follows. The CR2-coding sequence, derived from pBS/EA13X2 (16), was modified by site-directed mutagenesis (13) by insertion of a stop codon immediately following SCR-2 as shown in Fig. 1. Two new amino acids, Asp-Pro, were created at the 5' end of the CR2 gene by insertion of a BamHI linker. The CR2-coding region was then inserted in the proper reading frame and orientation in pRIT12 (26) by using the BamHI and SalI restriction sites. A baculovirus expression vector was constructed to produce CR2 containing SCR-1 and -2 (CR2-SCR 1+2) in insect cells. A cDNA clone containing the carboxyl-terminal SCR-20 of complement murine factor H (FH) (12), pGEM/MFH20, kindly provided by Dennis Vik (Scripps Clinic), was modified by the polymerase chain reaction to create a 225-bp fragment containing an XhoI-murine FH SCR 20-stop codon-XbaI nucleotide sequence. The nucleotide sequence of this fragment was confirmed by dideoxy sequencing and then inserted into pBS/EA13 which had been digested with XhoI and XbaI. This plasmid, designated pBS/CRFH, was then digested with EagI (New England BioLabs), blunt ended with DNA polymerase (Klenow), and modified with BamHI linkers (Pharmacia). After digestion with BamHI and XbaI, the CRFH fragment was inserted in the proper orientation in the baculovirus transfer vector pVL1393. CR2FH recombinant baculoviruses were produced by cotransfection of Sf9 cells with pVL1393/CRFH and Autographa californica nuclear polyhedrosis virus DNA as previously described (29).

Purification of recombinant CR2 chimeras. PA-CR2 was isolated from Escherichia coli (NM522) transfected with pRIT/SCR1+2. Cells were grown to the mid-log phase and then subjected to osmotic shock (25). The periplasmic extracts were collected by centrifugation and brought to 0.12 U of aprotinin per ml, 2 mM phenylmethylsulfonyl fluoride, 5 mM EDTA, 0.05% sodium azide, 0.5 U of leupeptin and pepstatin per ml, and 30 mM Tris-HCl (pH 8.3). Extracts (approximately 800 ml) were incubated overnight at 4°C on a rocker with 2 ml of immunoglobulin G (IgG)-Sepharose (Pharmacia). The affinity resin was then washed three times with 100 ml of 10 mM Tris-HCl (pH 8.3) containing 150 mM NaCl and 0.05% Tween 20 and then twice with the same buffer without detergent. Bound PA-CR2 was eluted with 50 mM diethylamine (pH 11.5) and immediately neutralized with 1 M glycine-HCl (pH 2.5). IgG affinity-purified PA-CR2 was then diluted fivefold in water and incubated overnight at 4°C with 4 ml of C3b-Sepharose, kindly provided by Richard Discipio (Scripps Clinic). The C3b resin was extensively washed with 10 mM Tris-HCl (pH 7.5) containing 10 mM NaCl, and the bound PA-CR2 was eluted with 10 mM Tris containing 450 mM NaCl. For virus inhibition studies, residual endotoxin was removed by the use of De-Toxi Gel as recommended by the manufacturer (Pierce Chemical Co., Rockford, Ill.). CR2FH was isolated from Spinner cultures of Sf9 cells infected with recombinant virus as follows. Sf9 cells in medium containing 1% fetal bovine serum were infected with a CR2FH recombinant virus at a multiplicity of infection of 4.0. At 72 h postinfection, the cells were removed by centrifugation at 3,000 rpm for 15 min and the supernatant was dialyzed extensively against 10 mM Tris-HCl (pH 7.4). The supernatant was then incubated with 1 ml of C3b-Sepharose per 250 ml of supernatant on a rocker at 4°C for 24 h. The C3b-Sepharose was subsequently washed extensively in 10 mM Tris-HCl (pH 7.4), and the CR2FH was eluted with 450 mM NaCl in 10 mM Tris-HCl (pH 7.5).

Ligand binding studies. An enzyme-linked immunosorbent assay (ELISA) was developed to detect binding of PA-CR2 to C3dg aggregates. A 1-μg sample of PA-CR2, CR2FH, or PA alone was dried onto 96-well plates (Immobilon II; Dynatech) in 0.1 M bicarbonate buffer (pH 8.8). Nonspecific binding sites as well as the IgG binding sites on PA were blocked by the addition of phosphate-buffered saline (PBS) containing 5% nonfat dry milk (BLOTTO) and 2 mg of human gamma globulins (Pierce) per ml for 2 h at 37°C. Immobilized receptor was then incubated for 1 h at 37°C with twofold serially diluted aggregated C3dg in PBS containing 0.5% BLOTTO. After the receptor-ligand mixture was washed with PBS containing 0.1% Tween 20, 100 μl of a 1:200 dilution of an anti-C3dg monoclonal antibody (Cytotech, San Diego, Calif.) in PBS–0.5% BLOTTO was added for 1 h at 37°C. The plates were washed with PBS–0.5% BLOTTO and then incubated for 1 h at 37°C with 100 μl of a 1:3,000 dilution of peroxidase-labeled goat anti-mouse Ig (Kirkegaard & Perry Laboratories) per well. The wells were again washed and then developed with substrate [2,2-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid (ABTS)]. Absorbance was monitored at 405 nm with a Titertek II ELISA plate reader (Flow Laboratories, Inc., McLean, Va.). Binding of recombinant CR2 to EBV gp350/220 was measured by using fluorescent microspheres (Baxter, Chicago, Ill.) bearing soluble EBV gp350/220 as previously described (23).

Inhibition of EBV infection in vitro and in vivo by CR2. The ability of fusion or chimeric forms of CR2 to inhibit EBV-induced B-cell transformation in vitro was determined as previously described (19). Briefly, 200 μl of EBV-containing B95-8 culture supernatants were incubated for 90 min at 4°C with various amounts of PA or PA-CR2 and then 6×10^5 PBL in complete RPMI 1640 medium were added for an additional 60 min at 4°C. The cells were then washed and cultured in 96-well plates in the presence of 0.2 μg of cyclosporin A per ml for 14 days. Outgrowth of transformed B-cell colonies was monitored by light microscopy at low-power magnification. For in vivo experiments, 2-month-old C.B.17/SCID (SCID) mice were reconstituted by intraperitoneal injection of 4.0×10^7 PBL from an EBV-seronegative donor. On day 13 postreconstitution, three mice each were injected with 200 μl of EBV alone or with 200 μl of EBV following preincubation for 90 min at 4°C with various amounts of PA, PA-CR2, or CR2FH in a total volume of 1.4 ml. Mice were examined daily for evidence of tumor-associated clinical signs of disease. The presence of abdominal tumors of human B-lymphoid origin was confirmed by autopsy. Serum samples were collected from the tail vein at weekly intervals starting at 14 days postinfection with EBV for ELISA of human Ig as an indicator of the extent of EBV-induced B-cell proliferation (26a).

RESULTS

Expression and isolation of CR2 chimeras. Initial studies indicated that truncated forms of the CR2 extracellular domain expressed in bacteria or in insect cells were unstable in these cells, thus limiting their ability to be functionally analyzed. To overcome this problem, CR2 containing SCR-1 and -2 was expressed in E. coli as a fusion protein with S. aureus PA (Fig. 1). A two-step affinity purification procedure was developed to isolate the fusion protein to near homogeneity. Periplasmic extracts of transfected NM522 cells were affinity purified on IgG-Sepharose and then bound to C3b-Sepharose at low ionic strength. As seen in Fig. 2 (lane 1), a CR2-PA fusion protein with an apparent molecular weight of 43,000 as well as a series of low-molecular-weight proteins (approximately 32,000 to 36,000) were present in the eluate from the IgG column. The low-molecular-weight proteins did

A. PROTEIN A-CR2 EXPRESSION VECTOR

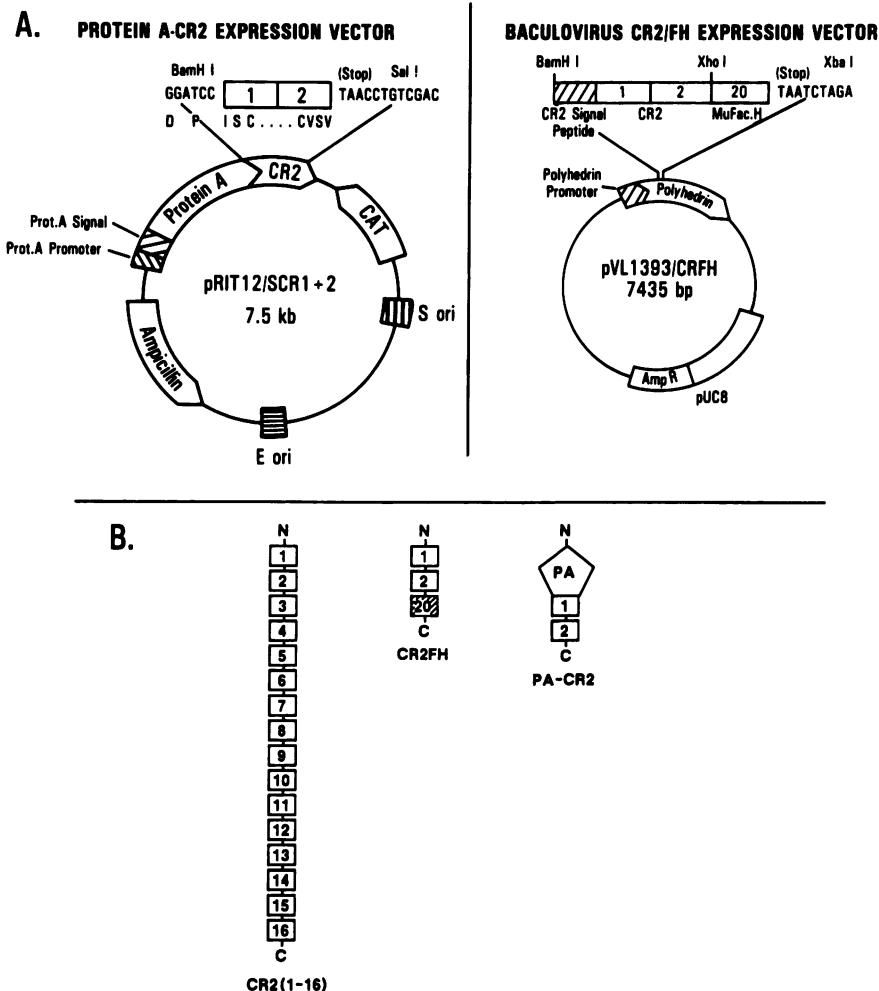**BACULOVIRUS CR2/FH EXPRESSION VECTOR**

FIG. 1. PA-CR2 and CR2FH expression vectors (A) and schematic representation of soluble forms of recombinant CR2 proteins (B). The SCR of CR2 and murine FH are represented by numbered boxes.

not bind to immobilized C3 (Fig. 2, lane 2). In contrast, the 43-kDa PA-CR2 fusion protein, which is very close to the predicted size of PA plus the SCR-1 and -2 segments, was eluted from the C3b resin with high-salt buffer (Fig. 2, lane 3). In parallel studies, CR2FH, produced in insect cells, was isolated from infected Sf9 culture supernatants by C3b affinity chromatography (Fig. 2, lane 4). The isolated CR2FH chimera migrated as two bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a major band at approximately 27 kDa and a minor band at 29 kDa. Both protein bands were immunoprecipitated by the OKB7 anti-CR2 monoclonal antibody but not by the HB5 anti-CR2 monoclonal antibody which recognizes SCR-3 to -4 of CR2 (15) (data not shown). The difference in molecular weight of these two proteins is likely due to differences in N-linked glycoslyation since the nucleotide sequence of the CR2FH cDNA contains three predicted sites for posttranslational modification.

Ligand binding properties of PA-CR2, CR2FH, and CR2(1-16). To assess the functional activity of PA-CR2, we also analyzed the binding of the receptor to glutaraldehyde-aggregated C3dg in an ELISA. One microgram of PA alone or PA-CR2 was coated onto ELISA plates and then reacted
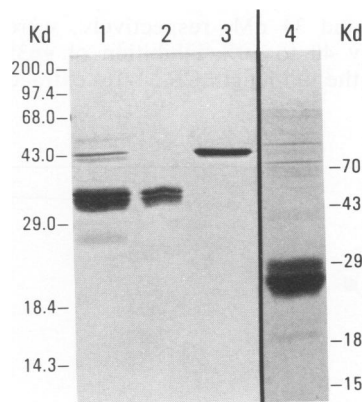
FIG. 2. SDS-PAGE analysis of PA-CR2 and CR2FH. Periplasmic extracts of *E. coli* expressing PA-CR2 were chromatographed on an IgG-Sepharose column (lane 1). PA-CR2 was eluted from the IgG column by high-pH buffer and then purified on a C3b-Sepharose column. The nonbinding fraction and high-salt eluate fraction from the C3b-Sepharose column are shown in lanes 2 and 3, respectively. CR2FH was isolated on C3b-Sepharose and eluted with high-salt buffer (lane 4). Samples were electrophoresed on an SDS-15% polyacrylamide gel which was stained with Coomassie blue.
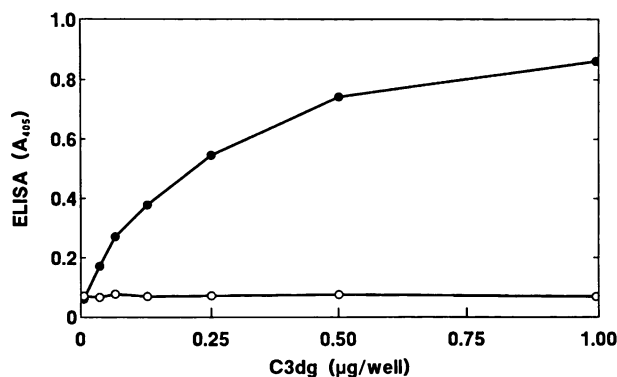
FIG. 3. Binding of PA-CR2 to C3dg. Various amounts of aggregated C3dg were incubated with 1 μg of immobilized PA-CR2 (●) or PA (○) in an ELISA. C3dg binding was detected by an anti-C3d monoclonal antibody.

FIG. 5. Inhibition of EBV gp350/220 binding to Raji B-lymphoblastoid cells by PA-CR2. Fluorescent microspheres coated with gp350/220 were incubated with various amounts of PA-CR2 (●) or PA (○) prior to addition of Raji B cells. Binding of ligand-coated microspheres to B cells was assayed by flow cytometry.

with various amounts of C3dg and then monoclonal antibody to C3dg. As shown in Fig. 3, C3dg showed dose-dependent binding to PA-CR2 but not to PA alone. In parallel studies, the ability of CR2FH to bind to aggregated C3dg was examined and similar dose-dependent binding to this complement fragment was observed (Fig. 4). PA-CR2 binding to EBV gp350/220 was next examined by flow cytometry. gp350/220-coated fluorescent microspheres were incubated with various amounts of PA-CR2 or PA alone prior to the addition of Raji B-lymphoblastoid cells bearing 30,000 CR2 sites per cell. The percentage of cells with greater than three microspheres per cell was quantitated by flow cytometry. As shown in a representative experiment (Fig. 5), PA-CR2 efficiently blocked EBV gp350/220 binding to Raji cells. Approximately 30 nM PA-CR2 was capable of causing 50% inhibition of gp350/220 binding to B cells, while 500 nM PA-CR2 caused greater than 90% inhibition of ligand binding. PA alone at all doses had minimal inhibitory activity.

Further gp350/220 inhibition experiments were done with PA-CR2, CR2FH, and the full-length CR2(1-16) molecule to determine the relative EBV ligand binding activity of these molecules (Table 1). Similar amounts of PA-CR2 and CR2FH, 10 and 34 nM, respectively, were required for approximately 40 to 50% inhibition of gp350/220 binding compared to the full-length CR2(1-16) extracellular domain,
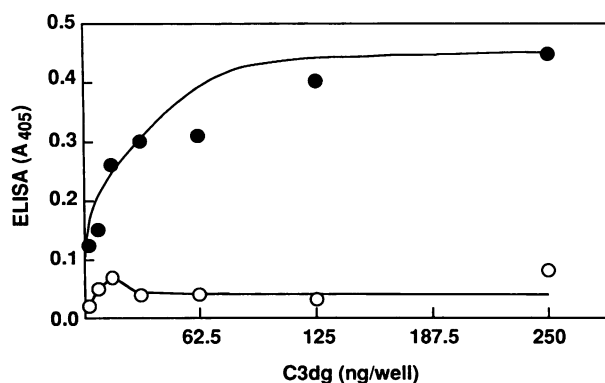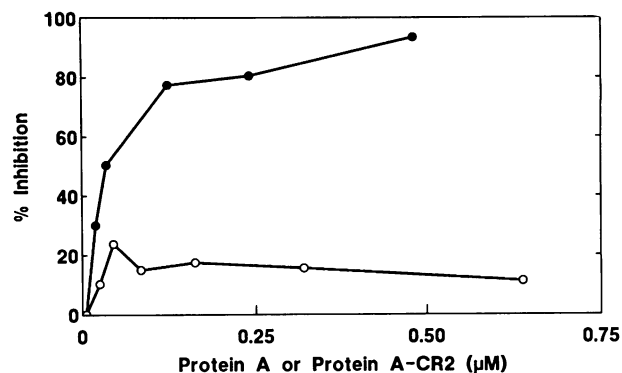
which required 38 nM. These results indicate that the majority of the EBV binding region of CR2 is located in SCR-1 and SCR-2 and that association of the receptor with non-CR2 sequences such as FH and PA does not appear to significantly alter ligand binding.

Effect of CR2 chimeras on EBV infection in vitro. The ability of PA-CR2 to competitively block EBV infection of adult PBL was assessed by outgrowth of EBV-transformed B-cell colonies in vitro. EBV was preincubated with various amounts of PA-CR2 or PA alone prior to the addition of PBL. After the initial incubation with B cells, both EBV and recombinant soluble CR2 were removed by washing and the cells were cultured for 14 days in the presence of cyclosporin A to allow outgrowth of EBV-transformed cells (2). As shown in Fig. 6, PA-CR2 caused dose-dependent inhibition of EBV infection as measured by outgrowth of transformed B-cell colonies. In these experiments, approximately 1 μM of PA-CR2 inhibited EBV infection of human B cells by 50%, while comparable amounts of PA alone had no effect on EBV-induced B-cell transformation.

Inhibition of EBV infection in vivo by recombinant CR2 chimeras. EBV infection of SCID mice reconstituted with PBL from EBV-seronegative donors results in the rapid development of aggressive, fatal lymphoproliferative disor-

FIG. 4. Binding of CR2FH to C3dg. Various amounts of aggregated C3dg were incubated with 1 μg of immobilized CR2FH (●) or bovine serum albumin (○), and C3dg binding was detected in an ELISA.

TABLE 1. Relative inhibitory activity of recombinant CR2 proteins[a]

Ligand	Inhibitor (nM)	% of cells with gp350/220	% Inhibition
gp350/220	None	79.9	0
Bovine serum albumin	None	4.1	NA[b]
gp350/220	PA-CR2 (10)	49.4	40.3
gp350/220	CR2FH (34)	42.4	49.4
gp350/220	CR2FH (180)	11.1	85.5
gp350/220	None	80.7	0
gp350/220	CR2(1–16) (38.1)	37.6	53.6

[a] gp350/220-coated fluorescent microspheres were reacted with soluble CR2 recombinant proteins and then incubated with Raji B-lymphoblastoid cells. The percentage of cells associated with three or more microspheres was determined by flow cytometric analysis. CR2(1–16) is the complete extracellular domain of the receptor produced in insect cells.
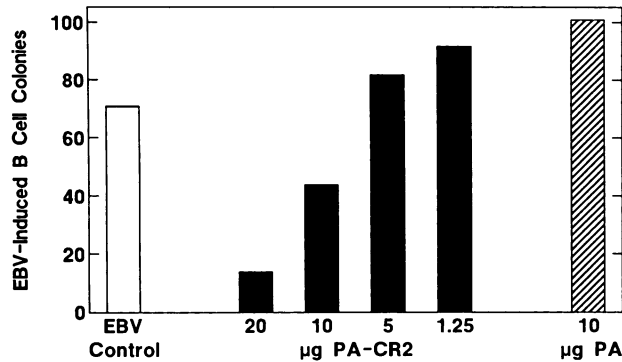
[b] NA, not applicable.

FIG. 6. Inhibition of EBV-induced B-cell transformation by PA-CR2. EBV-containing culture supernatants were preincubated with various amounts of PA-CR2 or PA prior to the addition of human peripheral blood mononuclear cells. After incubation with the cells, virus and soluble CR2 samples were removed by washing and the cells were cultured in the presence of cyclosporin A for 14 days. Outgrowth of transformed cell colonies containing greater than 25 cells per colony was enumerated by light microscopy at low magnification.
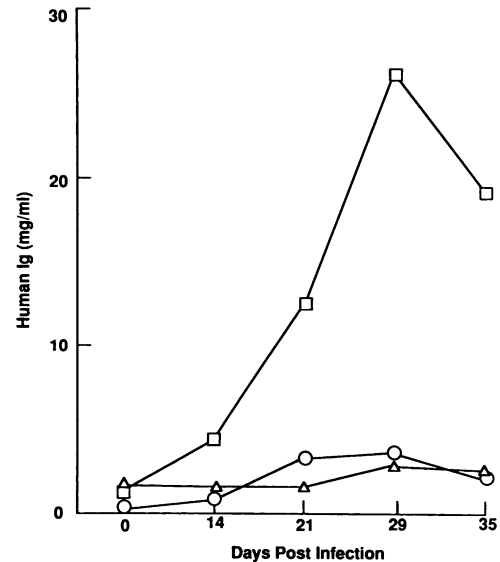

FIG. 7. Effect of soluble CR2 on EBV-induced Ig synthesis in SCID/human chimeric mice. Three SCID/human chimeric mice each were injected with EBV (□) or saline (△) or EBV plus 20 μg of PA-CR2 (○). Total human Ig synthesis was quantitated by an ELISA at various times after infection.

ders of human B-cell origin (3). SCID/human chimeric mice (C.B.17 mice) were injected intraperitoneally with EBV, resulting in clinical signs of disease (hunching, ruffled fur, lethargy, and tachypnea) on day 41 postinfection. Autopsies and histocytochemical analysis on day 42 revealed abdominal tumor masses in three of three mice. In contrast, each of the three mice that received saline or EBV which had been preincubated with 20 or 6.5 μg of PA-CR2 showed no signs of disease up to day 42, and only one of three mice injected with EBV and 2 μg of PA-CR2 showed evidence of abdominal tumor formation at autopsy. PA (20 μg) treatment of EBV alone had no tumor inhibitory effect (data not shown).

Tumor development in SCID/human chimeric mice is characterized by rapidly increasing serum levels of human Ig, reaching as much as 80 mg/ml at the time of clinical illness (26a). In these experiments, SCID/human chimeric mice infected with EBV alone had peak Ig titers of 26 mg/ml on day 29 postinfection, while uninfected (saline) SCID/human chimeric mice had approximately 3.5 mg of Ig per ml (Fig. 7). SCID/human chimeric mice injected with EBV which had been preincubated with 20 μg of PA-CR2 had Ig titers nearly identical to those of mice injected with saline alone.

DISCUSSION

The selected tropism by EBV for B cells and perhaps epithelial cells is in large part determined by a specific receptor on these cells, designated CR2 (CD21), which also is a receptor for the complement C3dg and C3d fragments. In these studies, the ligand binding domain of CR2 was expressed as a soluble protein so that its structural and functional properties in free solution could be analyzed. In previous studies, soluble CR2 containing 16 SCR produced in insect cells was shown to bind to EBV gp350/220 and C3dg (17) and to block EBV-induced B-cell transformation in vitro (23). However, further attempts to express truncated forms of the extracellular domain of CR2 in insect cells in order to analyze the ligand binding region of the receptor resulted in proteins which were produced in very low amounts and were thus unsuitable for further structure or function studies (22).

In studies presented here, CR2-SCR 1+2 was expressed as a soluble chimeric protein. The bacterially expressed PA-CR2 was produced at 300 to 500 μg/liter of culture, while the CR2FH produced in insect cells was isolated at 2 mg/liter from Sf9 cells. The lower yield of the bacterial fusion protein is likely due to partial degradation of the protein before it is transported into the bacterial periplasmic space. Both CR2 chimeric receptors retained C3dg binding activity as demonstrated by binding to C3b-Sepharose columns at low ionic strength and to purified C3dg in an ELISA of normal ionic strength. These results are consistent with two previous reports in which SCR-1 and SCR-2 were expressed on the surface of non-B cells (4, 15). However, one of these studies (4) failed to demonstrate binding of CR2-SCR 1+2 to soluble aggregated C3dg, and the authors concluded that SCR-3 and SCR-4 may have an additional role in C3dg binding. Our studies clearly showed that recombinant forms of soluble CR2-SCR 1+2 are capable of binding to either immobilized C3 or soluble C3dg. A possible explanation for the discrepancy in these studies is that in the studies by Carel et al. (4), CR2-SCR 1+2 was expressed on the cell surface immediately adjacent to the transmembrane domain of the receptor. This form of expression may have restricted proper access of SCR 1+2 to C3dg or perhaps altered its conformation.

In studies presented here, the CR2-SCR 1+2 protein also competitively blocked EBV gp350/220 binding to B cells. This chimeric form of CR2 displayed gp350/220 binding activity similar to that of the full-length form of CR2— approximately 30 nM was required to achieve 50% inhibition of binding. The observed gp350/220 inhibitory activity of the chimeric CR2 protein is consistent with the previously determined affinity of gp350/220 for CR2(1-16) (K_d = 3.2 nM) (17). These studies thus indicate that SCR 1+2 of CR2 contains the majority of the EBV and C3dg binding activity. The remaining SCR of CR2 may contribute a minor component of the EBV and C3dg binding activity or possibly extend the ligand binding domain away from the cell surface.

PA-CR2 caused dose-dependent inhibition of EBV-induced B-cell transformation as indicated by abrogation of outgrowth of B-cell colonies in vitro. The inability of PA-CR2 to completely block EBV infection may be due to the fact that EBV gp350/220 has a high affinity for CR2 and thus even a single unblocked viral envelope protein may be sufficient to permit virion attachment. In parallel studies, preincubation of EBV with PA-CR2 blocked the ability of the virus to promote Ig synthesis and initiate tumor development in SCID mice reconstructed with human PBL. In the in vivo system, soluble PA-CR2 was able to completely abrogate EBV-induced B-cell lymphoproliferation, in contrast to the in vitro system, in which partial inhibition was obtained. The increased efficiency in inhibitory activity by PA-CR2 in the in vivo assay may be due to the use of a multiplicity of infection which was 60-fold lower than that used in the in vitro system. Whether soluble CR2 can be used to block EBV-induced lymphoproliferative disease by injection prior to addition of the virus into these animals remains to be determined. The development of a suitable animal model for EBV-induced lymphoproliferative disease as well as expression of soluble forms of the EBV/C3d receptor (CR2) may allow the development of therapeutic rationales for the treatment of EBV infection in humans. Previous reports indicate that some polyclonal B-cell hyperplasias are amenable to acyclovir therapy (9, 10), implying that EBV replication plays a role in the pathogenesis of EBV-induced lymphoproliferative disorders. In the spontaneous model of EBV-induced lymphoproliferative disorders in SCID mice, the EBV genome is present in both episomal (latent) and replicating (linear) forms. Spontaneous B-cell tumors arising in SCID mice also contain EBV structural antigens (28). Under such circumstances involving viral replication, the use of soluble receptor may be of potential therapeutic value.

ACKNOWLEDGMENTS

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