The Journal of Immunology
RESEARCH ARTICLE | FEBRUARY 01 1991

Expression of CR2 (the C3dg/EBV receptor, CD21) on normal human peripheral blood T lymphocytes. FREE

E Fischer; ... et. al

J Immunol (1991) 146 (3): 865–869.
https://doi.org/10.4049/jimmunol.146.3.865

0022-1767/91/1463-0865$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 865–869, No. 3, February 1, 1991
Printed in U.S.A.

EXPRESSION OF CR2 (THE C3dg/EBV RECEPTOR, CD21) ON NORMAL HUMAN PERIPHERAL BLOOD T LYMPHOCYTES[1]

ELISABETH FISCHER, CATHERINE DELIBRIAS, AND MICHEL D. KAZATCHKINE[2]

From INSERM U28 and Unité d'Immunopathologie, Hôpital Broussais, 75014 Paris, France

The expression of CR2 (the C3dg/EBV receptor, CD21) on normal human T lymphocytes was investigated using purified peripheral blood T cells and indirect immunofluorescence with biotinylated anti-CR2 mAb and streptavidin-phycoerythrin. Thirty to 40% of normal peripheral blood T lymphocytes expressed CR2 Ag. The cells expressed three nonoverlapping epitopes of CR2. The specificity of the staining for CR2 epitopes was demonstrated by the ability of unlabeled anti-CR2 mAb but not of anti-CR1 mAb of the same isotype to compete for the binding of biotinylated anti-CR2 mAb to T cells. The intensity of staining of T lymphocytes with anti-CR2 mAb was approximately 10-fold lower than that of peripheral blood B cells. CR2 was immunoprecipitated from purified T lymphocytes as a single protein of apparent M_r 145,000. The presence of CR2 on normal human T lymphocytes suggests that the receptor may modulate the function of T cells in the immune response and the susceptibility of the cells to infection by lymphocytotropic viruses.

CR2 (CD21) is a 145-kDa integral transmembrane single-chain glycoprotein that functions as cellular receptor for the surface-bound C3dg and C3d fragments of human C3 (1, 2). CR2 is also the B lymphocyte receptor for EBV, as demonstrated by the ability of anti-CR2 antibodies to inhibit binding to and infection of B cells by EBV and by the acquired susceptibility to EBV infection of stably transfected murine L cells or K562 cells expressing CR2 (3–8). The primary structure of CR2 has been determined through analysis of nucleotide sequences of cDNA clones isolated from human tonsillar and Raji lymphoblastoid cDNA libraries (9, 10). The CR2 gene encodes a 20-amino acid signal peptide, a 954-residue extracellular domain, a 24-amino acid transmembrane region, and a 34-amino acid cytoplasmic domain. The extracellular domain is composed of 15 tandem short consensus repeat sequences, which are homologous to those described in the CR1 gene and in other genes encoding for C3/C4 binding proteins located in the RCA cluster on chromosome 1 (11, 12). Analysis of CR2 deletion mutants has demonstrated that the amino-terminal two short consensus repeat sequences are required and sufficient to mediate the binding of C3dg and of the gp350/220 envelope glycoprotein of EBV (13).

Human CR2 is expressed on all mature B lymphocytes and on follicular dendritic cells (14–16). It has also been found on certain epithelial cells and on rare T lymphoblastoid cell lines, and it has recently been described on thymocytes (17–22). The present study indicates that 30 to 40% of normal human peripheral blood T lymphocytes express CR2. The amounts of CR2 expressed by T lymphocytes are approximately 10-fold lower than those present on peripheral blood B cells.

MATERIALS AND METHODS

Antibodies. BL13 (23) and HB5 (American Type Culture Collection, Rockville, MD) (15) are IgG1 and IgG2a mAb that specifically recognize human CR2. The IgG1 mAb BD6 against CR2 was a gift from Dr. Wijdenes (CTS, Besançon, France). mAb BD6 was obtained by fusing the nonsecreting myeloma cell line X63AG8653 with spleen cells from a mouse immunized with cells of the Daudi B lymphoblastoid cell line. J3D3 is an IgG1 mAb directed against human CR1 (24). mAb were purified from ascites using contraprecipitation with octanoic acid (25). mAb were biotinylated using the biotinylation kit from Amersham (Les Ulis, France). FITC-conjugated anti-CD3 mAb Leu-4, anti-CD4 mAb Leu-3a and Leu-3b, anti-CD8 mAb Leu-2a, anti-CD20 mAb Leu-16, and anti-IL-2R mAb were obtained from Becton Dickinson (Mountain View, CA). IgG1 anti-β-galactosidase mAb was a gift from Dr. C. Kanellopoulos (University of Paris VII). Rabbit anti-mouse Ig antiserum and IgG2a UPC 10 and IgG1 MOPC 21 mouse myeloma proteins were purchased from Cappel (Cochranville, PA).

Cells. PBMC were isolated from citrated blood of healthy donors by centrifugation on Ficoll-Hypaque gradients (Pharmacia, Uppsala, Sweden). Purified T lymphocytes were $CD2^+$ cells obtained from PBMC by rosetting with AET[3]-treated SRBC. The cells contained more than 95% $CD3^+$ cells and less than 1% $CD20^+$ cells. For mitogen stimulation experiments, T lymphocytes were cultured (5×10^5 cells/ml) in RPMI 1640 containing 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FCS (GIBCO-BRL, Cergy, France), in the presence of Con A (10 μg/ml) (Sigma Chemical Co., St. Louis, MO) or PHA (1 μg/ml) (Wellcome, Beckenham, UK), for 6 days.

Thymocytes were prepared from a human thymus obtained from a child undergoing cardiac surgery and were purified by rosetting with AET-treated SRBC.

The EBV^+ Burkitt lymphoma cell line Raji of the pre-B phenotype and EBV^- BJAB Burkitt lymphoma cells (kindly provided by Dr. G. Lenoir, International Agency for Research on Cancer, Lyon, France) were maintained in RPMI 1640 containing antibiotics and 10% heat-inactivated FCS.

The monocytic cell lines U 937 and Mono Mac 6 were obtained through N. Haeffner-Cavaillon (INSERM U28 Paris) and Dr. Ziegler-Heitbrock (Inst. Für Immunologie München, Germany).

Cell staining and flow cytometry. For one-color immunofluorescence staining, 5×10^5 cells in PBS, containing 2% normal human serum and 0.1% sodium azide, were sequentially incubated with 2 μg of biotinylated mAb and with SAPE (Becton Dickinson), for 30 min at 4°C, or incubated with appropriate concentrations of FITC-

Received for publication July 12, 1990.
Accepted for publication November 9, 1990.
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 Institut National de la Santé et de la Recherche Médicale (INSERM) and Association de Recherche sur le Cancer (ARC-6766), France.
[2] Please address all correspondence and requests for reprints to Michel D. Kazatchkine, INSERM U28 and Unité d'Immunopathologie, Hôpital Broussais, 75014 Paris, France.

[3] Abbreviations used in the text: AET, 2-aminoethylisothiouronium bromide hydrobromide; SAPE, streptavidin phycoerythrin.

mAb. In some experiments, staining with biotinylated antibodies and SAPE was amplified by addition of biotinylated anti-streptavidin antibody and of a second layer of SAPE, as previously described (26). Two-color immunofluorescence staining was performed by simultaneously incubating the cells with FITC mAb and biotinylated mAb, followed by SAPE. Stained cells were fixed with 1% paraformaldehyde, and cytofluorometric analysis was performed on gated viable lymphocytes by using a FACSCAN flow cytometer (Becton Dickinson). Fluorescence data were collected by using logarithmic amplification. Percentages of positive cells were calculated by computer analysis based on negative controls in which first antibody was omitted or replaced by isotype-matched biotinylated mAb.

Immunoprecipitation. For immunoprecipitation of CR2 from T lymphocytes and Raji cells, 2×10^8 T lymphocytes that had been purified by two consecutive rosetting steps and 2×10^7 Raji cells were surface-labeled with 1 mCi and 0.25 mCi of $Na^{125}I$ (Amersham), respectively, using Iodogen (Pierce, Beijerland, The Netherlands)-coated tubes. The cells were washed four times with PBS containing 0.1 M potassium iodine and 0.1 M cysteine and were incubated in 10 mM Tris, 150 mM NaCl, 0.5 mM EDTA, pH 7.4, containing 1% Nonidet P-40, 2 mM PMSF, and 20 mM α-iodoacetamide, for 20 min at 2°C. After centrifugation at 40,000 × *g* for 20 min at 4°C, solubilized membrane glycoproteins in supernatants were collected and incubated twice with preformed precipitated complexes of anti-β-galactosidase mAb and rabbit anti-mouse Ig antibodies, for 1 h at 4°C, as previously described (27). Precleared supernatants were incubated with preformed complexes of HB5 and BL13 mAb and rabbit anti-mouse Ig antibodies, for 13 h at 4°C. Specific immunoprecipitates were thoroughly washed, as described (27), and electrophoresed on 7.5% acrylamide gels in the presence of SDS under nonreducing conditions. Radioactive proteins were detected by autoradiography using Hyperfilm MP films (Amersham).

RESULTS

Expression of CR2 epitopes by human peripheral blood T lymphocytes. The expression of antigenic determinants of CR2 by normal human peripheral blood T lymphocytes was investigated by indirect immunofluorescence and flow cytometry, using purified T cells and biotinylated anti-CR2 mAb. Expression of the epitope recognized by mAb BL13 on normal T lymphocytes is illustrated in Figure 1. An average of 40% (ranging from 38 to 47%) $BL13^+$ cells were found among purified T lymphocytes from six healthy donors by using amplified immunofluorescent staining. An average of 32% (10 to 64%) $BL13^+$ cells were found among T lymphocytes from 14 healthy donors by using conventional indirect immunofluorescence with biotinylated mAb BL13; similar percentages of $CR2^+$ cells were found when purified T lymphocytes were analyzed by two-color immunofluorescent staining using FITC-anti-CD3 and biotinylated BL13 antibodies (Fig. 2). Two-color staining of cells from four healthy donors indicated that two thirds of $BL13^+$ T lymphocytes stained with anti-CD4 antibody and one third stained with anti-CD8 antibody (data not shown). Purified peripheral blood T lymphocytes expressed the epitopes defined by three mAb directed against human CR2, BL13, HB5, and BD6 (Fig. 3). A relatively higher proportion of cells stained with mAb HB5 than with mAb BL13 and BD6, whether the cells were only stained with biotinylated anti-CR2 mAb or whether the cells were analyzed by two-color immunofluorescence with FITC-anti-CD3 mAb, biotinylated anti-CR2 mAb, and SAPE. The BL13, HB5, and BD6 epitopes are nonoverlapping epitopes of CR2, as observed in binding experiments in which the unlabeled anti-CR2 mAb were allowed to compete with the biotinylated form of each of the three antireceptor antibodies for binding to CR2 on Raji cells

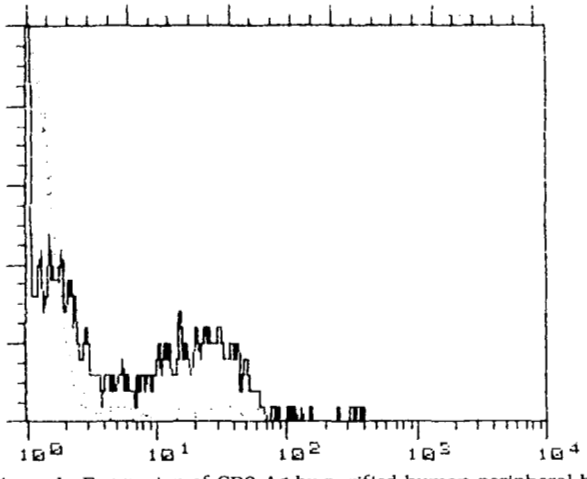

Figure 1. Expression of CR2 Ag by purified human peripheral blood T lymphocytes. Cells were stained with biotinylated mAb BL13 followed by SAPE, biotinylated antistreptavidin antibodies, and SAPE. Control cells (*dotted line*) were stained with the same reagents, omitting the anti-CR2 mAb.

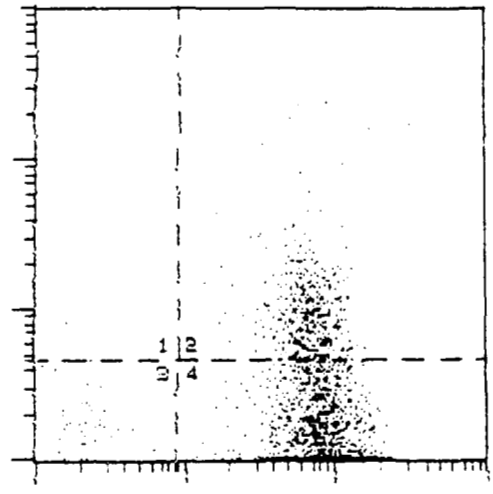

Figure 2. Expression of CR2 Ag by purified human peripheral blood T lymphocytes. Two-color immunofluorescence analysis with FITC-anti-CD3 mAb (*abscissa*) and biotinylated mAb BL13 and SAPE (*ordinate*).

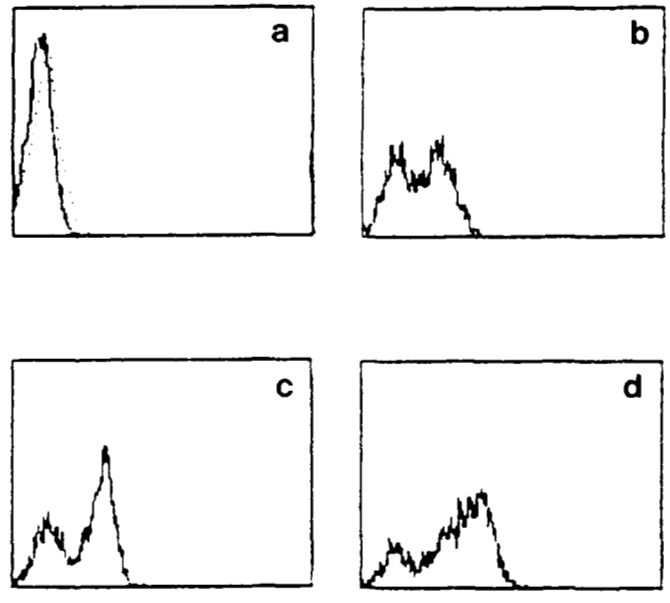

Figure 3. Expression of CR2 epitopes by purified human peripheral blood T lymphocytes. Cells were stained with biotinylated anti-CR2 mAb BL13 (*b*), BD6 (*c*), and HB-5 (*d*), followed by a single layer of SAPE. Controls (*a*) were cells stained with biotinylated IgG1 mAb MOPC 21, IgG2a mAb UPC 10, followed by SAPE.

(data not shown). No staining of the monocytic cell lines U 937 and Mono Mac 6 or of BJAB cells was observed with the biotinylated anti-CR2 mAb.

No staining was observed when purified T cells were incubated with the biotinylated isotypic controls IgG1 mAb MOPC 21 and IgG2a mAb UPC 10 (Fig. 3).

The specificity of staining for CR2 Ag was further demonstrated by the ability of an excess of unlabeled mAb BL13 to inhibit the binding of biotinylated BL13 to T lymphocytes, as shown for $CD4^+$ cells in Figure 4. In contrast, no inhibition of staining with biotinylated BL13 was achieved with unlabeled anti-CR1 mAb J3D3 of the same isotype. Similar results were obtained with $BL13^+$ $CD8^+$ T cells (data not shown).

The intensity of staining of peripheral blood T lymphocytes with anti-CR2 mAb BL13 and HB5 did not differ much from that of the staining of human thymocytes with these antibodies (Fig. 5). It was approximately 10-fold lower than the intensity of staining of peripheral blood B cells, as assessed by two-color immunofluorescence with biotinylated BL13 and FITC-Leu-16 antibodies; thus, the mean channel fluorescence intensity of staining with biotinylated BL13 was 107 and 9.4 arbitrary units for B lymphocytes and T lymphocytes from the same donor, respectively.

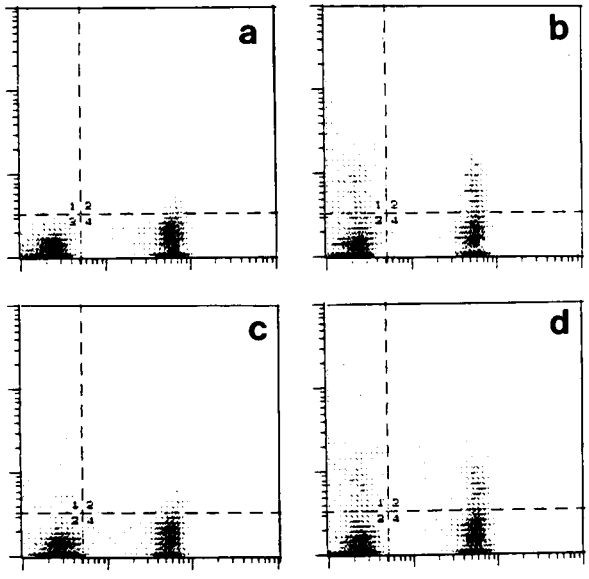

Figure 4. Specificity of the staining of $CD4^+$ T lymphocytes with anti-CR2 mAb BL13. Two-color immunofluorescence analysis of purified T lymphocytes (*abscissa*, green color; *ordinate*, red color). *a*, Staining with FITC-Leu-3a and -3b and SAPE alone; *b*, staining with FITC-Leu-3a and -3b and with biotinylated BL13, followed by SAPE; *c*, same staining as in *b*, in the presence of a 60 molar excess of unlabeled BL13; *d*, same staining as in *b*, in the presence of a 60 molar excess of unlabeled anti-CR1 mAb J3D3.

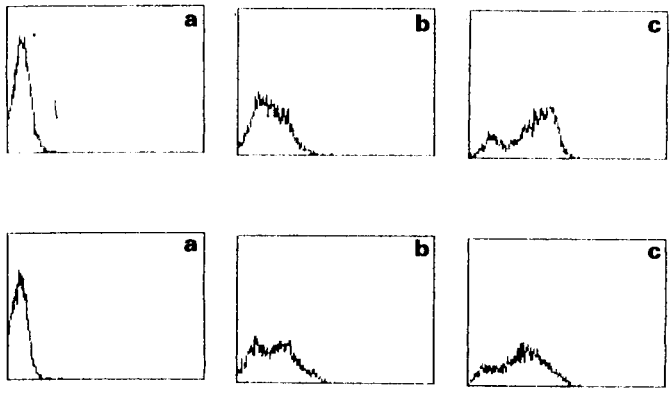

Figure 5. Comparative staining for CR2 Ag of purified human peripheral blood T lymphocytes (*upper*) and purified human thymocytes (*lower*). Indirect immunofluorescence using biotinylated anti-CR2 mAbs. *a*, Control cells stained with SAPE alone; *b*, staining with mAb BL13 followed by SAPE; *c*, staining with mAb HB5 followed by SAPE.

Culture of purified T lymphocytes for 6 days in the presence of optimal concentrations of PHA or Con A resulted in down-regulation of the expression of CR2 Ag. CR2 expression decreased within the first 2 days of culture, before cell division, which occurred from day 3, suggesting that the loss of CR2 is associated with blastic transformation of the cells. The percentage of $BL13^+$ T cells decreased from 20% to 4% and 8% by day 5 in cultures containing PHA and Con A, respectively, whereas the percentage of $CD25^+$ cells increased in culture from <5% to 85% and 65%, respectively (Fig. 6). Thus, activation of human T lymphocytes is associated with decreased expression of CR2.

Immunoprecipitation of CR2 from human peripheral blood T lymphocytes. CR2 was immunoprecipitated from ^{125}I-labeled peripheral blood T lymphocytes. The cells were purified by two successive rosetting steps and contained less than 0.3% $CD3^-$ $BL13^+$ or $HB5^+$ cells. Immunoprecipitated CR2 from T lymphocytes migrated with an apparent M_r of 145,000, similar to that of CR2 from Raji cells (Fig. 7).

DISCUSSION

In the present study, a fraction of human normal peripheral blood T lymphocytes, representing approximately one third of the cells, were found to express the C3dg/EBV receptor, CR2 (CD21). The results extend to T cells the cellular distribution of the immunoregulatory molecule CR2, which was considered to be almost exclusively restricted to the B cell lineage (1, 2, 14, 15).

Previous phenotypic studies that did not find the CD21 Ag on T lymphocytes have been carried out by indirect immunofluorescence examination of unfractionated PBMC. In the present study, we have investigated the expression of CR2 Ag on T lymphocytes by using purified peripheral blood T cells and biotinylated anti-CR2 mAb. This approach has proven to be highly sensitive for the detection and quantitation of low density cell surface Ag (26). Purification of T cells by rosetting with SRBC may have enriched the preparation in T lymphocytes expressing high amounts of CD2. $CR2^+$ T lymphocytes repre-

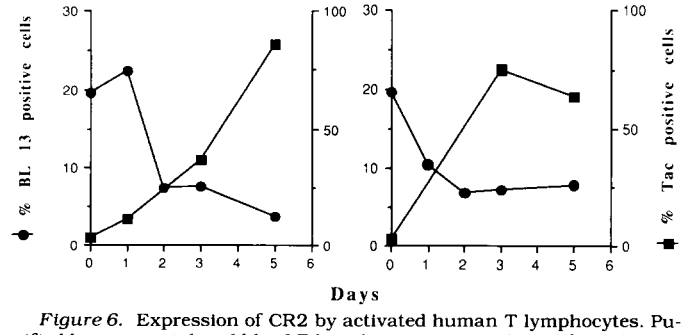

Figure 6. Expression of CR2 by activated human T lymphocytes. Purified human peripheral blood T lymphocytes were cultured for 6 days in the presence of 1.0 μg/ml PHA (*left*) or 10 μg/ml Con A (*right*). The expression of CR2 and of the Tac Ag was assessed by one-color immunofluorescent staining, using biotinylated mAb BL13 and FITC-anti-CD25 mAb.

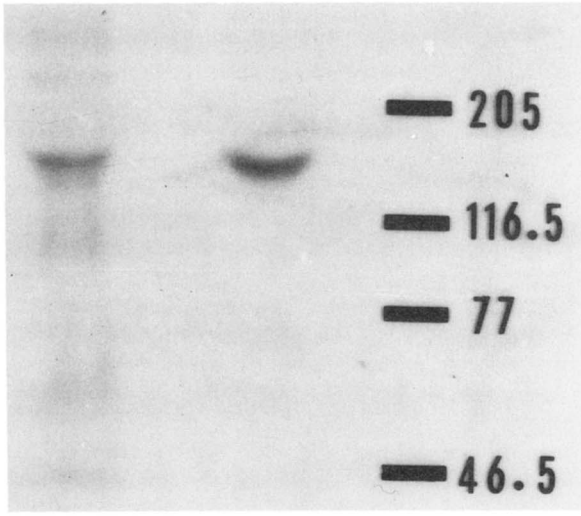

Figure 7. Autoradiograph of SDS-PAGE (7.5% polyacrylamide gel) of ^{125}I-labeled membrane proteins that had been immunoprecipitated with HB5 and BL13 mAb from Raji cells (*right lane*) and from purified human peripheral blood T lymphocytes (*left lane*). Immunoprecipitation was performed with 20×10^6 Raji cells and 200×10^6 T lymphocytes. No band at 145 kDa was seen in solubilized membranes that had been immunoprecipitated with control anti-β-galactosidase mAb.

sented 30 to 40% of peripheral blood T cells, depending on whether the binding of mAb was detected using SAPE alone or an enhancing four-layer revealing system. The specificity of the staining for CR2 Ag was demonstrated by the selective ability of unlabeled anti-CR2 mAb to compete for the binding of biotinylated anti-CR2 to T cells. T lymphocytes expressed three nonoverlapping epitopes of CR2. The mean intensity of staining of T cells for CR2 Ag was comparable to that of the staining of human thymocytes and approximately 10-fold lower than that of the staining of peripheral blood B cells, suggesting expression of approximately 1,000 to 3,000 receptors/cell, assuming 20,000 to 40,000 CR2 molecules/B cell (28). Both $CD4^+$ and $CD8^+$ T lymphocytes expressed CR2 in a ratio of 2 to 1.

The CR2 molecule was immunoprecipitated from purified T lymphocytes using two mAb directed against nonoverlapping epitopes of the molecule. T cell CR2 immunoprecipitated as a single molecular species of apparent M_r of 145,000, similar to that of CR2 that was immunoprecipitated from Raji cells. Immunoprecipitated CR2 could not originate from contaminating B cells, because non-T $CR2^+$ cells represented no more that 6×10^5 cells in the total number of purified T cells that were electrophoresed.

Two lines of evidence are presently available with regard to the expression of CR2 on cells of the T lineage. 1) 15 to 60% of human thymocytes were reported to express CR2 Ag (22); $CR2^+$ cells include immature thymocytes, because $CD1^+$ thymic cells strongly stained with anti-CR2 mAb. Staining of thymocytes with mAb HB5 was more intense than with mAb anti-B2, an observation that was previously made on peripheral blood B cells with mAb directed to the same epitopic clusters (23) and that is in agreement with our findings of a relatively more intense staining of peripheral blood T lymphocytes with mAb HB5. 2) Several human T lymphoblastoid cell lines express CR2, including Molt-4 (19), Jurkatt (20), and HPB-ALL (21); in addition, infection with HLTV-1 of a human T cell line resulted in acquired expression of CR2 by the cells (29). The $CR2^+$ T cell line Molt-4 cannot be infected with EBV (19). However, reports of an EBV genome$^+$ lymphoepithelioma-like carcinoma of the thymus (30), of EBV viral DNA-containing $CD4^+$ T cell lymphomas in patients with chronic EBV infections (31), and of the immortalization of human cord blood $CD4^+$ T cells after transfection of EBV DNA (32) suggest that some T lymphocytes may be susceptible to infection and/or transformation by EBV. Binding studies using fluoresceinated EBV have also suggested that a subset of $CR2^-$, $CD8^+$ cells may bind the virus (33). The observation that nasopharyngeal carcinoma cells harbor the EBV genome, although they express low amounts of CR2, indicates that high levels of CR2 expression are not required for the establishment of latent EBV infection (34). In addition, the low efficiency of infection with EBV of stably transfected murine L cells and of K562 cells, as compared with B lymphoblastoid cell lines, indicates that as yet unidentified factors other than CR2 may determine the tropism of EBV for certain cells such as B lymphocytes (6, 7).

CR2 on B lymphocytes plays an Ag-dependent enhancing role in the immune response by triggering proliferation of preactivated cells (35–37) and augmenting calcium signals induced in response to cross-linking of sIgM (38). At present, the functional role of CR2 on T cells in modulating the immune response is unknown. We found that mitogen-induced activation of T cells is associated with a dramatic decrease in the expression of CR2, a finding analogous to the observations of the loss of CR2 expression on B cells after activation with PWM or anti-μ (39, 40). Expression of CR1 (CD35) on the small subset of peripheral blood T cells that bear the receptor (41) also decreases after mitogen-induced activation (E. Fischer, unpublished observations).

It is possible that CR2 on T lymphocytes may facilitate the binding to the cells of C3-bearing ligands for T cell surface receptors. Thus, recent evidence indicates that CR2 enhances the binding to and infection of a $CD4^+$, $CR2^+$ T cell line by HIV1 opsonized with complement[4]. The ability of CR2 on T cell lines to bind surface-fixed C3 fragments emphasizes the potential role of the receptor on T cells in the regulation of the immune response and of infection with lymphocytotropic viruses.

Acknowledgments. We thank Dr. J. Wijdenes (Besançon, France) for kindly providing anti-CR2 mAb BD6. The secretarial assistance of A. Vioux is gratefully acknowledged.

REFERENCES

1. **Cooper, N. R., M. D. Moore, and G. R. Nemerow.** 1988. Immunobiology of CR2, the B lymphocyte receptor for Epstein-Barr virus and the C3d complement fragment. *Annu. Rev. Immunol. 6:85.*
2. **Ahearn, J. M., and D. T. Fearon.** 1989. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol. 46:182.*
3. **Nemerow, G. R., R. Wolfert, M. E. Mc Naughton, and N. R. Cooper.** 1985. Identification and characterization of the Epstein-Barr virus receptor on human B lymphocytes and its relationship to the C3d complement receptor (CR2). *J. Virol. 55:347.*
4. **Fingeroth, J. D., J. J. Weis, T. F. Tedder, J. L. Strominger, P. A. Biro, and D. T. Fearon.** 1984. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc. Natl. Acad. Sci. USA*

[4] **Boyer, V., C. Desgranges, E. Fischer, M. D. Kazatchkine.** Complement mediates HIV1 infection of a human T cell line in a CD4- and antibody-independent fashion. *Submitted for publication.*

81:4510.

5. **Frade, R., M. Barel, B. Ehlin-Henrikson, and G. Klein.** 1985. Gp 140, the C3d receptor of human B lymphocytes, is also the Epstein-Barr virus receptor. *Proc. Natl. Acad. Sci. USA 82:1490.*
6. **Ahearn, J. M., S. D. Hayward, J. C. Hickey, and D. T. Fearon.** 1988. Epstein-Barr virus (EBV) infection of murine L cells expressing recombinant human EBV/C3d receptor. *Proc. Natl. Acad. Sci. USA 85:9307.*
7. **Carel, J. C., B. Frazier, T. J. Ley, and V. M. Holers.** 1989. Analysis of epitope expression and the functional repertoire of recombinant complement receptor 2 (CR2) in mouse and human cells. *J. Immunol. 143:923.*
8. **Cantaloube, J. F., M. Piechaczyk, A. Calender, G. Lenoir, A. Minty, D. Carrière, E. Fischer, and P. Poncelet.** 1990. Stable expression and function of EBV/C3d receptor following genomic transfection into murine fibroblast L cells. *Eur. J. Immunol. 20:409.*
9. **Weis, J. J., L. E. Toothaker, J. A. Smith, J. H. Weis, and D. T. Fearon.** 1988. Structure of the human B lymphocyte receptor for C3d and the Epstein-Barr virus and relatedness to other members of the family of C3/C4 binding proteins. *J. Exp. Med. 167:1047.*
10. **Moore, M. D., N. R. Cooper, B. F. Tack, and J. R. Nemerow.** 1987. Molecular cloning of the cDNA encoding the Epstein-Barr virus/C3d receptor (complement receptor type 2) of human B lymphocytes. *Proc. Natl. Acad. Sci. USA 84:9194.*
11. **Klickstein, L. B., W. W. Wong, J. A. Smith, J. H. Weis, J. G. Wilson, and D. T. Fearon.** 1987. Human C3b/C4b receptor (CR1): demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristic of C3/C4 Dinding proteins. *J. Exp. Med. 165:1095.*
12. **Caroll, M. C., E. M. Alicot, P. J. Katzman, L. B. Klickstein, J. A. Smith, and D. T. Fearon.** 1988. Organization of the genes encoding complement receptors type 1 and 2, decay-accelerating factor, and C4-binding protein in the RCA locus on human chromosome 1. *J. Exp. Med. 167:1271.*
13. **Lowell, C. A., L. B. Klickstein, R. H. Carter, J. A. Mitchell, D. T. Fearon, and J. M. Ahearn.** 1989. Mapping of the Epstein-Barr virus and C3dg binding sites to a common domain on complement receptor type 2. *J. Exp. Med. 170:1931.*
14. **Iida, K., L. Nadler, and V. Nussenzweig.** 1983. Identification of the membrane receptor for the complement fragment C3d by means of a monoclonal antibody. *J. Exp. Med. 158:1021.*
15. **Tedder, T. F., L. T. Clement, and M. D. Cooper.** 1984. Expression of C3d receptors during human B cell differentiation: immunofluorescence analysis with the HB-5 monoclonal antibody. *J. Immunol. 133:678.*
16. **Reynes, M., J. P. Aubert, J. H. M. Cohen, J. Audoin, V. Tricottet, J. Diebold, and M. D. Kazatchkine.** 1985. Human follicular dendritic cells express CR1, CR2, and CR3 complement receptor antigens. *J. Immunol. 135:2687.*
17. **Young, L. S., D. Clark, J. W. Sixbey, and A. B. Rickinson.** 1986. Epstein-Barr virus receptors on human pharyngeal epithelia. *Lancet 1:240.*
18. **Sixbey, J. W., S. U. Lemon, and J. S. Pagano.** 1986. A second site for Epstein-Barr virus shedding: the uterine cervix. *Lancet 2:1122.*
19. **Menezes, J., J. M. Seigneurin, P. Patel, A. Bourkas, and G. Lenoir.** 1977. Presence of Epstein-Barr virus receptors, but absence of virus penetration, in cells of an Epstein-Barr virus genome negative human lymphoblastoid T line (Molt 4). *J. Virol. 22:816.*
20. **Tatsumi, E., S. Harada, C. Kuszynski, D. Volsky, J. Minowada, and D. T. Purtilo.** 1985. Catalogue of Epstein-Barr virus (EBV) receptors on human malignant and nonmalignant hematopoietic cell lines. *Leuk. Res. 9:231.*
21. **Fingeroth, J. D., H. L. Clabby, and J. D. Strominger.** 1987. Characterization of a T-lymphocyte Epstein-Barr virus/C3d receptor (CD21). *J. Virol. 62:1442.*
22. **Tsoukas, C. D., and J. D. Lambris.** 1988. Expression of CR2/EBV receptors on human thymocytes detected by monoclonal antibodies. *Eur. J. Immunol. 18:1299.*
23. **Cohen, J. H. M., E. Fischer, M. D. Kazatchkine, J. Brochier, and J. P. Revillard.** 1986. Characterization of monoclonal anti-human-B-cell antibody BL13 as an anti-C3d-receptor (CR2) antibody. *Scand. J. Immunol. 23:279.*
24. **Cook, J., E. Fischer, E. Boucheix, M. Mirsrahi, M. H. Jouvin, L. Weiss, R. Jack, and M. D. Kazatchkine.** 1985. Mouse monoclonal antibodies to the human C3b receptor. *Mol. Immunol. 22:531.*
25. **Steinbuch, M., and R. Audran.** 1969. The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch. Biochem. Biophys. 134:279.*
26. **Cohen, J. H. M., J. P. Aubry, M. H. Jouvin, J. Wijdenes, J. Banchereau, M. D. Kazatchkine, and J. P. Revillard.** 1987. Enumeration of CR1 complement receptors on erythrocytes using a new method for detecting low density cell surface antigens by flow cytometry. *J. Immunol. Methods 99:53.*
27. **Fischer, E., M. D. Appay, J. Cook, and M. D. Kazatchkine.** 1986. Characterization of the human glomerular C3 receptor as the C3b/C4b complement type one (CR1) receptor. *J. Immunol. 136:1373.*
28. **Kazatchkine, M. D., and D. T. Fearon.** 1990. Deficiencies of human C3 complement receptors type 1 (CR1, CD35) and type 2 (CR2, CD21). *Immunodeficiency Rev. 2:17.*
29. **Schultz, T. F., A. Petzer, R. Staudere, A. Eigentler, and M. P. Dierich.** 1986. Expression of CR2 (C3d/EBV-receptor) on HTLV-1 transformed human T-cells. In *Proceedings of the 6th International Congress of Immunology.* National Research Council Canada, Ottawa, Canada, p. 188.
30. **Leyvraz, S., W. Henle, A. P. Chahinian, C. Perlmann, G. Klein, R. E. Gordon, M. Rosenblum, and J. F. Holland.** 1985. Association of Epstein-Barr virus with thymic carcinoma. *N. Engl. J. Med. 312:1296.*
31. **Jones, J. F., S. Shurin, C. Abramowsky, R. R. Tubbs, C. G. Sciotto, R. Wahl, J. Sands, D. Gottman, B. Z. Katz, and J. Sklar.** 1988. T-cell lymphomas containing Epstein-Barr viral DNA in patients with chronic Epstein-Barr virus infections. *N. Engl. J. Med. 318:733.*
32. **Stevenson, M., B. Volsky, M. Hedenskog, and D. J. Volsky.** 1986. Immortalization of human T lymphocytes after transfection of Epstein-Barr virus DNA. *Science. 233:980.*
33. **Sauvageau, G., R. Stocco, S. Kasparian, and J. Menezes.** 1990. Epstein Barr virus receptor expression on human $CD8^+$ (cytotoxic/suppressor) T lymphocytes. *J. Gen. Virol. 71:379.*
34. **Billaud, M., P. Busson, D. Huang, N. Mueller-Lantzch, G. Rousselet, O. Pavlish, H. Wakasugi, J. M. Seigneurin, T. Tursz, and G. M. Lenoir.** 1989. Epstein-Barr virus (EBV)-containing nasopharyngeal carcinoma cells express the B-cell activation antigen blast 2/CD23 and low levels of the EBV receptor CR2. *J. Virol. 63:4121.*
35. **Bohnsack, J. F., and N. R. Cooper.** 1988. CR2 ligands modulate human B cell activation. *J. Immunol. 141:2569.*
36. **Carter, R. H., and D. T. Fearon.** 1989. Polymeric C3dg primes human B lymphocytes for proliferation induced by anti-IgM. *J. Immunol. 143:1755.*
37. **Hatzfeld, A., E. Fischer, J. P. Levesque, R. Perrin, J. Hatzfeld, and M. D. Kazatchkine.** 1988. Binding of C3 and C3dg to the CR2 complement receptor induces growth of an Epstein-Barr virus-positive human B cell line. *J. Immunol. 140:170.*
38. **Carter, R. H., M. O. Spycher, Y. C. Ng, R. Hoffman, and D. T. Fearon.** 1988. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. *J. Immunol. 141:457.*
39. **Roome, A. J., and C. L. Reading.** 1987. Frequency of B lymphocyte transformation by Epstein-Barr virus decreases with entry into the cell cycle. *Immunology 60:195.*
40. **Stashenko, P., L. M. Nadler, R. Hardy, and S. F. Schlossman.** 1981. Expression of cell surface markers after human B lymphocyte activation. *Proc. Natl. Acad. Sci. USA 78:3848.*
41. **Wilson, J. G., T. F. Tedder, and D. T. Fearon.** 1983. Characterization of human T lymphocytes that express the C3b receptor. *J. Immunol. 113:684.*