

Expression of EBV/C3d receptors on T cells: biological significance

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There is overwhelming evidence that a single polypeptide serves as a receptor for both the Epstein-Barr Virus (EBV), and for certain enzymatic fragments of C3. This receptor, termed CR2 (CD21), is known to be expressed on the surfaces of B cells, and a large body of evidence suggests that CR2, or related structures, are also expressed on cells of the T lineage. Here, Constantine Tsoukas and John Lambris review the studies of CR2 expression in T cells and offer some speculation on its possible biological significance.

As far back as eighteen years ago, Shevach *et al.*¹ described the cells from a patient with chronic leukemia that expressed both T-cell markers and C3 receptors. This observation was confirmed by Lin and Hsu² and by Toben and Smith³, who described additional cases of T-cell leukemias that displayed C3 receptors. In addition, West and Herberman⁴ demonstrated that Molt-4, an established T-lymphocytic leukemia line, also bore C3 receptors. The phenomenon is not restricted to leukemic T cells: normal immature thymocytes also express C3 receptors^{5,6}.

These early studies, although intriguing, did not clearly delineate the precise nature of the C3 receptors

expressed on T cells and thymocytes. More recent experiments, using specific reagents, provide direct evidence that human thymocytes react with anti-CR2 monoclonal antibodies (mAbs), which immunoprecipitate a molecule of 145 kDa from thymocyte membrane lysates⁷. This is consistent with the known apparent molecular weight of CR2 on B cells⁸. It is noteworthy that a battery of anti-CR2 mAbs against different CR2 epitopes display differential binding to thymocytes, in spite of equivalent reactivity with B cells⁹. Furthermore, thymocytes react with the Epstein-Barr virus (EBV), the binding of which can be inhibited using anti-CR2 antibodies, aggregated C3, or antibodies to the viral

gp350 molecule, which EBV uses to bind to CR2 (Ref. 9). Anti-CR2 antibodies and EBV react with thymocytes of immature phenotype, specifically cells that express CD1 and are double-positive for CD4 and CD8; properties of cortical immature thymocytes^{7,9}.

CR2, or antigenically related receptors, are also expressed on peripheral T cells^{10,11}. In one study using EBV as ligand, 50% of CD8⁺ T cells displayed specific virus binding that could be inhibited by anti-gp350 antibodies but was unaffected by anti-CR2 antibodies¹⁰; in a second study, 30–40% of peripheral CD4⁺ and CD8⁺ T cells reacted with a panel of anti-CR2 antibodies that recognize nonoverlapping epitopes on CR2 (Ref. 11). Generally, in these studies, the intensity of antibody staining was estimated to be about tenfold lower than on B cells, and immunoprecipitation analysis revealed a single protein of 145 kDa. Fingeroth *et al.*¹² determined the amino-terminal sequence of a protein purified from the human T-cell line HPB by anti-CR2 affinity chromatography, and found it to display significant differences from B-cell CR2.

Thus, thymocytes, peripheral T cells, and T-cell lines express CR2 or CR2-like molecules. The reactivities of these molecules with ligands or antibodies varies from that of B cells, suggesting that there might be structural differences between T- and B-cell CR2 molecules.

B-cell CR2

The gene encoding CR2 has been cloned^{13,14} and the elucidation of its genomic organization has revealed an array of related exons; 15 or 16 of these code for the extracellular segment of the receptor, with two additional exons coding for the transmembrane portion and another encoding the cytoplasmic region^{14,15}. At the protein level, this organization translates into a series of short consensus repeats (SCRs) that bear homology to each other and to SCRs found in related molecules, such as CR1 and other C3- and C4-binding proteins.

Human–mouse chimeric CR2 constructs and deletion mutants have been used to study the EBV- and C3-binding epitopes. These sites are located on the first and second SCRs respectively, and seem to be distinct^{16,17}, although Molina *et al.*¹⁸ have also provided data which argue in favor of an additional EBV-binding site in the second SCR. The EBV-binding site is dependent upon a specific CR2 conformation that appears to be controlled by amino acids in two discontinuous positions of the molecule¹⁹.

Similar technology has been used in the study of epitopes reactive with anti-CR2 mAbs. SCRs 1 and 2 are sufficient for the binding of antibody OKB7 (Refs 16,17). This antibody effectively blocks the binding of EBV to CR2, implying that the OKB7 and virus-binding sites are identical, overlapping, or conformationally related²⁰. The binding of EBV and OKB7 has been manipulated through substitution of specific residues and/or regions in SCRs 1 and 2: binding of EBV can be maintained in the absence of OKB7 reactivity, indicating that the two epitopes are not identical, but are overlapping and conformationally associated¹⁹. Epitopes bound by other anti-CR2 mAbs have been studied in less detail, but the results collectively indi-

cate that relatively small changes in the overall structure of CR2 may affect the binding of one antibody but not of another. Thus, the mAb HB5 reacts with a determinant formed by the third and fourth SCRs^{16,17}, whereas mAb B2 seems to require sites in SCRs 9–11 and 12–14 (Refs 16,17); mAbs 1C8 and 1F8 appear to need SCRs 12–15 for their binding^{17,21}.

T-cell CR2

It is clear that relatively small changes in the overall structure of CR2 could produce the various phenotypic profiles described for T cells, that is, EBV binding in the absence of anti-CR2 antibody reactivity, and differential binding among distinct anti-CR2 antibodies. What is the biological mechanism that produces these putative modifications in the CR2-like molecules of T cells?

It is possible that CR2 in T cells reflects the expression of CR2-related, but distinct, gene(s). These genes may differ from those that encode the B-cell CR2, such that binding of one ligand or mAb but not another occurs. For example, murine CR2, although capable of binding human C3d, cannot bind EBV and the anti-human CR2 mAb OKB7. This is due to single amino acid substitutions in two discontinuous regions of the primary structure of murine CR2 (Ref. 19). Another observation that supports the hypothesis of CR2-related genes is the receptor we have recently described on the T-cell line HSB2, which binds very strongly to EBV, reacts weakly with aggregated C3, but has no reactivity with any of a battery of anti-CR2 mAbs (Ref. 22). Furthermore, this receptor is functional: it allows infection by EBV, as demonstrated by the appearance of EBNA-1 transcripts²². Northern blotting analysis of HSB2 mRNA with a B-cell cDNA probe, under low-stringency hybridization, has revealed a 5.2 kb transcript²².

Another hypothesis is that splicing of other exons contributes to the differences between the T- and B-cell CR2 molecules. Holguin *et al.*²³ have isolated and located, in the human CR2 gene, exons that presumably represent the counterparts of the murine exons that code for the CR1/Crry-like sequence of the long form (5 kb) of murine CR2 mRNA. Although some of these exons have been described as pseudoxons²³, it is a formal possibility that they may provide the basis for alternative splicing and structural differences in the final mRNA products in T cells.

Finally, variation in the post-translational modifications, for example in glycosylation, of T- and B-cell CR2 molecules could also account for the differential binding of antibodies. Immunoprecipitation and SDS gel-electrophoretic analysis of Jurkat cell lysates with antibody HB5 has revealed a protein that migrates at a higher apparent molecular weight (155 kDa) compared to that from B cells (145 kDa) (manuscript submitted for publication). This difference seems to be due to differential glycosylation since, upon Endoglycosidase F treatment, T- and B-cell immunoprecipitates are of the same size (115 kDa).

Infection of T cells by EBV

EBV can infect thymocytes, as shown by the expression of EBNA-1 in cells cultured with the virus and

analysed by Western blotting of membrane lysates⁹. Similarly, in HSB-2 and Jurkat T cells cultured with EBV, EBNA-1 transcripts can be detected by PCR analysis²². In the case of Jurkat cells, detection of EBNA-1 transcripts is more variable (manuscript submitted for publication). Other investigators were unable to detect EBV infectivity in either Molt-4 leukemic T cells or CD8⁺ peripheral T cells, in spite of the ability of these cells to bind the virus^{10,24,25}; however, EBNA-1 was analysed by nuclear staining, a much less sensitive technique.

One explanation for the variability in infection of T cells could be that CR2, although required, is not by itself sufficient for infection to occur – other factors, such as additional cell surface receptor(s) or intracellular components, may be required. There is evidence that a glycoprotein, gp85, which is expressed in the EBV envelope, is involved in viral infectivity. Antibodies to gp85 neutralize infection by inhibiting the fusion between the cellular membrane and viral envelope, but do not interfere with the attachment of the virus to the host cell²⁶. Experiments where non-B cells have been transfected with the CR2 gene indicate that even though EBV can bind and internalize, it cannot infect the cells efficiently^{17,27}. Thus, an alternative explanation, albeit not mutually exclusive of the above, is that intracellular components, such as nuclear transcription factors, are necessary for the production of EBV antigens and efficient infection – variability in the expression of such factors from cell type to cell type and/or during various stages of cell cycle or cell differentiation may explain differences in infectivity. There is some evidence to suggest that cellular factors expressed in some tissues, but not in others, interact synergistically with EBV-coded transactivators to determine the viral state²⁸.

Physiological significance

The biological significance of expression of CR2-like molecules on cells of the T lineage is not understood. The expression of the receptor on immature thymocytes suggests its involvement in differentiation^{7,9}. This is consistent with data on B cells, in which CR2 expression appears at the immature B-cell stage but is downregulated upon activation and differentiation to the plasma cell stage⁸. CR2 has been shown to interact with CD23 in the regulation of IgE production²⁹. CD23 is a protein that is expressed both as a transmembrane molecule, on several types of cells including thymic epithelial cells, and as a soluble product³⁰. In view of the evidence that CD23, synergistically with interleukin 1 (IL-1), induces thymocyte maturation³¹, it can be argued that the CR2 molecules on immature thymocytes play an important role in T-cell development.

Anti-CR2 antibodies and polyvalent CR2-ligands prime B cells for antigen-receptor-induced proliferation and intracellular Ca²⁺ elevation^{8,32}, and EBV induces generation of phosphatidylinositols, increases free cytosolic Ca²⁺ and PKC translocation in B cells³³. A synergistic proliferative effect is induced in thymocytes by EBV and IL-2 (Ref. 9). Such a phenomenon was not reproduced with monovalent C3 as the

ligand. These findings have parallels in B-cell studies: enhancement of B-cell proliferation is seen when polyvalent C3d/C3dg is added to pre-activated B cells, whereas monovalent ligand inhibits this effect^{34,35}. Similarly, C3 fragments can inhibit antigen- and mitogen-induced T-cell proliferation, the production of cytotoxic T cells, and IL-2-dependent growth^{36,37}, and aggregated C3 can act as a costimulator for helper T cells along with IL-2 (Ref. 37). It is possible that soluble C3 fragments or intact aggregated molecules may interact with CR2-like receptors, found on peripheral T cells¹¹, in order to produce these biological effects. However, in view of the fact that T cells also express CR1 (Ref. 34), the possibility that C3 may mediate its effects via CR1 cannot be excluded. Delibrias *et al.*³⁸ have shown that, similar to B cells³⁹, some CR1 molecules on the human T-cell line HPB are associated with CR2 in the form of CR1–CR2 complexes and anti-CR2 mAbs can induce an increase in intracellular free calcium concentration.

Significance in pathology

The presence of CR2 or CR2-like receptors on T cells raises the possibility that these lymphocytes may be subject to EBV-targeting *in vivo*. In fact, recent reports have described several cases of patients with EBV-genome-positive T-cell lymphomas^{40–46}. One patient, after clinical EBV infection, developed a Kawasaki-like disease with the interesting characteristic of circulating T cells that carried EBV genome⁴⁰. Jones *et al.*⁴¹ described three different cases of patients with EBV-positive T-cell lymphomas who had primary EBV infections before the development of the lymphoma⁴¹. More recently, EBV-positive nasal T-cell lymphomas have been described in five patients with lethal midline granulomas which also expressed EBNA-1, EBNA-2 and LMP (Ref. 42). These T-cell tumors may be the result of EBV-induced transformation of peripheral EBV-receptor-bearing T cells or of immature T cells in the thymic environment. Alternatively, thymic T cells that emigrate to the periphery prematurely could be viral targets. In nine cases of EBV-positive, CD2⁺ T-cell lymphomas, the T-cell antigen receptor (TCR) genes were found to be in their germline orientation, suggesting that the viral targets were indeed immature T cells⁴⁴. The incidence of EBV-positive T-cell cancers may not be a rare event; it might be underestimated and further studies are required to establish its true frequency and significance.

The expression of CR2 or CR2-like molecules may also render T cells susceptible to other viruses, such as retroviruses. Although the CD4 glycoprotein is the primary receptor for HIV-1, recent reports have suggested that other molecules, such as CR2, might be involved in HIV-infection of T cells^{47,48}. In the presence of complement and antibodies to HIV-1 envelope glycoprotein there is enhanced infection of CD4⁺ T-cells which can be inhibited by anti-CD4 and anti-CD8 antibodies⁴⁷. Opsonization of HIV-1 with complement also amplifies infection of T cells under low doses of HIV-1 infectivity⁴⁸; this phenomenon is CD4-independent and anti-CR2 mAb-inhibitable⁴⁸.

The future

CR2 or CR2-like receptors are expressed on cells of the T lineage. This opens a new and exciting phase in complement and EBV research, and raises several important questions. For example, what is the structural relationship of the T-cell CR2 to that of the well-characterized B-cell receptor? What is the functional significance of CR2 on T cells and thymocytes? Is this receptor responsible for EBV- or retrovirus-mediated pathology? These, as well as other important issues need to be addressed in order to understand the significance of these receptors to immune functions.

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