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Interaction between CD4 and class II MHC molecules mediates cell adhesion

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The CD4 glycoprotein is expressed on T-helper and cytotoxic lymphocytes which are restricted to class II major histocompatibility complex (MHC) antigens on target cells. Antibody inhibition studies imply that CD4 acts to increase the avidity of effector-target cell interactions. These observations have led to the speculation that CD4 binds to a monomorphic class II antigen determinant, thereby augmenting low affinity T-cell receptor-antigen interactions. However, no direct evidence has been presented indicating that CD4 and class II molecules interact. To address this issue, we have used a vector derived from simian virus 40 (SV40) to express a complementary DNA (cDNA) encoding the human CD4 glycoprotein. When CV1 cells expressing large amounts of the CD4 protein at the cell surface are incubated with human B cells bearing MHC-encoded class II molecules, they are bound tightly to the infected monolayer, whereas mutant B cells which lack class II molecules fail to bind. Furthermore, the binding reaction is specifically inhibited by anti-class II and anti-CD4 antibodies. Thus, the CD4 protein, even in the absence of T-cell receptor-antigen interactions, can interact directly with class II antigens to function as a cell surface adhesion molecule.

The SV40-derived recombinant vector used to express the CD4 protein is essentially identical to that used previously to produce large amounts of the influenza haemagglutinin glycoprotein in CV1 cells. The cDNA sequences are transcribed under the control of the SV40 late promoter and replace the viral sequences which normally encode the capsid proteins required to package the lytic virus. The CD4 cDNA sequences are inserted between the HpaII (346-base pair (bp) and the
Fig. 3 Binding of lymphocytes to CVI cells infected with SV40, SV40-RA and SV40-CD4 virus stocks. a. Binding of radiolabelled Raji lymphocytes to mock, SV40, SV40-RA and SV40-CD4 infected monolayers. b. Binding of class II-positive (Raji, T5-1, JY, Daudi) and class II-negative (RJ2.2.5, 6.1.6, Rania and Nacer) B lymphocytes to monolayers of CVI cells expressing HA (solid bars) or CD4 (hatched bars). c and d. Antibody inhibitions of lymphocyte binding to SV40-CD4 infected cells. Monoclonal antibodies used were, for panel c: W6/32 (anti-class I), 100 μg ml⁻¹; anti-Leu3A (CD4-specific), 10, 50, 80 μg ml⁻¹. For panel d: LB3.1 (DR-specific), 10 and 35 μg ml⁻¹; and B7.21 and ST1.8 (DP-specific), 10 and 35 μg ml⁻¹. Methods. a. Raji B lymphocytes (1×10⁶ cells) were incubated in methionine-free DMEM containing 200 μCi ml⁻¹ [³⁵S]methionine for one hour. Binding of the radiolabelled Raji cells to infected monolayers was carried out as described above (see legend Fig. 2), followed by extensive washing. Monolayers and bound lymphocytes were then removed from dishes by trypsinization, dissolved in scintillation fluid and radioactivity (cpm bound) was determined. b. The binding of radiolabelled B lymphocytes to monolayers of CVI cells infected with either the SV40-haemagglutinin or the SV40-CD4 recombinant viruses was quantitated as above. Results represent the average of several determinations. However, in this case, the background (cpm bound to wild-type SV40-infected monolayers) was subtracted and binding to SV40-RA-infected cells was normalized for comparisons among cell lines. c. CVI cells infected with the SV40-CD4 recombinant virus and radiolabelled Raji B cells were preincubated with the W6/32 (100 μg ml⁻¹) or anti-Leu3A monoclonal antibodies (10, 50, 80 μg ml⁻¹) for two hours after which the binding assay was performed. d. Fab fragments of the monoclonal antibodies were employed. They were prepared by pepsin treatment (Sigma, 10 mg ml⁻¹ in 0.1 M sodium acetate, pH 4.5) of IgG for 16 h at 37°C followed by the addition of 2M Tris base. The pH₃ of the molecule(s) was removed by incubation with 0.2 ml of 10% protein A-Sepharose. Inhibition of binding was carried out as above.

then performed with a number of different B lymphoblastoid cell lines, some of which express class II antigens, some of which do not. All B cells examined displayed binding to monolayers expressing the HA. However, only those B cells which express class II antigens, that is Raji, Daudi, JY, and T5-1, bound to cells producing the CD4 glycoprotein. In contrast, immunoselected mutants of Raji and T5-1, which do not express class II antigens (RJ2.2.5, ref. 18, and 6.1.6, ref. 19, respectively) failed to bind to CD4-producing cells. Furthermore, other class II negative B cell lines (Rania and Nacer), derived from patients afflicted with severe combined immunodeficiency disease (SCID)²⁰, behaved similarly (Fig. 3b). Importantly, these mutant cell lines, although similar in phenotype, were isolated independently. RJ2.2.5 and 6.1.6 were generated by mutagenesis in vitro whereas the cell lines isolated from SCID patients arose spontaneously. Therefore, these cell lines are unlikely to contain a common pleiotropic mutation affecting loci other than class II. The Daudi cell line VIN, which express class II but not class I antigens (because of a defect in β₂-microglobulin expression), bound to cells expressing the CD4 molecule. Thus, the expression of class II antigens on the surface of B lymphocytes correlates with the ability of these cells to attach to monolayers of cells which express the CD4 glycoprotein. Notably, when binding was quantified using radiolabelled lymphocytes (Fig. 3b), higher levels of B lymphocyte binding to cells expressing the CD4 molecule was coincident with higher levels of class II expression (Raji > T5-1 > JY > Daudi), as determined by FACS analysis (data not shown).

The results of antibody inhibition studies confirmed the specificity of the CD4-class II interaction. The binding of class II positive B lymphocytes was completely inhibited by saturation with the anti-Leu3A monoclonal (Fig. 3c) or by a mixture of class II-specific antibodies (Fig. 3d). When either the DR-specific monoclonal antibody, LB3.1 (ref 22, 23) or DP-specific antibodies, B7.21 (ref 22, 24) or ST1.8 (J. C. Gorga, personal communication), were used alone, binding was only partially reduced (40-60%). Similar partial inhibitions were achieved using lower concentrations of a mixture of these class II monoclonal antibodies (see Fig. 3d). In contrast, a class I-specific antibody, W6/32 (Fig. 3c), failed to inhibit the binding activity and the binding of Raji cells to CVI cells expressing the HA could not be blocked with any of the reagents used in these studies.

Taken together, these data demonstrate that the adhesion of lymphocytes bearing class II antigens to fibroblasts expressing the CD4 glycoprotein is a consequence of direct interactions between these two molecules. Furthermore, because adhesion mediated by these molecules is observed in a heterologous system (in the absence of any T-cell receptor-antigen interactions), the binding of CD4 and class II molecules can be independent of such interactions. We presume that the high level of CD4 expression obtained in this study was crucial for demonstrating this high affinity, stable interaction. At more physiological levels of expression, it is likely that CD4 and class II antigens help to mediate low-affinity, transient interactions among lymphocytes. Under such circumstances, these molecules must work in parallel with other specific and accessory adhesion molecules to allow functional cell-cell interactions.

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