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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. 1  Indirect immunofluorescent labelling of CVI cells infected with the SV40-C4D recombinant virus. a, CVI cells were typically prepared for infection by plating in 60 mm tissue culture dishes at a density of approximately 4.5 x 10^4 cells in Dulbecco's modified Eagle's medium (DMEM) and 1% foetal calf serum (FCS). The medium was removed 8-10 h later and cells were incubated with 0.25 ml of an SV40-C4D (or an SV40-HA) high titre virus stock (multiplicity of infection of 10 plaque-forming units per cell) for 1.5-2 h at 37°C. 5 ml of DMEM-10% FCS was then added and virus was grown at 37°C for 36-60 h at which time the experiments were performed. For immunofluorescent labelling, SV40-C4D infected CVI cells grown on coverslips were fixed using 3.7% formaldehyde in phosphate-buffered serum and permeabilized with 0.1% Triton X-100 as previously described 11. Indirect immunofluorescent staining was carried out using a C4D-specific monoclonal antibody (anti-Leu3A, Becton-Dickinson) followed by goat anti-mouse IgG conjugated with rhodamine (Cappel Laboratories). b, SDS-polyacrylamide gel electrophoresis of C4D protein immunoprecipitated from extracts of CVI cells infected with SV40-C4D viral vectors. SV40-infected cells were immunoprecipitated with anti-Leu3A (lane 1). SV40-C4D infected cells were immunoprecipitated with OKT8 (lane 2), OKT4 (lane 3), and anti-Leu3A (lane 4).

Methods. CVI cells, infected for 42 h with wild-type SV40 or recombinant SV40-C4D virus stocks, were incubated for 30 mins in DMEM lacking methionine and labelled for one hour with [35S]methionine (100 μCi per 60 mm plate; 1,000-3,000 Ci mmol⁻¹, Amersham) in 0.25 ml DMEM lacking methionine. Cells were lysed in 1% Nonidet P-40, 10 mM Tris (pH 8.0) and aliquots of the cell extracts were immunoprecipitated in NET-GEL (150 mM NaCl, 5 mM EDTA, 10 mM Tris (pH 8.0), 0.25% gelatin) using a non-specific (OKT8) or C4D-specific monoclonal antibodies anti-Leu3A (4 μg) and OKT4 (5 μg) followed by incubation with rabbit anti-mouse serum and Protein-A Sepharose. The precipitated polypeptides were then washed extensively in NET-GEL+0.5M NaCl, NET-GEL+1% NP40 and 0.1% SDS and finally in 10 mM Tris-HCl containing 0.1% NP40. The proteins were then solubilized by boiling in Laemmli sample buffer and separated on discontinuous SDS-polyacrylamide gels (0.1% SDS; 10% polyacrylamide; 30:0.8:1 acrylamide:Bis) and visualized by fluorography at −70°C using Kodak XAR-5 film.

BamHI (2,533-bp) sites with conversion of the HpaI site to SalI. The recombinant viral genome, along with a defective helper virus 2, is introduced into the recipient fibroblasts by transfection using DEAE-Dextran and chloroquine as facilitators 26 as previously described 26. Serial passage on freshly growing monolayers provides a high titre virus stock which can subsequently be used to produce large amounts of protein in more than 90% of the cells.

To establish that the protein expressed from the recombinant vector was authentic in its structure, antigenicity and cellular localization, immunoprecipitation and staining by immunofluorescence using C4D-specific monoclonal antibodies, anti-Leu3A and OKT4, were performed. The immunofluorescent labelling pattern obtained is shown in Fig. 1a. The strong perinuclear staining is typical of that seen when glycoproteins synthe-sized in the endoplasmic reticulum are transported to the cell surface via the Golgi apparatus. A diffuse surface staining (Fig 1a) is apparent on approximately 25% of the infected cells and probably corresponds to those cells in the population which were infected early during the course of the experiment and which are likely to be expressing the most protein at the cell surface. This population expresses approximately 10-15 x the number of C4D molecules at the cell surface, as does the human T lymphocyte cell line, HPB-ALL, as determined by cytofluorographic analyses using the anti-Leu3A monoclonal antibody (data not shown). Preliminary evidence suggests that the yield of C4D purified from these cells is in the range of 1.0 μg per dish (5-6 x 10^5 cells per 10cm plate). Extracts of cells infected with the recombinant virus and labelled with [35S]methionine contained a protein with a relative molecular mass of 55K, which was specifically immunoprecipitated with both the OKT4 and the anti-Leu3A mAbs (Fig. 1b, lanes 3 and 4).

A lymphocyte binding assay was used to study C4D-class II interactions (Figs 2 and 3). CVI cells were infected with wild-type SV40 virus or with a recombinant virus producing either the influenza HA or the C4D glycoprotein. At 42 hours post-infection, the infected monolayers were incubated with B cells and examined. The results of a typical experiment using the B lymphoblastoid cell line, Raji, which expresses high levels of class II antigen(s) is shown in Fig. 2 and quantified in Fig. 3a. B lymphocytes, like erythrocytes, adhere to CVI cells expressing the HA via sialic acid receptor sites present on that molecule (Fig. 2b). A similar binding phenomenon was observed when Raji cells were incubated with CVI cells infected with the SV40-C4D recombinant (Fig. 2c) but not with cells infected with a wild type SV40 virus (Fig. 2d). Similar experiments were...
Fig. 3  Binding of lymphocytes to CVI cells infected with SV40, SV40-HA and SV40-CD4 virus stocks. a. Binding of radiolabelled Raji lymphocytes to mock, SV40, SV40-HA and SV40-CD4 infected monolayers. b. Binding of class II-positive (Raji, T1-3, JY, Daudi) and class I-negative (RJ2.2.5, 6.1.6, Rania and Nacera) 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 LB3.1 + ST1.8 (DP-specific), 10 and 35 μg ml⁻¹.

Methods. a. Raji B lymphocytes (1-2 x 10⁵ 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 (c.p.m. 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 quantified as above. Results represent the average of several determinations. However, in this case, the background (c.p.m. bound to wild-type SV40-infected monolayers) was subtracted and binding to SV40-HA-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 pepin 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 Fab portion 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 TS-1, bound to cells producing the CD4 glycoprotein. In contrast, immunoselected mutants of Raji and TS-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 Nacera), derived from patients afflicted with severe combined immunodeficiency disease (SCID), behaved similarly (Fig. 3b).

Importantly, these mutant cell lines, although similarly 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, which expresses 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 those 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 were coincident with higher levels of class II expression (Raji > TS-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 (refs 22, 23) or DP-specific antibodies, B7.21 (refs 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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