
Carolyn Doyle and Jack L. Strominger

*J Immunol* 2010; 184:5935-5938; ;

http://www.jimmunol.org/content/184/11/5935.citation
Interaction between CD4 and class II MHC molecules mediates cell adhesion

Carolyn Doyle & Jack L. Strominger

Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138, USA

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\(^1\)–\(^5\). Antibody inhibition studies imply that CD4 acts to increase the avidity of effector-target cell interactions\(^6\)–\(^8\). 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\(^9\)–\(^11\). 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)\(^12\) to express a complementary DNA (cDNA) encoding the human CD4 glycoprotein\(^13\). 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\(^12\). 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 10^5 cells in Dulbecco's modified Eagle's medium (DMEM) and 10% 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 immunofluorescence 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 to increase the virus. Indirect immunofluorescent staining was carried out using a CD4-specific monoclonal antibody (anti-Leu3A, Becton-Dickinson) followed by goat anti-mouse IgG conjugated with rhodamine (Cappell Laboratories). b, SDS-polyacrylamide gel electrophoresis of CD4 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 CD4-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 Laemml sample buffer and separated on discontinuous SDS-polyacrylamide gels (0.1% SDS; 10% polyacrylamide; 30-0.8% acrylamide-Bis) and visualized by fluorography at 70°C using Kodak XAR-5 film.

 BamHI (2,533-bp) sites with conversion of the HpaII site to Sall. The recombinant viral genome, along with a defective helper virus 4, is introduced into the recipient fibroblasts by transfection using DEAE-Dextran and chloroquine as facilitators 5 as previously described 6. Serial passage on freshly growing monolayers provides a high titre virus stock which can subsequently be used to produce large amounts of protein. To 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 CD4-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 uptake 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 corresponds to those cells in the population which were infected early during the course of the experiment and which are also likely to be expressing the most protein at the cell surface. This population expresses approximately 10-15% of the number of CD4 molecules at the cell surface, as does the human T lymphocyte cell line, HB/ALL, as determined by cytofluorographic analyses using the anti-Leu3A monoclonal antibody (data not shown). Preliminary evidence suggests that the yield of CD4 purified from these cells is in the range of 1-10 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, that 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 CD4-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 CD4 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. 2a). Similar experiments were
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 Naceria), derived from patients affected with severe combined immunodeficiency disease (SCID), 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, 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 was 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. 3c). 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.

We are grateful to Drs R. S. Accolla, D. Pious, C. Grisselli and B. Lisowska-Groszpieber for providing mutant and patient cell lines, to Dr R. Axel for the CD4 cDNA clone and to Drs M.-J. Gething and J. Sambrook for the SV40 vector system. We also thank Drs M. S. Krangel, J. C. Gorga and D. R. Johnson for advice and critical reading of the manuscript and S. Liang for assistance in initial experiments. This work was supported by NIH and the American Foundation for AIDS Research to J.L.S. and a National Research Service Award to C.D.

Received 24 September; accepted 5 October 1987.