A Monoclonal Antibody Which Blocks Infection with Feline Immunodeficiency Virus Identifies a Possible Non-CD4 Receptor

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Monoclonal antibody vpg15 detects a 24-kDa cell surface protein on feline cells permissive for infection with feline immunodeficiency virus (FIV). The antibody blocks infection of FIV-susceptible cells, and expression of the vpg15 marker is decreased in FIV-infected cells in vitro. These results suggest that the antibody may recognize an FIV receptor distinct from CD4.

Since its discovery in 1986 (24), feline immunodeficiency virus (FIV) has been established as a common and important infection of the domestic cat and as a cause of serious immune impairment (19, 20, 37). In addition, FIV is emerging as an increasingly relevant model for human AIDS, since the virus is T lymphotropic and the pathogenesis of experimental FIV infection closely parallels that of human immunodeficiency virus (HIV) infection in humans (2, 3, 23, 38).

Given the role of the CD4 helper T-cell marker as a receptor for HIV infection (12, 21), the question of whether FIV utilizes the feline homolog of CD4 (fCD4) (1) is of fundamental interest. The fCD4 marker was implicated by the observed decrease in the ratio of fCD4+ to fCD8+ cells in FIV infection (2, 3, 17, 23, 34) and the reduced expression of fCD4 following FIV infection of T cells in vitro (35). However, although fCD4+ T cells are highly susceptible to FIV infection, we found no correlation between the expression of fCD4 on cells and their ability to support the replication of FIV (35). Furthermore, FIV infects ostensibly fCD4- cells, such as renal cortical cells (38), macrophages (5), astrocytes (13), and fCD8+ T cells (4). We now describe the isolation and characterization of a monoclonal antibody that is capable of blocking FIV infection and recognizes a feline cell surface marker distinct from fCD4 which may comprise all or part of a receptor for FIV.

Monoclonal antibody vpg15 was selected from a panel raised by immunization of BALB/c mice with purified FIV virions grown in feline thymocytes. The antibody reacted only weakly by enzyme-linked immunosorbent assay (ELISA) against disrupted FIV particles and did not recognize any virus-specific components by immunoblotting (using FIV-infected cell lysates as described previously [18]). However, the antibody was found to react with the surface of uninfected cells, including cell types susceptible to FIV infection, such as the antigen-specific T-cell line Q201 (35), the renal cortical cell line CRFK (11), primary feline astrocytes, and the large granular lymphoma cell line MCC (6) (Fig. 1a). Two-color flow-cytometric analysis of feline peripheral blood cells showed that a small subset of both fCD4+ (1) and fCD8+ (22) cells also reacted with vpg15. In addition, vpg15 stained peritoneal macrophages (isolated by peritoneal lavage with phosphate-buffered saline [PBS] as described elsewhere [30]) and neutrophils (Fig. 2b). Cell surface iodination and immunoprecipitation identified a vpg15 ligand of 24 kDa. Cells (107) in 200 μl of PBS were labeled with 125I, using iodobeads as described previously (26). The washed cells were then disrupted in 200 μl of lysis buffer (100 mMol of Tris [pH 7.4] per liter, 140 mMol of NaCl per liter, 1 mMol of EDTA per liter, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 0.5% deoxycholate, 1% aprotinin). Agarose beads linked to sheep anti-mouse immunoglobulin (Sigma, Poole, United Kingdom) were washed in PBS, pelleted, and incubated with vpg15 (50 μl of agarsol to 100 μl of antibody) for 30 min. The lysate of surface-iodinated cells (50 μl) was then added and incubated at 4°C for 2 h. After washing, the immune complexes were eluted from the beads by boiling and analyzed by gel electrophoresis and autoradiography as previously described (18). As can be seen from Fig. 1b, FIV-susceptible cell lines such as Q201 T cells, CRFK cells, and MCC cells all expressed the 24-kDa protein which was specifically recognized by the vpg15 antibody. However, neither the nonfeline cell line U937 (31), which is nonpermissive for FIV, nor the feline lymphoma line 3201 (29), which is relatively resistant to infection (33, 35), expressed detectable levels of the 24-kDa ligand.

The first indication that the vpg15 antibody might recognize a receptor for FIV came from the observation that expression of the vpg15 marker is consistently decreased on FIV-infected cells (Fig. 2c). Moreover, activation of feline T cells with concanavalin A (ConA) and interleukin-2 (IL-2) increased their susceptibility to FIV infection concomitantly with expression of the vpg15 marker. Peripheral blood lymphocytes from a specific-pathogen-free cat were cultured at 5 × 106 cells per ml and stimulated with ConA (7.5 μg/ml). Twenty-four hours later, the cells were washed and then incubated in medium containing IL-2 (100 IU/ml). As shown in Fig. 3a, expression of the vpg15 marker increased from 3% on day 0 to 52% on day 7. Infection with the Glasgow-8 strain of FIV (FIV/GLA-8) (18) was attempted on day 0, 3, or 6 after T-cell activation. Only the day 3 and 6 cultures became infected, as shown by the production of FIV p24Ag (Fig. 3b).

In view of the correlation between vpg15 marker expression and susceptibility to FIV infection, we investigated whether the antibody could block de novo infection. Three

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FIG. 1. (a) Flow cytometric analysis of vpg15 marker expression on cells permissive and nonpermissive for FIV infection. Permissive cells include feline T cells (Q201), feline renal cortical cells (CRFK), feline astrocytes (kindly provided by L. R. Whalen and A. R. Mihajlov, Colorado State University), and feline large granular lymphoma cells (MCC). 3201 feline thymic lymphosarcoma cells are not readily infectable (35), and the human monocyte line U937 is nonpermissive. Results are displayed as histograms of relative cell number (ordinate) versus fluorescence intensity (abscissa). Each histogram compares vpg15 marker expression (solid line) with isotype-matched control (broken line) and represents 10,000 events gathered in LIST mode. (b) Immunoprecipitation analysis of surface-iodinated cells with either vpg15 (lanes 1 to 5) or an isotype-matched anti-ICD8 monoclonal antibody (lanes 6 to 10). The cell lines presented are MCC (lanes 1 and 6), CRFK (lanes 2 and 7), Q201 cells (lanes 3 and 8), 3201 (lanes 4 and 9), and U937 cells (lanes 5 and 10). The 24-kDa protein precipitated by vpg15 is indicated on the left. Positions of size markers (lanes Mr) are indicated on the right in kilodaltons.

cultures, each containing $5 \times 10^6$ Q201 cells, were incubated with isotype-matched monoclonal antibodies in 1 ml of medium at $37^\circ$C for 30 min. The monoclonal antibodies used were vpg30 (anti-fCD4), vpg15, and a monoclonal antibody against cat immunoglobulin A (IgA). All antibodies were of the IgG1 isotype, as determined by a commercial isotyping kit (Innotech, Antwerp, Belgium), and were added to a final dilution of ascites fluid of 1/200. A fourth culture was incubated in medium without antibody. Each culture was centrifuged at 1,000 rpm for 5 min, the supernatant was discarded, and the cells were resuspended in culture fluid derived from FIV/GLA-8-infected T cells with antibody as in the first incubation. The cells were incubated with the virus at $37^\circ$C for 30 min before being washed twice and resuspended in normal T-cell medium containing a maintenance solution of antibody (1/500 dilution of ascites fluid). Mainte-

FIG. 2. (a) Two-color flow cytometric analysis of vpg15 marker expression on ICD4+ lymphocytes and ICD8+ lymphocytes. Leukocytes were prepared from EDTA-anticoagulated blood by whole blood lysis and processed for flow cytometry. Primary antibody (anti-fCD4 or anti-fCD8) was detected by using phycoerythrin-conjugated F(ab')2 fragment of sheep anti-mouse IgG (abscissa); vpg15 marker expression was detected with fluorescein isothiocyanate-conjugated vpg15 antibody (ordinate). Lymphocytes were selected by gating on a plot of forward scatter versus 90° side scatter (analysis gates indicate the position of isotype-matched control antibody). (b) Flow cytometric analysis of vpg15 marker expression on neutrophils (i) and peritoneal macrophages (ii). The solid line represents vpg15, and the broken line represents the isotype-matched control antibody. (c) Effect of FIV infection on vpg15 expression in two feline T-lymphocyte-derived cell lines. i, uninfected FcT (36) cells (solid line) compared with the FIV/PET-infected derivative FL4 (36) (broken line) (both lines kindly provided by J. K. Yamamoto, University of California, Davis); ii, uninfected (solid line) versus FIV-infected (broken line) Q201 cells. Results are displayed as histograms of relative cell number (ordinate) versus fluorescence intensity (abscissa).
nance antibody was added to the medium in these blocking experiments as in analogous blocking experiments with HIV (21). The cells were subcultured 3 days postinfection and were maintained in culture for a week. Samples of culture fluid were taken daily for measurement of p24\textsuperscript{agg} production by ELISA. As shown in Fig. 3c, only the vpg15 antibody blocked the increase in FIV p24\textsuperscript{agg}, which began at day 4 in the infected cultures. In contrast, the isotype-matched monoclonal anti-fCD4 antibody had no such effect. Similarly, two other anti-fCD4 monoclonal antibodies which recognize different epitopes (28a) had no effect on FIV infection (data not shown).

We extended our analysis to the Petaluma isolate of FIV (FIV/PET) (24), which has a broad host range, also infecting the CRFK cell line (25). CRFK cells were seeded onto 24-well plates at a concentration of 2 × 10\textsuperscript{5} cells per well and were incubated overnight. The following day, the medium was removed and the cells were incubated at 37°C for 30 min with twofold dilutions of vpg30 and vpg15, starting at 1/100. The cells were then incubated with a virus suspension culture fluid (derived from FIV/PET-infected CRFK cells) containing antibody solution at 37°C for 30 min. The cells were then washed twice, and medium containing maintenance antibody (1/5 dilution of antibody solution used to treat cells prior to infection) was added. The cultures were maintained for 4 days. FIV infection of the CRFK cells was monitored by immunocytochemical staining to detect p24\textsuperscript{agg} antigen as described elsewhere (14). The wells were examined by light microscopy, and the foci of infection expressing p24\textsuperscript{agg} antigen were counted. As shown in Fig. 3d, the vpg15 antibody caused a concentration-dependent extinction of FIV foci on CRFK cells.

The blocking effect of monoclonal antibody vpg15 was not attributable to direct neutralization of FIV. Sera from two FIV-infected cats and an uninfected specific-pathogen-free cat as well as the vpg15 ascites fluid were heat inactivated at 56°C for 30 min. Tenfold dilutions (starting at 1/10) of each antibody were then mixed with an equal volume of virus containing between 30 and 60 focus-forming units of FIV. After a 1-h incubation at 37°C, this inoculum was seeded onto a subconfluent monolayer of CRFK cells, incubated for 1 h, and then replaced with culture medium. Following a
4-day incubation, foci of infection were visualized by immunocytochemical staining for FIV p24\textsuperscript{core}. The FIV-infected cat sera neutralized FIV at titers of 10\textsuperscript{3} and 10\textsuperscript{4}. In contrast, neither the control serum from a specific-pathogen-free cat nor the vpg15 ascites fluid demonstrated neutralizing activity. Furthermore, lack of production of p24\textsuperscript{core} by vpg15-treated FIV-infected cells was not due to an inhibitory effect on the cells, since incubation of cell line Q201 with vpg15 had no effect on cell viability or proliferation (data not shown). Similarly, the blocking of infection was unlikely to have resulted from a nonspecific effect, since the isotype-matched control antibody recognizing fCD4 bound to the Q201 cells but had no effect on the course of infection. In the light of these results, we consider that the vpg15 marker may comprise a receptor, or an essential component of a receptor, for FIV.

The vpg15 antibody was the fortuitous product of immunizing mice with purified FIV virions and was found to recognize a cell-coded rather than a virus-coded protein. Retroviruses frequently incorporate in their envelopes cellular proteins which are derived from host cell membranes by budding (15, 28). It would seem possible that the vpg15 marker either was incorporated during the budding process or became attached to the virion surface through affinity for viral proteins. If the former explanation is correct, it is possible that the putative receptor molecule is not directly involved in viral attachment to the host cell membrane, since antibodies which block infection are not always directed against the viral receptor molecule (29a, 32a). Therefore, until the vpg15 marker is cloned and shown to confer susceptibility to FIV infection, we will not have definitive evidence that vpg15 recognizes a receptor for FIV.

However, this study demonstrates that FIV infection can be blocked by an antibody against a cell surface molecule which does not recognize FIV by either ELISA or immunoblotting and which is nonneutralizing. This blocking effect appears to be specific, since antibody vpg15 does not block infection of feline cells with feline leukemia virus (data not shown) or CD4-transfected feline cells with HIV-2 (22a). The vpg15 antibody detects a 24-kDa protein which shows no apparent cross-reaction with the fCD4 molecule (1). A non-fCD4 receptor for FIV has been implicated by previous studies which have shown that FIV can infect cells devoid of detectable fCD4 (4, 5, 13). While a role for CD4 as an alternative receptor or as a coreceptor for FIV cannot be formally ruled out and while there may be heterogeneity among FIV isolates in receptor specificity, our results indicate the possible existence of a novel non-CD4 receptor for FIV.

Further studies will be aimed at the molecular cloning of the gene encoding the vpg15 marker and the determination of its role in FIV infection. Characterization of the putative FIV receptor may also help to elucidate the mechanism by which FIV induces long-term depletion of fCD4\textsuperscript{+} cell numbers (2, 3, 17, 23, 34) and perhaps provide a useful clue to the putative CD4 coreceptors (8, 9) or CD4-independent entry mechanisms (7, 10, 16, 27, 32) which have been implicated in studies with HIV.

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