Neutralization and Receptor Recognition of Human T-Lymphotropic Retroviruses

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A. Introduction

Like other enveloped viruses, the outer membrane antigens of retroviruses present target antigens for neutralizing antibodies, and also play a key role in interacting with cell-surface receptors for initial stages of infection. We have investigated these attributes of the envelopes of human T-lymphotropic retroviruses (Weiss 1985; Wong-Staal and Gallo 1985) and our studies are briefly summarized here.

The viruses investigated are human T-cell leukemia virus type-1 (HTLV-1), the etiologic agent of adult T-cell leukemia-lymphoma (ATL), HTLV-2, first isolated from the leukemic cells of a patient with T-cell hairy leukemia (Kalyanaraman et al. 1982) and present in a proportion of intravenous drug abusers (Tedder et al. 1984), and human immunodeficiency virus (HIV-1, HTLV-3/LAV) the causative agent of AIDS.

B. Neutralizing Sera for HTLV-1 and HTLV-2

HTLV-1 and HTLV-2 are not readily amenable to quantitative titration of infectivity because the virions are largely cell-associated. We have therefore employed a viral pseudotype technique for detecting neutralizing antibodies and for probing receptors. The pseudotypes are virions of vesicular stomatitis virus enveloped by the glycoproteins of HTLV-1 or HTLV-2 (Clapham et al. 1984), a system originally devised for avian retroviruses by Zavada (1972). Pseudotypes possess the neutralization and host range properties of the retrovirus, while replicating as VSV to produce cytopathic plaques in cell monolayers that can be assayed in a simple, quantitative way.

Using stocks of VSV(HTLV-1), we found that sera of infected subjects, whether healthy or suffering ATL, possessed neutralizing titers ranging from 1:50 to 1:50000 (Clapham et al. 1984). In collaboration with H. Hoshino, we showed that VSV(HTLV-1) pseudotypes prepared with Japanese or American HTLV-1 isolates did not distinguish by neutralization titer between sera from Japanese and British West Jamaican subjects (Hoshino et al. 1985).

For HTLV-2 neutralization, we have used a single isolate from an American patient (Kalyanaraman et al. 1982) for pseudotype preparation, and observed high neutralization titers in the sera of 4% of British intravenous drug abusers (Tedder et al. 1984). There is a slight degree of cross-neutralization between HTLV-1 and HTLV-2, but the heterologous titers are 100- to 1000-fold lower than the homologous titers (Clapham et al. 1984).

C. Common and Variable Antigens for HIV-1 Neutralization

We have also detected neutralizing activity in sera of subjects infected with HIV-1 (Weiss et al. 1985a, 1986). In a longitudinal

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Table 1. Common and strain-specific neutralizing activities of human and rabbit sera to HIV-1 [a]

Serum	Neutralizing titer for VSV (HIV-1)						
Strain origin (% env divergence)	RF Haiti 15	IIIB USA 0	MN USA 10	ARV-2 USA 15	MA-2 UK 10	Rut Tanzania >20	Z129 Zaire >20
Human							
British control							
1	10	10	250	6250	50	250	50
2	50	10	250	1250	50	250	50
3	10	–	250	250	10	250	50
4	50	10	250	1250	50	50	250
5	50	10	10	1250	10	50	250
6	250	–	10	1250	10	250	250
Ugandan control							
1	50	10	50	1250	50	10	250
2	250	10	–	1250	10	250	–
3	50	10	10	6250	250	250	50
4	50	10	10	1250	250	250	–
Rabbit							
Preimmune	–	–	–	–	–	–	–
Anti-gp130	–	50	10	–	10	–	–

[a] Data from Weiss et al. (1986).

study of initially asymptomatic male homosexuals known to be infected with HIV-1 for at least 3 years (Weber et al. 1987), the continued presence and a slightly rising titer of neutralizing anti-HIV-1 appears to correlate with a relatively good prognosis. However, a stronger correlation was found with low or no anti-gag serum activity (measured by radioimmunoprecipitation) and progression to AIDS or AIDS-related complex (ARC). We do not, at present, know whether some of the neutralizing activity may be directed to gag antigens, though this appears unlikely for VSV(HIV-1) pseudotypes, which are thought to assemble only membrane glycoproteins of the retrovirus.

Using VSV(HIV-1) pseudotypes prepared with seven different isolates of HIV-1 from USA, Haiti, Europe and Africa, we examined the specificity and variability of neutralizing epitopes (Weiss et al. 1986). Sera from Ugandan and British subjects selected for capacity to neutralize the RF (Haitian) isolate, showed significant neutralizing activity for almost all HIV-1 strains (Table 1). Interestingly, the ARV-2 pseudotype stock appeared to be most sensitive to neutraliza-

tion, whatever the origin of the anti-HIV human serum. By contrast to the cross-neutralization observed with human sera, we found in collaboration with L. Lasky and P. Berman that a rabbit serum raised by Lasky et al. (1986) against a recombinant gp130 molecule specific to the outer envelope glycoprotein of HIV-1, neutralized the strain from which the recombinant was made (HTLV-IIIB), but only two of the other six strains. Even the ARV-2 isolate that was so sensitive to human sera was not significantly neutralized by the anti-gp130 rabbit serum. Tests with further rabbit and guinea-pig sera raised against gp130 confirmed the strain-specificity of this antigen (Weiss et al. 1986).

The antigen targets for neutralization of HIV-1 thus appear to include epitopes common to widely divergent HIV-1 strains as well as variable antigens specific to individual strains and more closely related isolates. The presence of common neutralization antigens between HIV-1 strains differing by 20% or more in the envelope gene (Alizon et al. 1986; Starcich et al. 1986) is a promising finding for the development of vaccines.

D. Receptors for HTLV and HIV

HTLVs and HIV share a common property of tropism for T4$^+$ lymphocytes in vivo (Weiss 1985; Wong-Staal and Gallo 1985). ATL is a T4$^+$ neoplasm, while the salient feature of AIDS is the depletion of T4$^+$ T-helper cells. However, other cell types can be infected in vitro by HTLV-1 (Clapham et al. 1983) and by HIV-1 (Levy et al. 1985; Gartner et al. 1986). We investigated to what extent the cellular tropisms of HTLV and HIV are determined by the cell surface receptors for initiating infection, and identified the T4 (CD4) antigen itself as an important component of the HIV-1 receptor (Dalgleish et al. 1984).

Using VSV pseudotypes with envelopes of HTLV or HIV, we found that HTLV-1 and HTLV-2 recognise receptors on diverse types of human cells and mammalian cells of many species. Productive infection of cells by HTLV-1 not only blocks receptor availability to VSV(HTLV-1) but also to VSV(HTLV-2), indicating that these two viruses use a common receptor (Weiss et al. 1985 b). The recognition of a common cell surface receptor for HTLV-1 and HTLV-2 accords with the slight cross-neutralization of these viruses already noted, as there may be a common epitope of the external glycoprotein that binds to the receptor. We do not at present know the biochemical nature of the HTLV receptor.

Pseudotypes of HIV-1 plate only on cells expressing T4 antigen (Dalgleish et al. 1984). These may be T-helper lymphocytes or monocytes, such as the U937 cell line. Using monoclonal antibodies (mAbs) to T4 antigen, we found that pseudotype infection, and also induction of multinucleated syncytia by HIV-1, could be blocked in T4$^+$ T cells (Dalgleish et al. 1984) and U937 cells (Clapham et al. 1987). Infection by HIV-1 (Dalgleish et al. 1984) and treatment by TPA (Clapham et al. 1987) caused concomitant disappearance of T4 antigen and HIV-1 receptor from the cell surface without affecting the HTLV receptor. Other groups have also observed that mAbs specifically blocked infection by HIV-1 (Klatzmann et al. 1984) and binding of labelled HIV-1 virions (McDougal et al. 1986). Preliminary studies also indicate that HIV-2 (LAV-2;

Clavel et al. 1986) reception is also blocked by certain anti-T4 mAbs. Thus, the T4 antigen acts as a specific receptor on lymphocytes and monocytes for binding HIV.

The precise epitopes on the T4 antigen recognized by HIV-1 remain to be determined. In collaboration with Q. Sattentau and P. C. L. Beverley, we have analyzed a series of 25 different mAbs raised against T4 for ability to block each other and HIV-1 syncytium induction (Sattentau et al. 1986). The mAbs that interfere with HIV-1 reception fall into three noncompeting groups. Two block HIV-1 completely and the third only weakly. Some mAbs, e.g., OKT4 and OKT4c, fail to compete with the HIV-1 receptor at all. These epitopes are thought to be located near to the transmembrane domain of the T4 molecule. Although there is some degree of variation in T4 epitope expression on T lymphocytes from different African and Caucasian individuals, this was not correlated with susceptibility to HIV-1 infection in vitro and did not affect those epitopes most important for T4 binding (Sattentau et al. 1986).

We next examined whether the T4 antigen would act as a functional receptor for HIV-1 if expressed ectopically on the surface of unusual cell types. In collaboration with P. Maddon, R. Axel, and J. S. McDougal, the cDNA for the human *T4* gene was introduced into various human and mouse cell types via a retrovirus vector carrying a neomycin resistance gene as a dominant selectable marker (Maddon et al. 1986). Our findings, summarized in Table 2, show that human cells such as the immature T-cell line HSB2, the Burkitt's lymphoma line, Raji, and the cervical carcinoma line, HeLa, become susceptible to HIV-1 infection and replication upon expression of T4. On the other hand, transfection and expression of the human *T4* gene to mouse cells, including L3T4$^+$ mouse cells (the murine equivalent of human T4$^+$ lymphocytes), does not result in infection of HIV-1 or of the VSV(HIV-1) pseudotype. HIV-1 virions did, however, bind specifically to the surface of T4 transfected mouse cells and not to control mouse cells. Therefore, we conclude that T4 antigen is sufficient for HIV-1 attachment to the surface of mouse cells, but that some other human component, present in HeLa and Raji

Table 2. Interaction of HIV-1 with cells transfected with the human T4 gene[a]

Cell type[b]		HIV-1 virion binding	Syncytium induction	VSV (HIV-1) infection	HIV-1 replication[c]
Human					
HSB2	T cell	+	+	+	+
Raji	B cell	+	+	+	+
HeLa	Carcinoma	+	+	+	+
Murine					
3DT	T cell	+	−	−	−
L	Fibroblast	+	−	−	−
NIH-3T3	Fibroblast	+	−	−	−

[a] Data from Maddon et al. (1986).
[b] All control cells (transfected with neo vector alone or neo + human T8 gene) were universally negative for all HIV-1 interactions listed in the table.
[c] HIV-1 replication was assayed by production of reverse transcriptase, immunofluorescence for viral antigens, and induction of syncytia in indicator T cells.

cells, is necessary for functional penetration following attachment. It appears that the binding of virions alone is insufficient to trigger endocytosis of the ligand-receptor complex on mouse cells.

E. Conclusions

Our studies of virus neutralization serve to distinguish different strains of human retroviral pathogens and may eventually provide prognostic tools and aid the development of vaccines. Our receptor studies serve to distinguish the cellular tropisms of HTLVs and HIVs, and have identified certain epitopes of the T4 surface antigen as the binding receptor for HIV. Infection of T4$^+$ lymphocytes and monocytes by HIV-1 largely explains the immunodeficiency underlying the opportunistic infections and neoplasms evident in AIDS. The binding of free envelope glycoprotein to uninfected T4$^+$ cells when shed by infected cells may further exacerbate the immunodeficiency, as T4 antigen plays a functional role in cell interactions in the immune system (Dalgleish 1986).

References

1. Alizon M, Wain-Hobson S, Montagnier L, Sonigo P (1986) Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. Cell 46:63-74

2. Clapham PR, Weiss RA, Dalgleish AG, Exley M, Whitby D, Hogg N (1987) Human immunodeficiency virus infection of monocytic and T-lymphocytic cells: receptor modulation and differentiation induced by phorbol ester. Virology 158:44-51

3. Clapham P, Nagy K, Weiss RA (1984) Pseudotypes of human T-cell leukaemia types 1 and 2: neutralization by patients' sera. Proc Natl Acad Sci USA 81:2886-2889

4. Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey M-A, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C, Rouzioux C, Klatzmann D, Champalimaud JL, Montagnier L (1986) Isolation of a new human retrovirus for West African patients with AIDS. Science 233:343-346

5. Dalgleish AG (1986) The T4 molecule: function and structure. Immunology Today 7:142-144

6. Dalgleish AG, Beverley PCL, Clapham PR, Crawford DH, Greaves MF, Weiss RA (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312:763-767

7. Gartner S, Markovits P, Markovits DM, Kaplan MH, Gallo RC, Popovic M (1986) The role of mononuclear phagocytes in HTLV-III/LAV infection. Science 233:215-219

8. Hoshino HH, Clapham PR, Weiss RA, Miyoshi I, Yoshida M, Miwa M (1985) Human T-cell leukemia virus type I: pseudotype neutralization of Japanese and American isolates with human and rabbit sera. Int J Cancer 36:671-675

9. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Blayney D, Golde D, Gallo RA (1982) A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. Science 218:571

10. Klatzman D, Champagne E, Chamaret S, Gruest J, Guetard D, Hercent T, Gluckman J-C, Montagnier L (1984) T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature 312:767–768

11. Lasky LA, Groopman JE, Fennie CW, Benz PM, Capon DJ, Dowbenko DJ, Nakamura GR, Nunes WM, Renz ME, Berman PW (1986) Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science 233:209–212

12. Levy JA, Shimabukuro J, McHugh T, Casavant C, Stites D, Oshino L (1985) AIDS-associated retroviruses (ARV) can productively infect other cells besides human T helper cells. Virology 147:441–448

13. Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R (1986) The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell 47:333–348

14. McDougal JS, Kennedy MS, Sligh JM, Cort SP, Mawle A, Nicholson JKA (1986) Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. Science 231:382–385

15. Sattentau QJ, Dalgleish AG, Weiss RA, Beverley PCR (1986) Epitopes of the CD4 antigen and HIV infection. Science 234:1120–1123

16. Starcich BR, Hahn BH, Shaw GH, McNeely PD, Modrow S, Wolf H, Parks ES, Parks WP, Josephs SF, Gallo RC, Wong-Staal F (1986) Identification and characterisation of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell 45:637–648

17. Tedder RS, Shanson D, Jeffries D, Cheingsong-Popov R, Clapham P, Dalgleish A, Nagy K, Weiss RA (1984) Low prevalence in the UK of HTLV-1 and HTLV-II infection in subjects with AIDS, with extended lymphadenopathy, and at risk of AIDS. Lancet II:125–128

18. Weber JN, Clapham PR, Weiss RA, Parker D, Roberts C, Duncan J, Weller I, Carne C, Tedder RS, Pinching AJ, Cheingsong-Popov R (1987) Human immunodeficiency virus infection in two cohorts of homosexual men: Neutralising sera and association of anti-gag antibody with prognosis. Lancet I:119–122

19. Weiss RA (1985) Human T-cell viruses. In: Weiss RA, Teich NM, Varmus HE, Coffin J (eds) RNA tumor viruses, vol 2. Cold Spring Harbor Laboratory, New York, pp 404–485

20. Weiss RA, Clapham PR, Weber JN, Dalgleish AG, Berman PW, Lasky LA (1986) Variable and conserved neutralization antigens of human immunodeficiency virus (HIV-1). Nature 324:572–575

21. Weiss RA, Clapham PR, Cheingsong-Popov R, Dalgleish AG, Carne CA, Weller IVD, Tedder RS (1985a) Neutralization of human T-lymphotropic virus type III sera of AIDS and AIDS-risk patients. Nature 316:69–71

22. Weiss RA, Clapham PR, Nagy K, Hoshino H (1985b) Envelope properties of human T-cell leukemia viruses. Current topics in microbiol. Immunol 115:235–246

23. Wong-Staal F, Gallo RA (1985) Human T-lymphotropic retroviruses. Nature 317:395–403

24. Zavada J (1972) Pseudotypes of vesicular somatitis virus with the coat of murine leukemia and of avian myeloblastosis viruses. J Gen Virol 15:183–191