HUMAN IMMUNE RESPONSE TO HTLV-III VIRUS INFECTION IN ACQUIRED IMMUNODEFICIENCY SYNDROME

ANNUAL/FINAL REPORT

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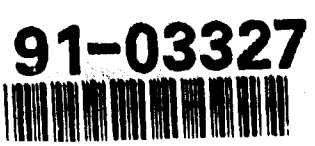

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This contract was awarded for a three year period to study aspects of the human cellular and humoral antibody responses to HIV-1 infections.

The major research findings during this study were:

1. The memory lymphocytes of HIV-1 infected individuals fail to respond to recall viral antigens including HIV-1 antigen early in the course of HIV-1 infections. This finding made aspects of these research investigations extremely difficult especially the study of T cell responses to HIV-1.
2. We generated human CD4+ and CD8+ CTL clones to novel epitopes on the HIV-1 gag protein.
3. We generated a human CD8+ CTL clone to a novel epitope on the envelope glycoprotein gp 160.
4. We establsihed that the sera of HIV-1 infected patients contain antibodies which enhance infection of FcR receptor bearing cells.
5. We demonstrated the antibody dependent enhancement of HIV-1 infection requires two receptors, Fc receptors and CD4.

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Summary

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3. We generated a human CD8+ CTL clone to a novel epitope on the envelope glycoprotein gp160.

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B. Foreword

1. Copyright material: NA

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5. For the protection of human subjects the investigators have adhered to policies of applicable Law 45CFR46.

6. The investigators have abided by the NIH Guidelines for Research involving recombinant DNA Molecules (April 1982) and the Administrative Practices Supplement.

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C. Body of Report

1. The memory T lymphocyte response of HIV-1 infected individuals to HIV-1 and other viral antigens is reduced.

Table 1. HIV induced proliferation of human peripheral blood mononuclear cells is determined by ^3H thymidine incorporation

Donor Ab pos	Mean[a] CPM	Stim Index	Donor Ab neg	Mean CPM	Stim Index
#2			#HJ		
Media	254	1	Media	187	1
HIV:5X	6,473	25	HIV 5X	192	1
1X	3,911	15	1X	210	1
.1X	1,136	4	.1X	192	1
.01X	537	2			
PHA	33,731		PHA	48,206	
#12					
Media	263	1			
HIV 5X	1,453	5			
X	682	2.6			
.1X	552	2.1			
PHA	82,106				

[a]Mean CPM of 4 microtiter wells each containing 2 x 10^5 cells/well in 100 ul. On day 0 an equal volume of medium alone or of infectious HIV virus grown in MOLT-3 cells and concentrated on a metrizamide gradient (provided by Dr. C. Bruck) was added. A ^3H thymidine pulse was performed on day 5, and cells were harvested on day 8 by a MASH harvester after overnight incubation.

This T cell response required concentrated preparations of HIV-1 virus. We determined that the PBMC of some HIV-1 infected donors were stimulated by concentrated live HIV-1, and that the PBMC of antibody negative donors did not respond, however, most HIV-1 infected individuals' PBMC did not respond to HIV-1 or influenza virus antigen.

Table 2: Stimulation of PBMC of HIV antibody positive donors

Stimulating Antigen	# Tested	Responders*	% Responders
Concentrated live HIV-1	30	13	43
gag p55	16	7	44
A/PR/8	12	5	42
PHA	26	25	96

*Responders to viral antigen were defined by a S.I. of $\geq$2.5:1. Responders to PHA were defined by S.I. >80:1.

PBMC were distributed into 96-well microplates. Concentrated, live HIV-1 or an HIV-1 gag p55 construct was added

over a range of concentrations. PHA was added after dilution to 1:200. The plates were then incubated at 37°C for 5 days, pulsed with [H^3] thymidine for 18 hours and then harvested. In the case of A/PR/8 the PBMC were pulsed with the live virus at a MOI of 10:1 for 1.5 hours.

We found that the HIV-1 antigen specific T cell proliferation responses could be enhanced if low concentrations of IL-2 were added on day 5 after addition of antigen, with addition of ^{3}H-thymidine on day 7 and harvesting on day 8, as shown in Fig. 1 (antigen ☐, media ▨).

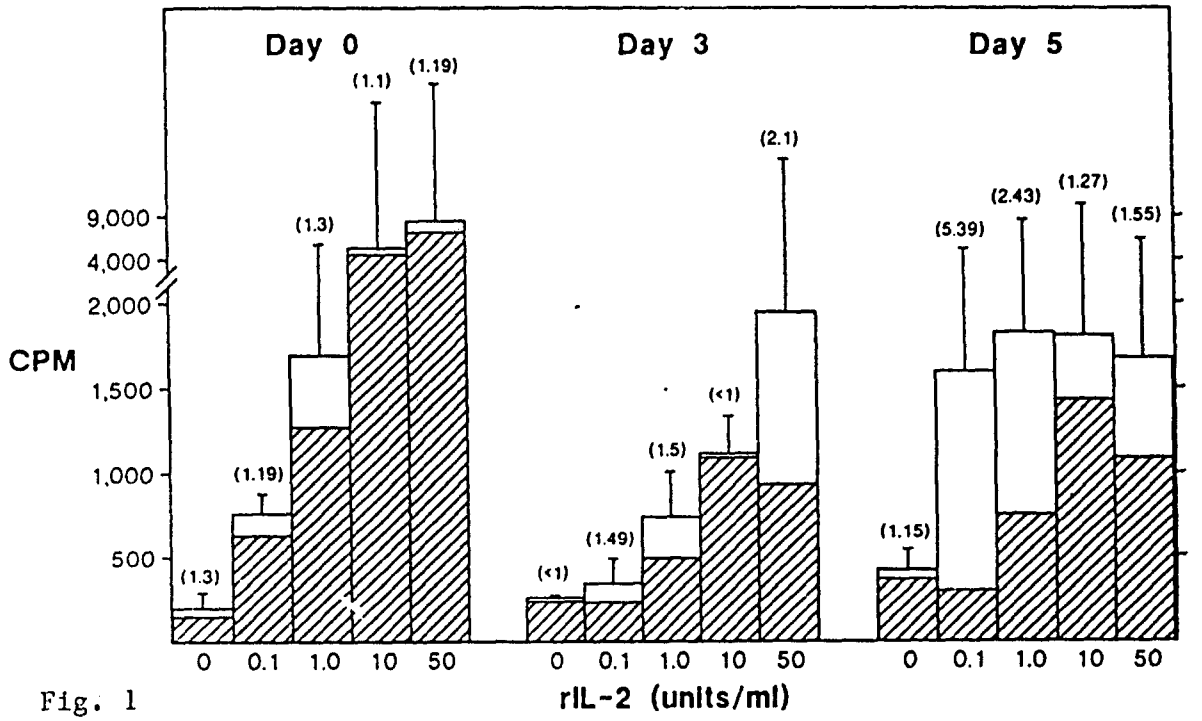


Fig. 1

The Figure 2 below demonstrates that the addition of 0.1-1.0 u/ml of IL-2 enhances the specific T cell responses to HIV-1 antigens, but does not affect the stimulation index of a non-responders PBMC.

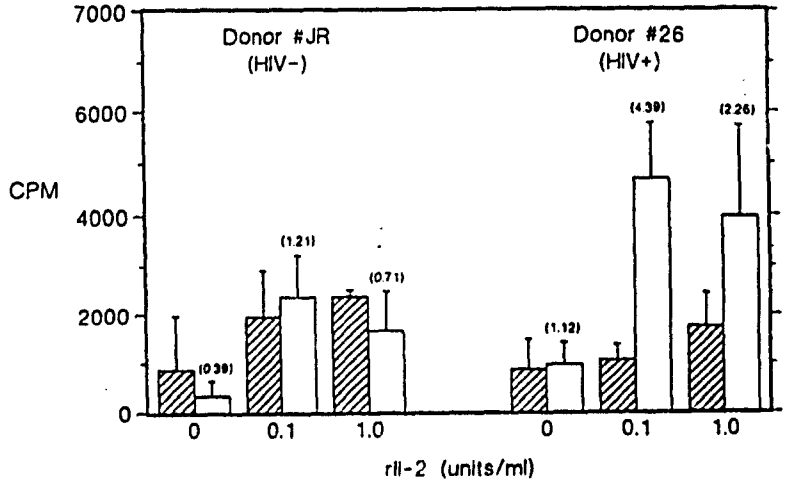


Fig. 2

There was also an increase in the specific response to baculovirus expressed p55 particles as shown below ▨ medium control, ▭ HIV-1 live virus antigen, ▦ p55 antigen, when 0.1 u/ml of IL-2 was added on day 5 after antigen to the PBMC of HIV antibody + donors (Fig. 3 below).

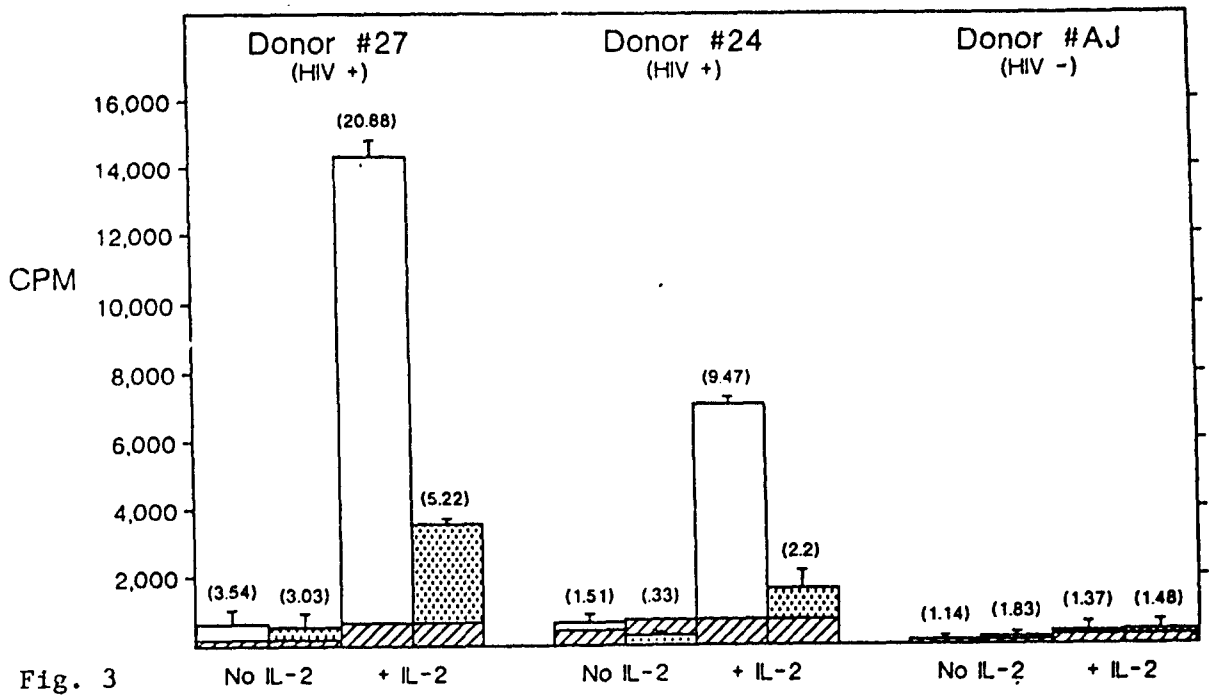


Fig. 3

These responses were dependent on IL-2 itself and not on some contaminating material in the recombinant IL-2 because the specific enhancement of T cell responses to virus antigen by the addition of IL-2 was blocked if specific antisera to IL-2 was added to the culture (data not shown).

We conclude that these results demonstrate an early loss in antigen specific T cell responses of HIV-1 infected individuals. These results were not due to a decreased number of CD4+ cells because these individuals were studied early in their HIV-1 infections, and there were no significant differences in the CD4+ cell numbers between the non-responders and the responders to viral antigen. The defect in response is not due to a lack of memory T cells, or to a failure by the antigen-specific T cell to be activated by viral antigen, because the defect was corrected by IL-2 which could only specifically enhance proliferation of viral antigen specific T cells after activation and upregulation of IL-2 receptors.

These results raise questions concerning the potential for immunotherapy with IL-2 of HIV-1 infected patients. To our knowledge this was only performed in a few patients with full blown AIDS before the cause of AIDS was defined. Our data suggest the possibility that IL-2 therapy might improve the antigen-

specific T cell memory and subsequent effector cell responses to HIV-1 infected individuals to recall antigens. The failure of the immune response to control infections is a hallmark of the acquired immunodeficient state which cause an inability of the host to eliminate reactivated infections unlike the immunocompetent host.

C2. Generation of CD4+ and CD8+ human T cell clones to HIV-1 gag

It was extremely difficult to generate HIV-1 specific T cell clones despite our success in doing this with other viruses, e.g., dengue and influenza. Undoubtedly this is due to the difficulty in generating antigen-specific T cell responses described above. This contract was requested for five years, but was funded for three years. After much effort we finally succeeded in generating human CD8+ and CD4+ T cell clones that lyse autologous target cells infected with Vac gag or pulsed with E. coli derived p24 antigen. Figure 4 below shows some results with clone #165.

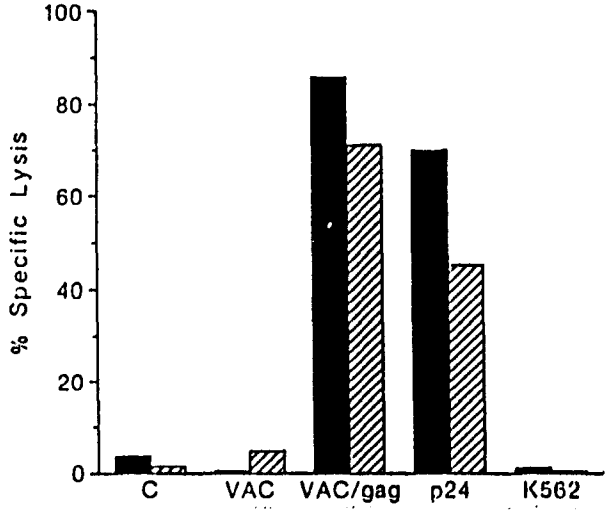


Fig. 4

This CTL clone is CD8+, and its HLA restriction pattern is being defined. We know that this is a novel HIV-1 specific clone because our donor does not have either B7 or A2, the HLA restricting antigens for the only other two HIV-1 gag specific epitopes that have been reported. Peptide mapping studies have also ruled out the two sites reported early, and detailed mapping is almost complete. The site that appears to be recognized by clone #165 is highly conserved.

C3. HIV-1 specific CD4+ CTL clones to gag protein.

We have also generated a long-lived clone of CD4+ CTL which recognizes autologous target cells infected with Vac/gag, or pulsed with p24 antigen.

This is the first report that we are aware of describing a human CD4+ CTL clone to HIV-1 gag. The only other CD4+ CTL reported is to an epitope in gp41 by Silaciano et al in a gp160

vaccinated, not infected, individual.

Table 3 below is a summary of the results in the literature and our results in the HIV-1 gag specific human CD4+ and CD8+ T cell responses.

Table 3

HIV-1 ag	CD4+	CD8+	HLA	Laboratory
gag	--	+	B2	Nixon
gag	--	+	B8	Gotch
gag	--	+	CW6	Ennis
gag	+	-	Class II	Ennis

C4. Human CD8+ CTL clone to gp160

Recently we established a CTL clone which specifically kills autologous target cells infected with Vac/gp 160. This also is a novel CTL clone because it comes from a donor whose HLA antigens do not contain the restrictive element for the only other CTL epitope on gp160 that has been published to date. Berzofsky's group has defined murine $H\text{-}2^d$ CD4+ and CD8+ T cell responses. The only human envelope specific CTL responses that have been published is by Plata, et al. which is restricted by HLA, unlike the CD+ T cell clone we have generated. Mapping of the epitope is not completed.

Table 4 below is a summary of the literature on human CTL responses to gp160.

Table 4

HIV ag	CD4+	CD8+	HLA	Laboratory
env	-	+	A2	Plata
env	+	-	DR4	Siliciani
env	-	+	Class I (non-A2)	Ennis

In conclusion, we have generated three new human CTL epitopes on HIV-1

C5. We established that the sera of HIV-1 infected individuals contains antibodies which bind to HIV-1 virus and enhances infection of Fc receptor T cells.

We were the first laboratory to report that the sera of HIV-1 infected individuals contained antibodies which bound to HIV-1 and enhanced infection by Fc receptor mediated entry. The infection was detected at subneutralizing concentrations of antibodies in human sera (Table below).

The effect was Fc receptor mediated because it was blocked by 1) addition of heat-aggregated human gamma globulin, and by 2) cleaving of the Fc from purified IgG, because the $F(ab')_2$ could neutralize virus but not enhance infection (Table 5 & 6 below).

TABLE 5. Effect of blocking of FcR on enhancement of HIV-1 infection.

Treatment	p24 (ng/ml)
HIV	65.2 ± 0.1
HIV + antiserum (10^{-5})	133.4 ± 14.7
HIV + AHG (1 mg/ml)	80.7 ± 1.8
HIV + antiserum + AHG	81.1 ± 4.4

TABLE 6. Infection enhancement activity in IgG and $F(ab')_2$ from HIV-1 seropositive serum.

Serum dilution equivalent*	p24 (ng/ml)	
	IgG	$F(ab')_2$
0*	96.0 ± 3.6 (1.0)	90.6 ± 1.6 (1.0)
10^{-3}	49.3 ± 1.2 (0.51)	
$10^{-3.5}$	72.7 ± 0.7 (0.76)	35.8 ± 1.3 (0.40)
10^{-4}	115.0 ± 1.5 (1.20)	46.8 ± 0.4 (0.52)
$10^{-4.5}$	174.2 ± 3.3 (1.81)	64.7 ± 2.7 (0.71)
10^{-5}	244.8 ± 6.0† (2.55)	69.4 ± 3.1 (0.77)
$10^{-5.5}$	159.4 ± 0.8 (1.66)	93.1 ± 2.5 (1.03)
10^{-6}	121.1 ± 3.9 (1.26)	81.0 ± 2.4 (0.89)

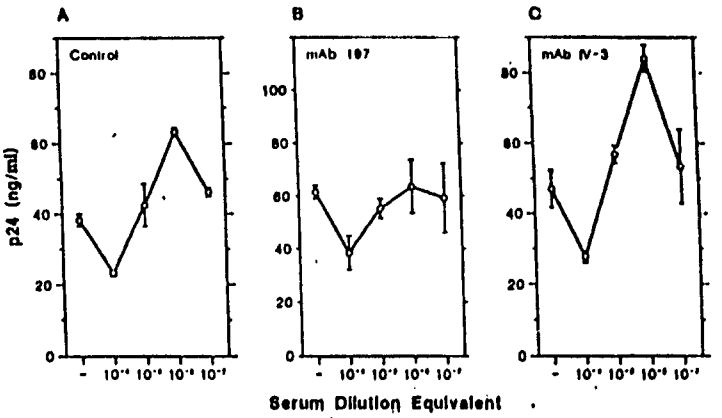
*HIV alone. †Peak enhancement.

C6. Two receptors are required for antibody dependent enhancement of HIV-1 infection: CD4 and Fc R.

To define the entry mechanism of HIV-1 complexed with anti-HIV-1 antibody, we attempted to determine the receptor molecules responsible for mediating enhancement of HIV-1 infection of monocytic cells. Monoclonal antibodies to FcRI for immunoglobulin infection. Furthermore, we demonstrate a requirement for the CD4 molecule in antibody-enhanced HIV-1 infection via FcR. Soluble CD4 prevented infection by HIV-1 antibody-treated virus, and enhancement of infection of virus-antibody complexes was abrogated by monoclonal antibody to CD4 (anti-Leu3a antibody). Treatment of human macrophages with an anti-CD4 antibody also inhibited antibody-enhanced HIV-1 infection of macrophages, supporting our contention that antibody-dependent enhancement of HIV-1 infection via FcR requires CD4 interaction with the virus glycoprotein.

The figure below shows that a monoclonal antibody to FcRI (mAb197) blocks antibody dependent enhancement. A monoclonal antibody to FcRII (mAb 1 V-3) does not.

Fig. 5



Effect of MAbs to $Fc_\gamma RI$ and $Fc_\gamma RII$ on infection enhancement in the presence of antibodies to HIV-1. U937 cells (10^6) were untreated (A) or pretreated with MAb 197 to $Fc_\gamma RI$ (B) or MAb IV-3 to $Fc_\gamma RII$ (C) at 5 μg/ml for 30 min at 4°C and then incubated for 2 h at 37°C with 10^5 $TCID_{50}$ of HIV-1 (MOI, 0.01) or HIV-1 complexed with various concentrations of purified IgG from an HIV-1 antibody-positive serum in the presence of each MAb at 5 μg/ml. The MAbs at this concentration saturate the binding sites of $Fc_\gamma Rs$ (7). After infection, the cells were washed and cultured. Supernatants were harvested on day 7 for determination of p24. Data are presented as the means ± standard deviations of p24 values from duplicate cultures. A repeated experiment showed similar results. The p24 yields from the culture of the cells which were infected in the presence of IgG antibody to HIV-1 diluted at the serum equivalent of 10^{-4} were significantly higher than those in the absence of IgG ($P < 0.001$) in treatment groups A and C but not in treatment group B.

CD4 interaction with the gp160 in the virus-antibody complex to infect the cell because sCD4 reduced infection of antibody complexed virus to very low levels, similar to its effect on blocking infection by free virus.

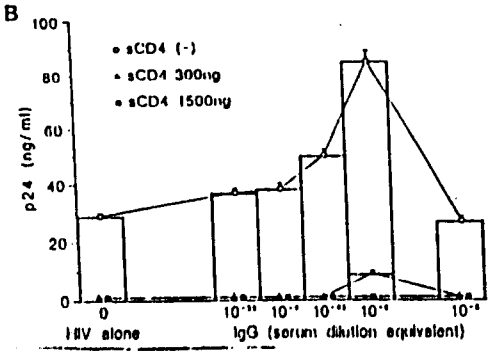


Fig. 6. Inhibition of infectivity of HIV-1 for U937 cells by sCD4.

C7. In conclusion, the research performed under this contract has provided new, important, basic information concerning specific human T cell and humoral responses to HIV-1 infection which will improve our understanding of:

1) the pathogenesis of HIV-1 infections and acquired immunodeficiency,

2) possible immunopathology mediated by CD4+ and CD8+ HIV-1 specific CTL,

3) the definition of new CD4+ and CD8+ T cell epitopes which may be important for vaccine development efforts,

4) the definition of infection-enhancing antibodies to HIV-1 in infected patients, thus stimulating the need to define such epitopes and to exclude them from vaccines, and

5) the demonstration that interaction by the gp160 in the antibody-complexed virus must interact with the CD4 in the cell, or to the endosome for antibody dependent enhancement of infection to occur. This may be relevant in other virus infections, e.g., dengue.

Publications

1. Takeda, A., Tuazon, C., Ennis, F.A. Antibody enhanced infection by HIV-1 via Fc receptor-mediated entry. Science 242:580-583, 1988.

2. Takeda, A., Tuazon, C., and Ennis, F.A. Immune enhancement of HIV-1 infection: Possible mechanism of Fc receptor mediated infection enhancement in Vaccines 89, eds R.A. Lerner, H. Ginsberg, R.M. Chanock and F. Brown, Cold Spring Harbor Laboratory Press, 149-153, 1989.

3. Takeda, A. and Ennis, F.A. FcR-mediated enhancement of HIV-1 infection by antibody. AIDS Res and Human Retroviruses 6:999-1004, 1990.

4. Takeda, A., Sweet, R.W. and Ennis, F.A. Model for enhancement of HIV-1 infection by antibody in Vaccines 90, Cold Spring Harbor Laboratory Press 333-337, 1990.

5. Takeda, A., Sweet, R.W., Ennis, F.A. Two receptors are required for antibody-dependent enhancement of HIV-1 neutralization and infection--enhancement by human monoclonal antibodies to gp120. J. Virology, in press, 1990.

6. Takeda, A., Robinson, J.E., Ho, D.D., Debouck, C., Ennis, F.A. Distinction of HIV-1 neutralization and infection--enhancement by human monoclonal antibodies to gp120. Submitted 1990.

7. Littaua, P., Oldstone, M.B.A., Takeda, A., Debouck, C., Moss, B., Wong, J., and Ennis, F.A. An HLA-C-restricted CD8+ CTL clone recognizes a highly conserved epitope on HIV-1 gag. J. Virology, in press, Aug. 1991.

8. Takahaski, K., Dai, L-C., Fuerest, T.R., Biddison, W.E., Earl, D.C., Moss, B., and F.A. Ennis. Characterization of a CTL epitope on HIV-1 gag gp41 restricted by HLA-31 and lysis of HIV-1 infected cells by a CD8+ CTL clone. Submitted for publication.

9. Littaua, R.A., Oldstone, M.B.A., Takeda, A., and Ennis, F.A. CD4+ CTL clone to a conserved epitope on HIV-1 p24: cytotoxic activity and secretion of IL-2 and IL-6. Submitted for publication.