TD-IAI04: HIV infection

The HIV, the immunodeficiency Virus, is a pathogenic retrovirus for human, and is the cause person of acquired immunodeficiency syndrome (AIDS). The syndrome is characterized by the progressive loss of a cellular subpopulation (CD4 T cells) associated with many opportunist infections such as appearance of the Kaposi sarcoma, tuberculosis, meningitis…

I. Identification of the target cells and the entry receptor

The most majority of the viruses, including retroviruses, are characterized by their cellular tropism. In a first part, one seeks to identify the target cells of the virus and the receptor which allows its entry. In figure 1 are presented certain immunological and virological parameters which evolve/change in the course of time after an infection by the HIV.

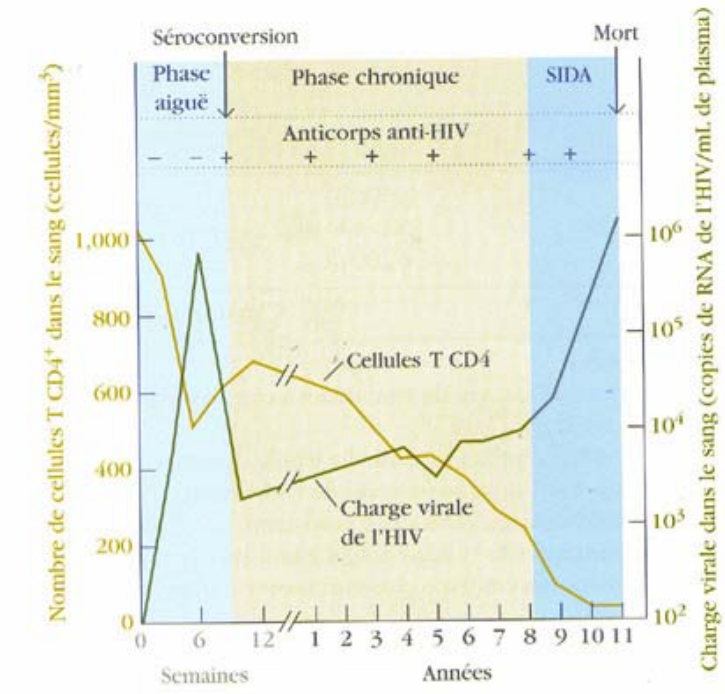


Figure 1: Evolution of the number of cells T $CD4^+$ and the viral load in blood.

Question 1. Can you comment the Figure 1 by describing the variations observed for the parameters?

During the infection of the cells by HIV, the infected cells can fusion between them (infected or not), resulting in the formation of syncitia. Several cellular types were incubated in the presence of virus and the formation of syncitia was observed. Also, the cells were incubated with an anti-CD4 or anti-CD5 monoclonal antibody coupled to a fluorochrome, and the percentages of fluorescent cells were determined by flow cytometry (Table 1).

Question 2. Which hypothesis can you emit concerning the cells and receptors necessary for the in vitro infection by HIV?

Table 1: Correlation between the infection by HIV and the expression of CD4 and CD5 receptors. CD4 is expressed by the auxiliary T lymphocytes and CD5 is a receptor expressed by all the T lymphocytes (according to Dalgleish A. G. and Al 1984 Nature 312:763)

Infected cells		Syncitia formation	% fluorescent cells	
Origin	Name		CD4	CD5
T cell Leukaemia	JM	+++	100	100
T cell Leukaemia	CEM	+++	100	100
T cell Leukaemia	HT/H4	+++	100	100
T cell Leukaemia	MOLT-4	+++	100	90
T cell Leukaemia	HSB2	-	0	80
T cell Leukaemia	GH1	+	80	90
Burkitt Lymphoma (B cells)	Raji	-	0	0
B Leukaemia	-	-	0	0
Promyelocytic Leukaemia	HL60	-	0	0
Monocytic Leukaemia	U937	++	70	0
Osseous Sarcoma	HOS	-	0	0
Cervical Carcinoma	Hela	-	0	0

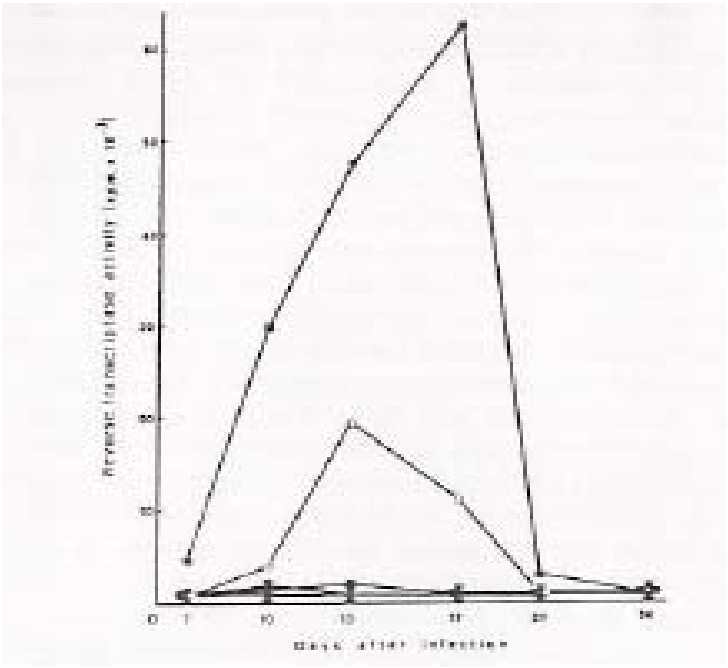
T cells from JM cell line were incubated in the presence of virus and blocking monoclonal antibodies against membrane receptors expressed by these cells. The inhibition of the syncitia formation is studied for each antibody tested (Table 2).

Table 2: Syncitia formation in the presence of several monoclonal antibodies.

Monoclonal antibodies	Specificity	Inhibition of syncitium formation (positives/total tests)
anti CD1	Cortical thymocytes	0/9
anti CD2	Pan-T	0/16
anti CD3	Pan-T	0/13
anti CD5	Pan-T + B-CLL	0/11
anti CD6	Pan-T	0/7
anti CD7	Pan-T	0/11
anti CD4	T helper	14/14
anti CD8	T cytotoxic	0/21
anti CD25	IL-2 receptor	0/9
anti CD26	Activation marker	0/4
	MHC class I	0/1
	MHC class II	2/8
	β2-microglobulin	0/2

Question 3. Do these results make possible to confirm the hypothesis?

In another experiment, lymphocytes T were cultivated in the presence of PHA (mitogen) during 3 days, then were incubated in the presence of anti-CD4 or anti-CHM class II monoclonal antibody, then were infected by HIV and were again cultivated in the presence of IL-2. The production of virus was measured in the culture supernatants (Figure 2). (According to Klatzman D. and Al 1984 Nature 312:767).



Measure of virus production according to time (reverse transcriptase activity in log.), in the presence of anti-MHC class II (black squares) or several anti-CD4 (round blacks and black triangles) monoclonal antibodies.

Question 4. According to these results, what is your conclusion about the nature of receptor(s) allowing the infection by HIV?

II. Identification of a co-receptor allowing the cell entry of HIV

The human and murine cells were transfected by a plasmid containing CD4 or CD8 human molecules, then were incubated in the presence of HIV. The attachment of the virus on the cells, syncitia formation and the viral production in the culture supernatants were then studied (table 3). (according to Maddon P.J. and Al 1986 Cell 47/333).

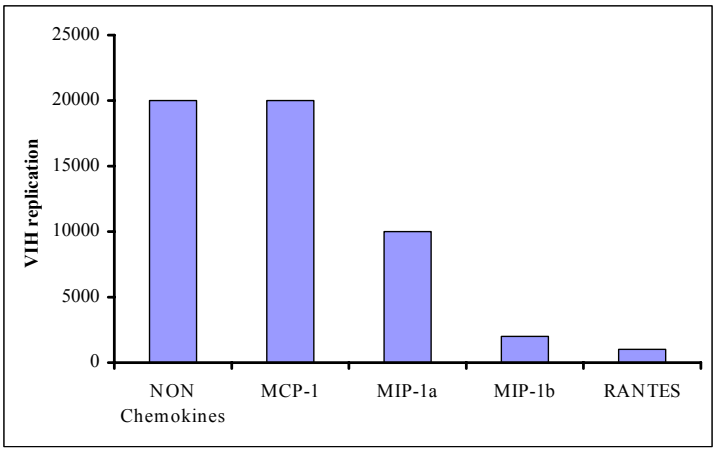
Table 3

Cell type	Attachment	syncitium	Virus production
Human			
JM (T CD4+)	+++	+++++	+
81666 (T CD4+)	+++	+++++	+
Raji (B cells)	-	-	-
Raji CD4+	+++	+++	+
Raji CD8+	-	-	-
Hela (carcinome)	-	-	-
Hela CD4+	+++++	+++++	+
Hela CD8+	-	-	-
Murine			
3DT (T cells)	-	-	-
3DT CD4+	+++	-	-
3DT CD8+	-	-	-
P815 (mastocytoma)	-	-	-
P815 CD4+	+++	-	-
P815 CD8+	-	-	-
3T3 (fibroblastes)	-	-	-
3T3 CD4+	+++	-	-
3T3 CD8+	-	-	-

Question 1. Which differences do you note between the infection of the human and murine cells?

Question 2. What can you conclude from these experiments? Which hypothesis can you emit?

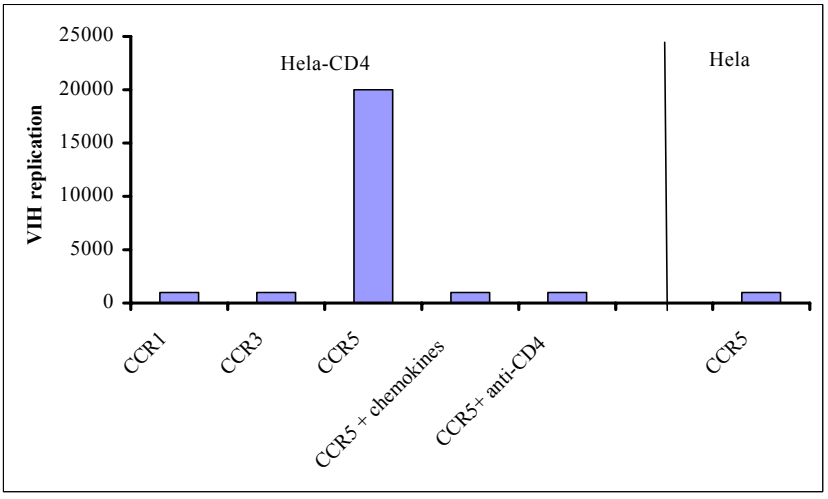
Researchers identified in the supernatants of culture from $CD8^+$ T lymphocytes some proteins which inhibit the replication of the virus. These proteins were characterized, they are the secreted proteins RANTES, MIP-1a and MIP-1b, which belong to the family of the chemokines. Their receptor is CCR-5, a transmembrane protein expressed by certain cells of the immune system among which monocytes and the T lymphocytes.



Lymphocytes were incubated with HIV in absence or in the presence of MCP-1, MIP-1a, MIP-1b or RANTES chemokines. After 4 days, the rate of replication HIV was studied for each condition. It is specified that the receptor of MCP-1 is CCR2.

Question 3. Comment the results of this figure. By which mechanisms does these chemokines influence the replication of the virus?

Hela cells were transfected by a plasmid allowing the expression of the human CD4 and one of the CCR1, CCR3 or CCR5 receptor, then were incubated with HIV, in presence or absence of the chemokines RANTES, MIP-1a, MIP-1b or anti-CD4 blocking antibody. Hela cells non expressing CD4 were also used as negative control. The rate of HIV replication was studied 4 days after the infection of the cells.

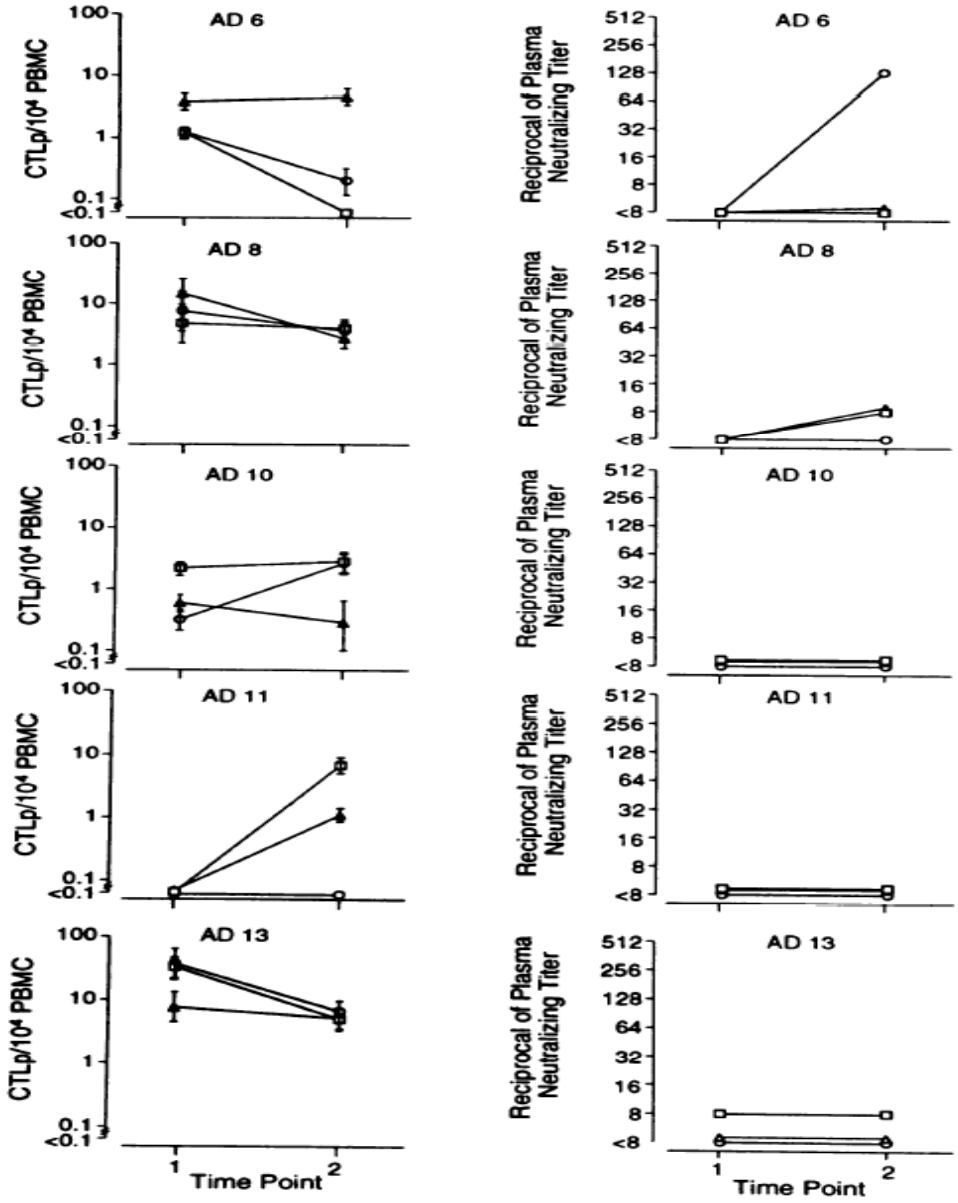


Question 4. According to these results, which receptor can it allow the entry of the virus?

III. The effects of the infection by the HIV on the immune system

The immunological and virological parameters were studied in several people infected by the HIV (2 time points). The first (time point 1) is close to the moment of the seroconversion (detection of anti-HIV antibody in the serum), the second (time point 2) is 3 to 6 months after the seroconversion. In the 1st experiment, the cytotoxic activities (CTLs) against (CTL) 3 were measured against viral proteins (gag, pol, and env). The authors also mesured the rate of neutralizing anti-HIV antibody from blood samples in 5 patients infected by HIV. (According to Koup R.A. et al. 1994, J Virol. 68: 4650)

CTL Neutralizing antibodies

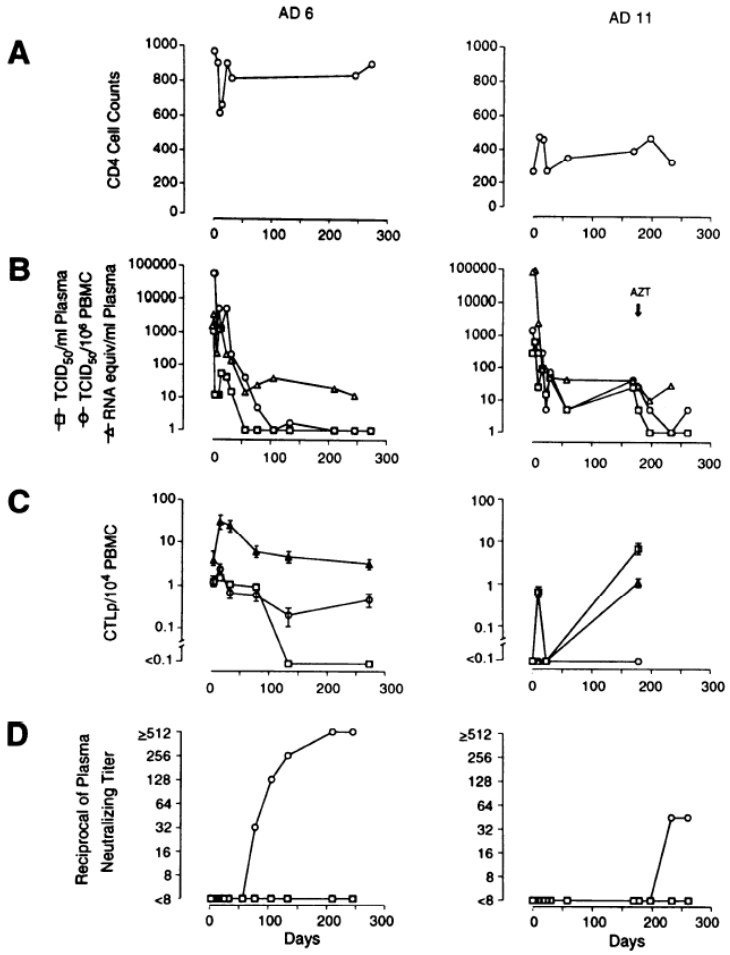


The CTL activities and the titles of neutralizing antibody were studied for 5 patients AD6, AD8, AD10, AD11 and AD13. Activities CTL were studied against viral proteins gag (round), pol (triangles) and env (square). The rates of neutralizing antibody were studied against 3 HIV stocks.

Question 1. Which experiment can one realize to determine the title in neutralizing antibody?

Question 2. Can you comment the results obtained? What is your analysis of immune response against HIV in this infection stage?

Patients AD6 and AD11 were more studied. At day 0, the patients presented the first symptoms and the seroconversion occurred at day 8 for AD6 and day 10 for AD11. T $CD4^+$ numbers (A), viral load in plasma (B), frequencies of anti-HIV CTL gag (round), pol. (triangles) and env (square) (C), and neutralizing antibody against 2 stocks of HIV (d) were studied.



Question 3. By comparing the results presented in this figure and the previous graphs of Figure 1, can you analyse the immune response during the HIV infection at the acute and chronic stages of the infection?