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Differential requirements for HIV-1 replication in naive and memory CD4 T cells from asymptomatic HIV-1 seropositive carriers and AIDS patients

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SUMMARY

One of the major routes for modulating HIV-1 expression by infected T cells is through the control of transcription initiation from the HIV-1 long terminal repeat (LTR), which is regulated either by its own viral gene products or by several cellular DNA-binding proteins induced during T cell activation. Previous work reported preferential HIV-1 infection and replication of memory CD4 T cells from infected individuals, which was explained either by a higher viral burden of this subset or by differences between naive and memory cells in the activation of the general transcription machinery involved in HIV-1 replication. In this work, we have studied HIV-1 replication by highly purified naive and memory CD4 T cells from asymptomatic seropositive carriers (ASC) and AIDS patients following different activation signals. Our results demonstrate that viral replication in memory cells from ASC was observed after mitogenic (phytohaemagglutinin (PHA) and/or phorbol myristate acetate (PMA)) recombinant tumour necrosis factor-alpha (rTNF-α) and CD3-mediated activation. In contrast, in naive subsets, early viral replication was almost exclusively observed upon CD3-mediated activation. AIDS patients are characterized by similar levels of viral replication in both subsets after PHA and soluble or immobilized anti-CD3 MoAb activation. However, naive subsets from AIDS patients still displayed differential requirements since they failed to replicate HIV-1 after treatment with PMA and rTNF-α. Taken together, these results provide evidence that HIV-1 replication in $CD4^+$ T cells from infected individuals is a function of the differentiation stage of the cells, the disease stage of the patient and the activation signal employed.

Keywords HIV-1 replication naive and memory CD4 T cells

INTRODUCTION

In AIDS, CD4-expressing cells, including T lymphocytes and cells of the monocyte/macrophage lineage, are selectively infected by HIV [1-4]. In addition, it has been shown that CD4 T cells represent the main cell type harboring HIV-1 among peripheral blood mononuclear cells (PMBC) of seropositive patients [5], though only small numbers of these cells have been demonstrated to be infected in a given patient [5-7].

A wide range of immunological abnormalities observed during HIV infection has been attributed to a selective dysfunction and destruction of the memory subsets of CD4 T cells, which have a higher viral burden than naive subsets [8] and preferentially replicate HIV after phytohaemagglutinin (PHA) stimulation [9]. Additionally, we have recently reported an important functional impairment of naive CD4 T cells concerning their proliferative and cytokine secretion abilities [10].

At present only partial data are available on the efficiency of both subsets from HIV-infected individuals in replicating HIV-1. In this study we analysed P24 (gag) antigen production in cultures of highly purified naive and memory CD4 T cells from asymptomatic seropositive carriers (ASC) and AIDS patients after activation signals associated or not to the reported functional impairments.

Our results point to differential requirements among naive and memory CD4 T cells from HIV-infected individuals in order to support HIV-1 replication, which depends not only upon the stimuli but on the disease status of the patient.

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PATIENTS AND METHODS

Study subjects

Six healthy blood donors, 11 HIV-1 ASC and five AIDS patients, were included in this study. Seropositive status was defined by the presence of serum antibodies to HIV-1 using an ELISA (ELAVIA I, Diagnostics Pasteur, Marnes la Coquette, France), and subsequently confirmed by Western blot (Lav-Blott, Diagnostics Pasteur).

The status of the patients studied in the present work is summarized in Table 1, depicting absolute and relative values of

Table 1. Immunological status of healthy donors and HIV-infected individuals

Patients	CD4 T cells (%)	CD4 T cells (absolute number/mm^3)	CD45RA^{high} (% of CD4 T cells)	CD45RA$^-$ (% of CD4 T cells)	CD29^{high} (% of CD4 T cells)	Serum p24 (pg/ml)	Treatment*
ASC group							
1	26	676	46·1	42·3	61·8	—	—
2	19	475	35·5	43·8	45·2	—	—
3	27	787	46·0	44·4	80·9	—	—
4	36	929	40·7	50·6	42·8	—	—
5	39	450	60·3	28·1	35·1	—	—
6	15	195	45·3	53·8	ND	—	AZT
7	20	509	33·0	57·0	43·4	—	AZT
8	13	326	21·9	65·6	53·1	—	—
9	43	553	27·4	64·3	31·3	—	AZT
10	38	1058	52·7	35·0	38·5	—	—
11	19	279	38·8	40·8	56·0	—	—
AIDS group							
1	16	54	18·9	65·5	71·7	—	AZT
2	7	82		49·4	49·4	425	AZT
3	15	181	38·1	42·4	57·1	—	AZT
4	15	123	20·1	37·5	ND	200	AZT
5	4	15	98·0	<2	98·0	—	AZT
Healthy donors (mean ± s.d.)	40·8 ± 5·3	807 ± 377	43·6 ± 9·3	45·8 ± 9·7	47·6 ± 14·3	—	—

* AZT, Patients receiving azydo-thymidine therapy.

CD4 T cells, levels of serum p24 antigen and treatment status for each patient. In addition, the percentage of CD45RA^{high}, CD45RA$^-$ and CD29^{high} cells within CD4 T cells for each patient is indicated. It should be noted that in patient AIDS-5, only double-positive cells were observed (CD45RA^{high}/CD29^{high}); thus, CD4 T cells from this patient were considered as memory or primed cells [11]. A second series of three control donors and three asymptomatic, previously untreated patients, all displaying >400/mm^3 CD4 T cells, was studied to better define the role of the different stimuli in supporting viral replication.

Isolation of the CD4 T cell subpopulations

PBMC were separated from heparinized venous blood by centrifugation over Ficoll-metrizoate density gradient (Lymphoprep, Nycomed, Oslo, Norway) and finally resuspended in 5% fetal calf serum (FCS) RPMI 1640 (Gibco, Grand Island, NY).

PBMC were partially depleted of monocytes/macrophages by plastic adherence for 1 h at 37°C. The adherent cells were recovered by scraping, and resuspended in RPMI 1640 culture medium with 10% pooled normal AB human serum. In some cases, these cells were used as a source of accessory cells (APC). which were irradiated at 30 Gy before culture.

For prepurification of CD4$^+$ T cells, non-adherent cells were incubated for 45 min at 4°C with saturating concentrations of the following MoAbs: anti-CD8, anti-CD19, anti-CD14 (Dakopatts AS, Denmark), anti-CD16 and anti-CD56 (Immunotech, Marseille, France). Cells were then washed twice and resuspended in 5% FCS RPMI 1640, containing the appropriate numbers of sheep anti-mouse-coated immunomagnetic beads (Dynabeads, Dynal, Norway), so as to achieve a 10:1 bead-to-cell ratio. After incubation for 90 min at 4°C, rosetted and non-rosetted cell fractions were separated by the application of a magnet to the outside of the tube for 3 min and the unbound cells, enriched for CD4 T cells, were carefully aspirated and washed twice in 5% FCS RPMI 1640. Then, CD4 T cells were incubated with FITC-labelled anti-CD4 MoAb and PE-labelled anti-CD45RA MoAb (Coulter, Hialeah, FL), and sorted on an EPICS 752 flow cytometer. Sorting was controlled by gating through a combination of forward angle and 90° light scattering to screen for lymphocytes, followed by restricted gates of fluorescence intensity to obtain the CD4$^+$ CD45RA^{high} and CD4$^+$ CD45RA$^-$ subsets, eliminating CD45RA^{low} and double positive cells.

Purity of the subsets was always evaluated at the end of sorting assays. In all the experiments described in this study, the purity of the cell preparations was >96% for both the CD4$^+$ CD45RA$^+$ and the CD4$^+$ CD45RA$^-$ subsets. Moreover, cell samples obtained after sorting were found to contain <1% of contaminating non-CD4 cells.

Additional experiments were performed in order to avoid any previous signal delivered by prior incubation with anti-CD4 and anti-CD45RA MoAbs. In these cases naive and memory CD4 T cells were isolated from enriched CD4 T cell fraction by a second round of binding to immunomagnetic beads using anti-CD45RO MoAb to negatively select naive subsets (CD45RO$^-$) and anti-CD45RA MoAb to negatively select memory cells (CD45RA$^-$). In this case, no sorting was performed and each of these cell fractions was more than 95% pure.

Culture conditions

Sort-purified CD4$^+$CD45RA^{high} and CD4$^+$CD45RA$^-$ cells derived from both healthy donors and HIV-1-infected individuals were cultured in RPMI 1640 supplemented with human recombinant IL-2 (rIL-2) at a final concentration of 50 U/ml (a kind gift of Roussel, UCLAF, France), 10% heat-inactivated pooled human AB serum (Jacques Boy SA, Paris, France),

glutamine 4 mM (GIBCO) and penicillin-streptomycin (GIBCO). Cultures were performed in 96-well flat-bottomed plates (Costar) at 2×10^4 cells/well, to which one of the following was added: PHA (Sigma, St Louis, MO) at a final concentration of 5 μg/ml, PMA (phorbol 12-myristate 13-acetate; Sigma) at a final concentration of 10 ng/ml, anti-CD3 MoAb (Dakopatts, Denmark) at a final concentration 1 μg/ml and recombinant tumour necrosis factor-alpha (rTNF-α) at a final concentration of 20 ng/ml (a generous gift of Dr F. Arenzana-Seisdedos, Unité d'Immunologie Virale, Institut Pasteur, Paris, France). As a control, sort-purified cells were cultured in rIL-2 containing culture medium at a final concentration of 50 U/ml.

In a second series of three normal control donors and three asymptomatic patients, naive and memory cells were negatively isolated as described above and stimulated with: (i) PHA or PMA + rIL-2 as above; (ii) PHA at 5 μg/ml + PMA at 10 ng/ml; (iii) sCD3 at 1 μg/ml + autologous APC at 2×10^4 cells/well; and (iv) sCD3 + PMA at 10 ng/ml. In the case of immobilized anti-CD3, negatively selected naive and memory cells as described above were incubated in 96-well plates precoated with anti-CD3 MoAb at 10 μg/ml.

Culture supernatants were collected every 4 days, frozen at −80°C for subsequent assays and replaced by fresh rIL-2 containing culture medium when indicated. Weekly restimulation was carried out for the cultures extended beyond 7 days. Cellular viability was checked by trypan blue exclusion test and the extension in time of the different cultures was established as a function of this parameter (< 15% of dead cells). Thus, cultures with PHA + IL-2 were prolonged until 35 days without significant cell death whereas cultures with soluble anti-CD3 plus rIL-2 could not be extended after 12 days. Whatever the stimulus employed, no differences could be found in terms of cell death between naive and memory cells, which never exceeded 15%.

^{3}H-thymidine incorporation assay

Purified CD4 T cell subsets derived from healthy donors and HIV-1-infected individuals were cultured in 96-well flat-bottomed plates at 2×10^4 cells/well in a total volume of 0·2 ml and stimulated as specified above. All cultures were performed in quadruplicate, and for each experiment a control containing the same number of cells cultured in the presence of culture media was performed.

At days 3 and 6 of cultures at 37°C in the presence of 5% CO_2, ^{3}H-thymidine incorporation was determined by adding 1 μCi of ^{3}H-thymidine (CEA, Paris, France) to each well for the last 8 h of culture. Plates were harvested with a PHD 200 cell harvesting system (Cambridge Technology, Cambridge, MA) and radioactivity was measured by means of a scintillation beta-counter. Results observed at day 6 are represented in tables as a mean of quadruplicate cultures.

HIV-1 antigen assay

Culture supernatants from cultured CD4 T cell subsets were taken every 4 days and frozen at −80°C until assayed. Aliquots of 0·1 ml were thawed and assessed for levels of p24 antigen, which was used as a measure of viral replication, by means of a solid-phase antigen-capture ELISA (ELAVIA 1, Diagnostics Pasteur), which detects mainly p24 antigen. This assay was performed as specified by the manufacturers. A standard curve derived from known p24 antigen dilutions was used for quantitative transformation of optical density data. The lower detection limit of the test is 50–70 pg/ml, whereas we considered as positive those results exceeding 75 pg/ml. Quantitative values were expressed as follows: < 200 pg/ml (+), 200–500 pg/ml (++), 500–1000 pg/ml (+++) and values higher than 1000 pg/ml (++++).

Statistical analysis

The non-parametric two-sided Wilcoxon–Mann–Whitney rank test was used for statistical comparison of ^{3}H-thymidine uptake between patient groups, and the Spearman rank correlation between ^{3}H-thymidine uptake and p24 antigen levels.

RESULTS

Upon PHA plus rIL-2 stimulation, HIV-1 preferentially replicated in the memory subset of the ASC group, whereas comparable levels in both subsets from AIDS patients were observed

After PHA plus rIL-2 stimulation, high levels of p24 antigen production, beginning at day 6 and achieving maximal values at day 11, were detected in memory subsets of 7/11 ASC patients (Table 2). Viral replication was never observed when isolated naive and memory CD4 T cells from ASC and AIDS patients were cultured in the exclusive presence of rIL-2, which was used as a control (data not shown). In contrast, naive subsets from ASC patients remained negative for p24 production, for at last 2 weeks and in most cases for 3 weeks of culture. Nevertheless, when cultures of naive CD4 T cells were continued for an additional period (from day 21 to day 35), high production of p24 was observed in 5/9 ASC patients studied, irrespective of p24 production in respective memory subsets. Interestingly, for one ASC patient (ASC-2) who failed to produce p24 antigen in memory cells, high levels of p24 could be detected in naive cells beyond 21 days of culture.

In contrast, PHA induced high levels of p24 antigen in both subsets from all AIDS patients studied, which was detected earlier (day 4) than the respective subsets from ASC patients.

A selective decrease in the proliferative capacities of naive subsets from ASC as well as AIDS patients was observed (Table 2), but no significant correlation between ^{3}H-thymidine incorporation and p24 production could be demonstrated.

Preferential viral replication in memory CD4 T cells from ASC and AIDS patients after PMA or TNF-α plus rIL-2 stimulation

It has been well established that phorbol esters and rTNF-α efficiently induce HIV-1 replication in several '*in vitro* systems' [12–17].

However, the capacities of these stimuli to induce HIV-1 production in freshly purified naive and memory CD4 T cell subsets from ASC and AIDS patients have not yet been investigated. In cultures with PMA plus rIL-2, high levels of p24 production were almost exclusively observed in memory subsets from four out of 11 ASC and in all AIDS patients, with values exceeding 1000 pg/ml (Table 3). In contrast, naive subsets of both groups remained negative for p24 detection up to 21 days of culture except for naive cells from one out of four AIDS patients studied.

A selective decrease in the proliferative responses of naive subsets from ASC patients was observed when compared with healthy donors, and no significant correlation with p24 production could be demonstrated.

Table 2. ^{3}H-thymidine incorporation and p24 antigen production by CD4 T cell subsets from HIV-infected individuals after phytohaemagglutinin (PHA) + rIL-2 stimulation*

	Naive (CD45RA$^+$) CD4 T cells				Memory (CD45RA$^-$) CD4 T cells			
	^{3}H-thymidine incorporation	Levels of p24 antigen† (days of culture)			^{3}H-thymidine incorporation	Levels of p24 antigen (days of culture)		
Patients	(ct/min × 10^{-3})	4–12	13–21	22–35	(ct/min × 10^{-3})	4–12	13–21	22–35
ASC group								
1	79·5	–	+++	++++	65·9	++++	++++	++++
2	32·8	–	–	++++	24·0	–	–	–
3	101·2	–	–	++++	44·0	++	++++	++++
4	50·6	–	–	++++	74·8	++++	++++	++++
5	69·9	–	–	ND	84·8	–	–	–
6	5·3	–	++	++++	5·1	++++	++++	++++
7	95·7	–	–	ND	46·1	++++	++++	++++
8	69·3	–	–	–	96·5	++++	++++	++++
9	68·8	–	–	–	35·3	–	–	–
10	14·9	–	–	–	54·2	–	–	–
11	31·0	–	–	–	40·8	+++	+++	–
AIDS group								
1	105·1	++++	++++	++++	73·3	++++	++++	ND
2	ND	++++	++++	ND	ND	++++	++++	ND
3	26·3	+++	++++	ND	38·3	++++	++++	ND
4	58·6	++++	++++	ND	32·5	++++	++++	ND
5					ND	++++	++++	ND
Healthy subjects (±s.d.)	120·5±20·2	–	–	–	40·8±9·4	–	–	–

* Cells (2×10^4) were cultured in the presence of PHA (5 μg/ml) plus rIL-2 (50 U/ml), and thymidine uptake was measured at day 6. Data are expressed as a mean of quadruplicate samples in ct/min × 10^{-3}. As a control, cultures in the presence of rIL-2 alone were performed for each group and values were always less than $0{\cdot}38 \times 10^{-3}$ ct/min.

† Levels of p24 antigen are expressed as follows: < 200 pg/ml (+); 200–500 pg/ml (++); 500–1000 pg/ml (+++); and > 1000 pg/ml (++++).

ASC, Asymptomatic seropositive carrier; ND, not done.

In the presence of TNF-a, p24 production was observed exclusively in memory subsets from both groups of patients, though lower values of p24 antigen ranging from 75 to 650 pg/ml were obtained. In these cases, detectable p24 levels were achieved in the absence of significant ^{3}H-thymidine incorporation (Table 3).

In contrast to mitogens, the CD3-mediated stimulation pathway efficiently induced viral replication in naive cells

As a first step, we examined p24 production by naive and memory cells in the presence of sCD3 plus rIL-2. Significant levels of p24 antigen ranging from 75 to 750 pg/ml were observed exclusively in naive CD4 T cell subsets from five out of 11 ASC patients, whereas in one case (ASC-3) both naive and memory cells were capable of replication (Table 4). In contrast, when soluble anti-CD3 plus rIL-2 stimulation was performed in cultures from AIDS patients, high levels of p24 antigen were observed early (after 4 days of culture) in culture supernatants of memory cells of four out of five AIDS patients and in naive subsets of two out of four AIDS patients studied (Table 4).

In contrast to ASC patients, most AIDS patients showed significant values of ^{3}H-thymidine incorporation, predominating in naive subsets when compared with healthy donors. However, as shown in Table 4, no clear correlation between ^{3}H-thymidine incorporation and p24 production was observed.

In order to analyse the effect of other accessory signals (PMA or accessory cells) in CD3-induced p24 production and to exclude any previous signal that could be delivered by previous incubation wth CD4 and CD45RA, naive and memory cells from three additional asymptomatic patients were obtained by negative selection (Table 5).

Results shown in Table 5 indicate that all stimuli efficiently induced p24 production in memory subsets, whereas in the respective naive subsets this could only be observed with soluble anti-CD3 plus autologous accessory cells or immobilized CD3. In contrast, no p24 production was detected when these cells were stimulated by PHA + PMA or by PMA + sCD3, despite the fact that both stimuli induced significant thymidine incorporation (data not shown). The increased values of p24 production by CD4 cells in the presence of autologous irradiated APC were not explained by viral secretion of autologous APC, since controls including the same APC with sCD3, in the absence of CD4 cells, failed to produce p24 antigen.

DISCUSSION

In this work, we studied the ability of naive and memory CD4 T cell subsets from asymptomatic HIV-1-infected and AIDS patients to support HIV-1 replication following different activation signals.

Table 3. ^{3}H-thymidine incorporation and p24 antigen production by CD4 T cell subsets from HIV-infected individuals in the presence of phorbol myristate acetate (PMA) + rIL-2 and tumour necrosis factor-alpha (TNF-α) + rIL-2*

	Naive (CD45RA$^+$) CD4 T cells				Memory (CD45RA$^-$ CD4 T cells			
		Levels of p24 antigen†				Levels of p24 antigen		
Patients	^{3}H-thymidine incorporation (ct/min × 10^{-3}) for PMA	PMA 4–12 days	PMA 13–21 days	TNF-α, 6 days	^{3}H-thymidine incorporation (ct/min × 10^{-3}) (for PMA)	PMA 4–12 days	PMA 13–21 days	TNF-α, 6 days
ASC group								
1	12·8	−	−	−	13·0	++++	++++	+++
2	11·2	−	−	−	5·3	−	−	−
3	13·0	−	−	−	4·1	++++	++++	+++
4	6·6	−	−	−	8·2	−	−	++
5	19·7	−	−	−	2·7	−	−	−
6	1·4	−	−	−	1·5	++	+++	+
7	9·6	−	−	−	1·0	−	−	−
8	5·6	−	−	−	0·6	++	++++	++
9	11·0	−	−	ND	23·7	−	−	ND
10	11·0	−	−	ND	10·1	−	−	ND
11	8·7	−	−	ND	6·5	−	−	ND
AIDS group								
1	40·6	−	−	−	5·8	++++	++++	++
2	ND	++	+++	−	ND	++++	++++	−
3	20·8	−	−	−	7·7	++++	++++	++
4	17·8	−	−	−	8·7	++++	++++	+
5					ND	++++	++++	+++
Healthy subjects ± s.d.	23·1 ± 0·2	−	−	−	8·9 ± 5·8	−	−	−

* Cells (2×10^4) were cultured in the presence of PMA (10 ng/ml) or TNF-α (20 ng/ml) + rIL-2 (50 U/ml), and thymidine uptake was measured at day 6. Data are expressed as a mean of quadruplicate samples in ct/min × 10^{-3} (results of thymidine uptake for TNF-α were not significant and omitted in table).

† Controls and p24 antigen expression are the same as for Table 2.

ASC, Asymptomatic seropositive carrier; ND, not done.

Our results following PHA + rIL-2 stimulation point to preferential HIV-1 replication in memory subsets from ASC patients, although in the AIDS group, kinetics and values of p24 production were similar when we compared the two subsets during the first 3 weeks of culture.

A recent report by Schnittman *et al.* [8] lends supporting evidence to a preferential infection of memory subsets (four to 10-fold viral DNA in memory CD4 T cells) in HIV-1-infected individuals. Our results with PHA + IL-2 tend to agree with the report of these authors. According to them, early replication in memory cells could be explained by a greater burden of HIV-1 in this subset, and the late replication observed in naive cells may be due to the fact that more cycles of reinfection are needed to enable detection of HIV.

It has been reported that soluble anti-CD3 MoAb is capable of inducing transient but significant Ca^{++} mobilization [18], phosphatidyl inositol phosphate hydrolysis [19] and protein kinase C activation [20] in purified resting T cells. When this minimal activation signal through the CD3 pathway was used (sCD3 + rIL-2), we observed moderate viral replication almost exclusively in naive CD4 T cell subsets from six out of 11 ASC patients, with a virtual absence of ^{3}H-thymidine incorporation in most patients. These results suggest differential requirements to replicate HIV-1 irrespective of full activation, proliferation or the existence of several reinfection cycles. Further evidence is provided by results obtained with the three additional ASC patients studied, in which a preferential viral replication in naive cells was observed when stimulated through the CD3 pathway, as compared with the failure to induce early viral replication when naive cells are stimulated by PHA + PMA or by PMA + sCD3 (known to induce efficiently cell proliferation and viral replication). Results observed for AIDS patients confirm the differential capacity of naive and memory cells to support viral replication, since replication of HIV-1 in naive subsets could not be obtained for these patients through stimulation by PMA or rTNF-α, with the exception of one patient in whom low levels of virus production were obtained with PMA. However, in contrast to ASC patients, high p24 antigen levels were detected early in culture supernatants from both subsets of AIDS patients when PHA and soluble anti-CD3 MoAb were employed. In this respect, an increased number of infected cells and higher rate of viral replication associated with this stage of disease could explain these results [21,22].

PMA and TNF-α have been shown to mediate induction of viral replication through the NFkB enhancer, though it was recently demonstrated that the presence of this enhancer is not essential [23]. In light of present results, the possibility exists that naive cells are unable to induce functional NFkB binding sites

Table 4. ^{3}H-thymidine incorporation and p24 antigen production by CD4 T cell subsets from HIV-infected individuals after soluble anti-CD3 MoAb plus rIL-2 stimulation*

	Naive (CD45RA$^+$) CD4 T cells			Memory (CD45RA$^-$) CD4 T cells		
Patients	^{3}H-thymidine incorporation (ct/min × 10^{-3})	Levels of p24 antigen† (days of culture) 4–7	8–14	^{3}H-thymidine incorporation (ct/min × 10^{-3})	Levels of p24 antigen (days of culture) 4–7	8–14
ASC group						
1	0·5	−	−	1·4	+	−
2	0·6	++	−	0·3	−	−
3	9·2	+++	++++	0·7	+	−
4	2·2	−	−	7·1	−	−
5	0·4	−	−	0·5	−	−
6	1·8	−	−	0·9	−	−
7	0·9	+	++	1·0	−	−
8	0·4	+	+	0·5	−	−
9	0·6	++	+++	1·2	−	−
10	0·7	−	−	0·6	−	−
11	0·5	++	+	0·7	−	−
AIDS group						
1	38·2	++++	++++	0·3	−	−
2	ND	−	−	ND	++++	++++
3	3·6	−	−	1·2	++++	++++
4	2·7	++++	++++	0·4	++++	++++
5				ND	++++	++++
Healthy subjects ±s.d.	0·35±0·2	−	−	0·26±0·2	−	−

* Cells (2×10^4) were cultured in the presence of soluble anti-CD3 MoAb (1 μg/ml) plus rIL-2 (50 U/ml), and thymidine uptake was measured at day 6. Data are expressed as a mean of quadruplicate samples in ct/min × 10^{-3}.

† Controls and p24 antigen expression are the same as for Table 2.

Table 5. p24 antigen production by CD4 T cell subsets from HIV-infected individuals after treatment with immobilized anti-CD3 MoAb and different costimulatory signals for soluble anti-CD3 (accessory cell (APC) or phorbol myristate acetate (PMA)) and for phytohaemagglutinin (PHA) (PMA) stimulation*

		Soluble anti-CD3		Mitogens			
Patients	CD4$^+$ T cell subpopulation	+APC†	+PMA	PHA-PMA	PHA+rIL-2	PMA+rIL-2	Immobilized anti-CD3
ASC-12	Naive	++++	−	±	−	−	+
	Memory	++++	++++	++++	+++	+++	+++
ASC-13	Naive	++++	−	−	−	−	+
	Memory	+++	++	+++	++	++	++
ASC-14	Naive	++	−	−	−	−	+
	Memory	−	++	++	++	−	−

* Cells (2×10^4) were cultivated in triplicate with soluble anti-CD3 MoAb (1 μg/ml) in addition to APC (2×10^4) or PMA. PHA and PMA were always used at 5 μg/ml and 10 ng/ml, respectively. For immobilized CD3 stimulation, cells were negatively selected and cultivated in CD3 MoAb precoated plates as described in Patients and Methods. Supernatants for p24 assays were harvested at day 5 and 8, and only maximal values obtained are depicted. Expression of p24 values is the same as for Table 2.

† Control cultures with APC+sCD3 in the absence of CD4 cells remained negative for p24 expression.

and that other cis-acting sequences in addition to NFkB could be involved in inducing viral replication. In this respect, recent work [24] reported an anti-CD3-induced sequence-specific DNA-binding protein which recognizes an upstream regulatory element on HIV-2 long terminal repeat (LTR). In addition, human CD4 T cell clones have been reported to induce HIV-enhancer-dependent transcription after PMA stimulation, but not after rTNF-α and anti-CD3 treatment, suggesting different requirements for NFkB-mediated HIV replication between tumoral T cell lines and human CD4 T cell clones [12]. Moreover, it cannot be excluded that requirements for freshly purified CD4 T cell subsets from HIV-1-infected patients differ from those of lymphoblastoid T cell lines and human CD4 T cell clones.

As shown in Tables 2–5, if we analyse the results observed with the different stimuli employed, p24 antigen could be detected in all supernatants from naive subsets except for one ASC patient (ASC-10), indicating that naive cells harbored virus before cultures. However, it can be argued that differential requirements for viral replication observed between naive and memory cells reflect an early cell death of infected cells, depending on the stimuli. In this context, an active cell death process by apoptosis in CD4 T cells from infected patients has recently been reported, after PWM and superantigens [25] or CD3 MoAb [26]. However, in our experimental conditions cell viability was tested (trypan blue exclusion test) and no significant cell death was observed. These differences could be partially explained by the use of rIL-2 in most cultures (which is known to inhibit apoptosis) or use of other stimulation conditions (pokeweed mitogen (PWM) or superantigen).

In agreement with recent reports [8,9], detectable levels of p24 antigen in naive CD4 T cells from ASC patients were never observed before completion of phenotypic conversion of $CD45RA^{high}$ cells into $CD45RA^{-}$ cells. It can be argued that viral replication occurs only after naive cells have at least partially switched to memory cells and as viral burden is lower in naive cells [8], detection of p24 antigens in supernatants probably requires more cycles of reinfection. Since cell stimulation is necessary to obtain viral replication and since it invariably results in a switch from naive to memory cells, which in HIV-1-infected CD4 cells does not differ from normal CD4 cells [9], a definitive answer to this question cannot be provided.

The transactivation mechanism of HIV replication, governed through viral and cellular proteins acting on LTR, is only partially known. It can be hypothesized that naive cells, considered as virgin cells, have a differential regulatory mechanism compared with primed or memory cells, thus explaining the differential replication of HIV observed in the two subpopulations. The fact that these different requirements appear to be less stringent in AIDs patients might favour the idea that some down-regulatory mechanisms are lost when patients reach the AIDS stage. However, it cannot be excluded that the remaining CD4 T cells in AIDS could represent a selected subpopulation of CD4 T cells with a greater capacity for infectivity or viral replication.

Overall, these results suggest preferential replication of the HIV-1 virus in CD4 memory cells. This subset is known to display a higher viral burden and appears to replicate HIV when stimulated through the mitogenic or TCR pathway, as opposed to the preferential ability of naive cells to replicate virus upon CD3/TCR mobilization. Since a higher viral burden has been claimed to exist in memory cells, the non-cycling memory cells may constitute a reservoir of latently infected cells which, upon either polyclonal or antigen-specific activation, replicate the virus. On the other hand, naive cells appear to replicate virus through an antigen-dependent pathway. Activation resulting from antigen-specific stimulation of these clones might result in cell activation, viral replication and a switch of these cells to a memory phenotype, which could then be prone to replicate virus when stimulated through an antigen-specific or polyclonal pathway. This may lead to deletion of this clone and could explain the defective responses to recall antigens reported in these patients. If correct, this would have important therapeutic implications, by favouring treatments aimed at down-regulating immune responses so as to slow viral replication and subsequent CD4 destruction, and by discouraging treatments that would reactivate latently infected cells.

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