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HIV-induced deletion of antigen-specific T cell function is MHC restricted

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SUMMARY

When antigen-specific T cells are pulsed by antigen-presenting cells (APC) in the presence of HIV they are functionally deleted following subsequent exposure to syngeneic APC in the absence of HIV. Recombinant soluble HIV envelope (gp120) is able to induce a similar effect which, unlike that induced by HIV, is reversible. Neither HIV nor gp120 affect the ability to respond to IL-2. Thus it is only antigen-specific responses involving the T cell receptor pathways and CD4/MHC class II interaction that appear to be inhibited by HIV-1 and gp120. Furthermore, the functional impairment caused by HIV-1 is specific to the T cells that respond to the antigen in co-culture with HIV, as there is no apparent effect on 'bystander'-activated T cells specific for another antigen. Antigen-specific T cell lines may be deleted by a signalling mechanism which involves molecules other than gp120/CD4 but still requires MHC class II restriction.

Keywords HIV gp120 antigen presenting cells pathogenesis MHC restriction

INTRODUCTION

The immunodeficiency seen in HIV infection is specific in that defects in antigen-specific responses can be measured long before a decline in CD4 cell numbers has occurred [1–4]. These selective defects take place against a background of generalized immune activation of both T and B cells [5] which are still able to recognize and initiate strong immune responses to novel antigens including those of HIV [6]. Indeed, there is no quantitative defect in the humoral response to the viral antigens. We and others have demonstrated the selective interference of antigen-specific responses *in vitro* by showing that the presence of HIV, or gp120, alone prevents the stimulation of specific T cell lines during challenge with the appropriate antigen [7]. This interference appears to be CD4/MHC class II dependent and does not involve other activation pathways. As antigen-specific recognition by $CD4^+$ T cells occurs only in the context of self MHC class II molecules interacting with the T cell receptor complex [8], we have studied whether or not HIV functional deletion of T cell responses is also MHC restricted.

Antigen-presenting cells (APC) are readily infectable with HIV and may constitute a reservoir for HIV [9,10]. As very few CD4 cells (1 in 10^4–10^5) are infected in the early stages of HIV disease [11] and activation facilitates infection and virus replication [12,13], the selective defects seen in HIV infection may be due to the specific interaction between APC, antigen and T cells.

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Here we show that the ability of HIV to inhibit T cell responses is not only antigen specific but also MHC class II restricted.

MATERIALS AND METHODS

Media and antigens

RPMI 1640 (Flow Laboratories, Irvine, UK) was used during the generation and maintenance of T cell lines and for experiments. The medium was supplemented with L-glutamine 2 mM, 2-mercaptoethanol 5×10^{-5} M and 5% autologous plasma. For co-culture experiments, in which cells from donors F and D were stimulated together, 5% AB serum was used in the medium. Tetanus toxoid (TT) was purchased from the Massachusetts Health Laboratory (Boston, MA) and PPD from Statens Seruminstitut (Copenhagen, Denmark). HIV gp120 was a recombinant protein produced in CHO cells. Batch 2.7.68/66 was provided by Celltech (Slough, UK) through the MRC AIDS directed programme as an immunoaffinity purified preparation in 20 mM HEPES buffer, pH 7. Soluble CD4 was obtained from Smith Kline and French (King of Prussia, PA). Monoclonal anti-Leu-3a was provided by Beckton Dickinson (Palo Alto, CA).

T cell lines and antigen-presenting cells

T cell lines specific for TT and PPD (for the experiments with soluble gp120) and adherent cells were prepared as previously described [13,14]. Briefly, peripheral blood lymphocytes (PBL) were repeatedly stimulated with alternate cycles of antigen stimulation in the presence of autologous irradiated PBL as a

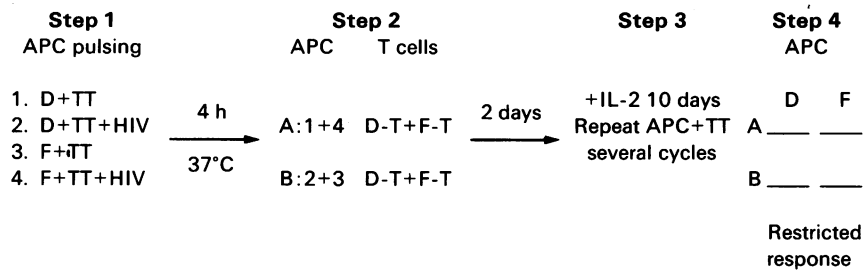

Fig. 1. The experimental protocol is illustrated in four steps. Step 1: pulsing of antigen-presenting cells (APC) with antigen, in the presence or in the absence of HIV; step 2: co-culture of APC (with or without HIV) with T cells from the two donors; step 3: expansion of co-cultured cells with IL-2 and with repeated cycles of antigen restimulation in the presence of irradiated peripheral blood lymphocytes (PBL) as APC; step 4: assay for phenotypic characteristics and functional response of T cells from the two donors. TT, tetanus toxoid.

source of APC, and for IL-2 expansion. T cell lines were generated from two donors, F (HLA type A2, 2; B5, 7; DR2 W6) and D (HLA type A3, 28; B12, 14; DR1,7). The two donors have diferent DR antigens and functional testing confirmed that there was no cross-presentation of TT.

Antigen-dependent T cell activation was measured both as IL-2 production and as proliferative response. IL-2 production was assayed by adding 0·1 ml spent medium from cultures containing 2×10^4 T cells and 10^5 autologous irradiated PBL incubated for 48 h to 10^4 phytohaemagglutinin (PHA) human blasts as IL-2 responsive indicator cells in 0·1 ml medium. After 1 day the indicator cells were pulsed with 0·5 μCi tritiated thymidine (4–5 Ci/mmol, Amersham, Amersham, UK) for 4 h, harvested and counted. Proliferative response was measured in cultures containing 2×10^4 T cells and 10^5 autologous irradiated PBL in the presence or in the absence of antigen. Two days later the cultures were pulsed and harvested as described above.

Experimental protocol

A co-culture experiment using two T cell line-APC systems which are functionally independent of each other by nature of their differing antigen-specific responses has been previously described [7]. Here only one antigen is used, and in the co-culture system T cell lines and APC were derived from two different donors. The experimental protocol is shown in Fig. 1. Adherent APC were pulsed with antigen (TT) in the absence or presence of HIV (strain HTLV-IIIB) at 100 TCID50 at 37°C for 4 h (step 1). After three washes to remove soluble antigen and free virus, APC were mixed with specific T cells as shown in Fig. 1, step 2 (co-cultures A and B). Co-cultures were then further expanded with IL-2 for 10 days. Three cycles of antigen-specific stimulation with irradiated normal PBL were performed during IL-2 expansion (step 3) before final testing for antigen-specific responses to TT presented by APC from either donor D or donor F (step 4). Virus replication was sought by daily observation looking for cytopathic effects and by performing the p24 antigen assay (Dupont) every 5–7 days.

Cytofluorometric analysis

T cells (2×10^5 cells/well) were stained with anti-Leu-3a at various concentrations in U-bottom microtitre plates for 30 min at 4°C. Cells were washed twice and pellets were resuspended in 10 μl goat Fab 2 anti-mouse IgG (Fluorescence conjugate 1/40 final dilution; Clonetech, Palo Alto, CA). After 30 min at 4°C the cells were washed twice and resuspended in phosphate-buffered saline (PBS) azide. The cells were analysed in a Becton Dickinson FACScan. Binding indicates the per cent of $CD4^+$ cells relative to background fluorescence of cells incubated with fluorescence antibody only, in the absence of anti-Leu-3a. Relative fluorescence intensity was determined as described by Leiserson [15].

Complement-dependent microlymphocytotoxicity test

This was performed as described [16]. Briefly, 1 μl lymphocytes at 2×10^6/ml were dispensed in a Terasaki microplate (Sterilin, Hounslow, UK) in wells containing 1 μl specific anti-HLA A2 or A3 antiserum (Fresenius AG, Münich, Germany). After 30 min at room temperature, 5 μl formalin (37% solution, pH 7·2) were dispensed in the well. Percentage of dead cells (those that have incorporated the dye) was evaluated under a phase contrast microscope. Eighty per cent killing or higher was scored as a positive reaction.

RESULTS

F and T lines specific for TT use different non-shared restriction elements

Donors F and D used to generate the two TT-specific T cell lines have different DR antigens. Accordingly we predicted no cross-stimulation by using APC from one individual and T cells from the other. Antigen-dependent T cell activation was measured by proliferation and by IL-2 production. The lack of cross-

Table 1. Lack of cross-presentation by different donor cells

T cell line	DR alleles	APC		Readout
F	2,w6	28 (3)	5 (4)	Proliferation
		16 (1)	2 (1)	IL-2 production
D	1,7	2 (2)	22 (3)	Proliferation
		1 (1)	24 (2)	IL-2 production

Effect of autologous and allogeneic antigen-presenting cells (APC) on activation of tetanus toxoid (TT)-specific T cell lines, measured as proliferative response of specific T cells and as IL-2 production by specific T cells, in the presence or in the absence (in parentheses) of antigen. Results are expressed as ct/min $\times 10^{-3}$ thymidine incorporation by T cells (proliferation assay) or by IL-2 responsive indicator phytohaemagglutinin (PHA) blasts (IL-2 production assay) as described in Materials and Methods.

Table 2. Selective functional loss of antigen-specific T cells is due to presentation of antigen by autologous antigen-presenting cells exposed to HIV

	Proliferative response				Phenotype	
	D ($A3^+$)		F ($A2^+$)		CDC with anti-HLA	
APC						
TT	−	+	−	+	A3	A2
Co-culture						
A	1·1	21·2	1·0	1·1	5%	95%
B	1·0	1·1	1·0	19·9	95%	5%

Co-cultures A and B (see Fig. 1) only respond in the presence of antigen presented by syngeneic and not allogeneic APC. T cell activation was measured as IL-2 production, and results indicate thymidine incorporation (ct/min $\times 10^{-3}$) by the IL-2-responsive phytohaemagglutinin (PHA) blasts used as indicator cells. The phenotypic characterization of T cells in co-cultures A and B was performed by using anti-HLA A2 and A3 antibodies in a conventional complement-dependent microlymphocytotoxicity (CDC) in Terasaki microplates [16].

Table 3. Blocking of inhibitory activity of anti-CD4 Leu-3a by incubation with soluble CD4

HIV antigen	−	+	+	+
Anti-CD4 (1 $\mu g/l$)	−	−	+	+
SCD4 (50 $\mu g/l$)	−	−	−	+
T line				
F-PPD	2±0·5	38±4 (0%)	4±0·2 (90%)	35±4 (8%)
F-TT	3±0·9	56±2 (0%)	9±0·6 (84%)	51±5 (9%)

PPD and tetanus toxoid (TT)-specific T cells (2×10^4) were stimulated with antigen in the presence of irradiated autologous peripheral blood lymphocytes (PBL) (5×10^4). Anti-Leu-3a at 1 μg/ml inhibited antigen-dependent proliferation, an effect which was abolished by pre-incubation of anti-Leu-3a with soluble CD4 at 50 μg/ml. Results are shown as ct/min $\times 10^{-3}$ thymidine incorporation ± s.d. Per cent inhibition of antigen-dependent proliferation is in parentheses.

presentation was confirmed functionally, as shown in Table 1. Therefore F and D lines, even if co-cultured, represent two functionally independent systems. The data also demonstrate that F does not have cells alloreactive to D in the TT-specific lines and *vice versa*.

Selective functional loss of antigen-specific cells

Following three cycles of TT antigen plus IL-2 stimulation, co-cultures A and B (see Fig. 1) were tested for response to TT presented by D or by F APC. T cell activation was evaluated as IL-2 production measured on IL-2-responsive PPD-specific human T cell blasts. Table 2 shows that co-culture A responded to TT presented by D APC only, suggesting that the T cells functionally detectable were of D origin. On the contrary, co-culture B responded to antigen presented by F APC, only.

The non-responsiveness to either APC could either be due to a functional deletion of the T cells which persist in the culture or to a physical loss of such T cells. A phenotypic analysis was

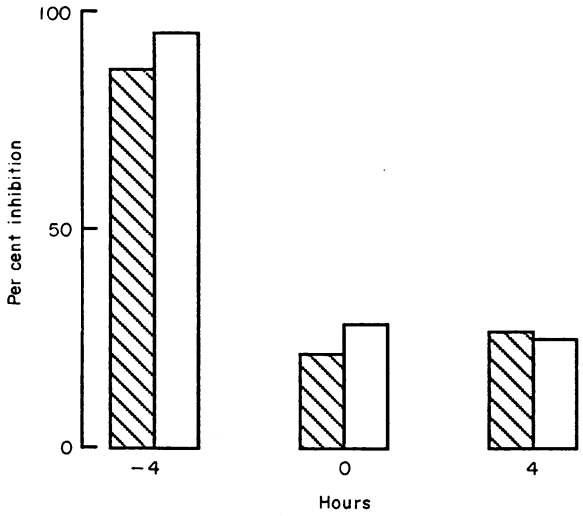

Fig. 2. Gp120 at suboptimal concentration for inhibitory activity (10 μg/ml) added at the initiation of the culture containing T cells, antigen-presenting cells (APC) and soluble antigen (time 0) or 4 h later (time +4) shows low inhibitory activity. Higher inhibitory activity of the same concentration of gp120 is observed when pre-incubated for 4 h with specific T cells, before addition of APC and antigen (time −4). Results are shown as per cent inhibition of maximal proliferative response of PPD specific line (▨) and of TT specific line (□).

therefore carried out on the co-cultures. A conventional complement-dependent microlymphocytotoxicity test (CDC) was carried out to quantify T cells from line D (HLA $A3^+$, $A2^-$) and from line F (HLA $A2^+$, $A3^-$). Table 3 also shows that in co-culture A T cells bearing the A3 antigen (95% killing) represent the large majority. The opposite is true for co-culture B. This indicates a physical loss of the T cells that are syngeneic to the APC pulsed with HIV as well as TT antigen.

Inhibitory activity of HIV gp120 on antigen-driven proliferation

The above data suggest that T cells responding specifically to TT antigen presented by APC exposed to TT and to HIV are functionally and physically deleted. By-stander T cells of an independent system are not affected. This could be explained by the effects of HIV replication after the transfer of HIV from infected APC to specific T cells which could not be demonstrated here. An additional mechanism by which HIV can interfere with the antigen-driven activation of $CD4^+$ T helper cells is by binding of gp120 either as a surface component of viral particles, or in a soluble form, to CD4. This has been extensively shown in the recent literature and reviewed by us [6]. The inhibitory activity of gp120 depends on its CD4 binding capacity and on an interaction with specific T cells rather than with APC during antigen pulsing (Fig. 2). When added to cultures containing specific T cells and antigen-pulsed APC at the initiation of the culture or 4 h later a subinhibitory dose of 10 μg/ml gp120 gives only a 20% inhibition, whereas when given to T cells 4 h before the addition of pulsed APC, 10 μg/ml gives a 90% inhibition of the response seen in the absence of adding gp120.

Figure 3 shows directly that gp120 does not interfere with the pulsing of APC with antigens such as PPD and TT, confirming that the main target of inhibitory activity by gp120 is the $CD4^+$ T helper cell.

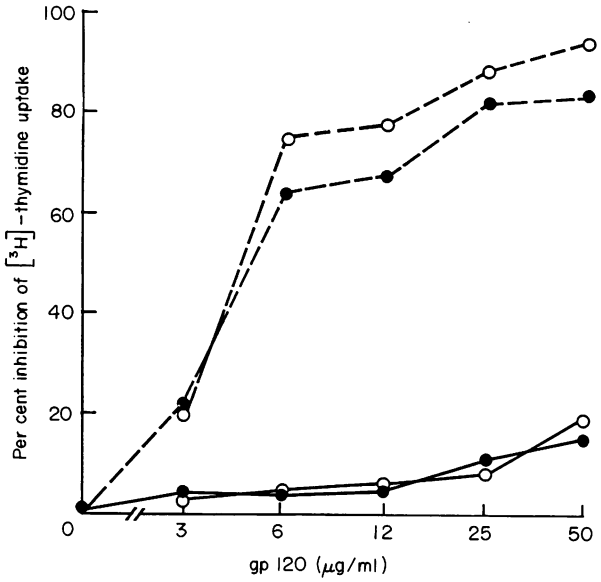

Fig. 3. Effect of gp120 on antigen pulsing of antigen-presenting cells (APC). APC (autologous inactivated peripheral blood lymphocytes (PBL)) were pulsed with PPD or with tetanus toxoid (TT) in the presence of gp120 at various doses (106 cells in 0·1 ml medium for 3 h at 37°C). After two washings, 2·5 × 104 APC were used to stimulate 2 × 104 T cells specific for PPD or for TT. Per cent inhibition is shown when gp120 was present during antigen pulsing only (——) or present in the proliferation assay (----). ●, FT-PPD; ○, FT-TT.

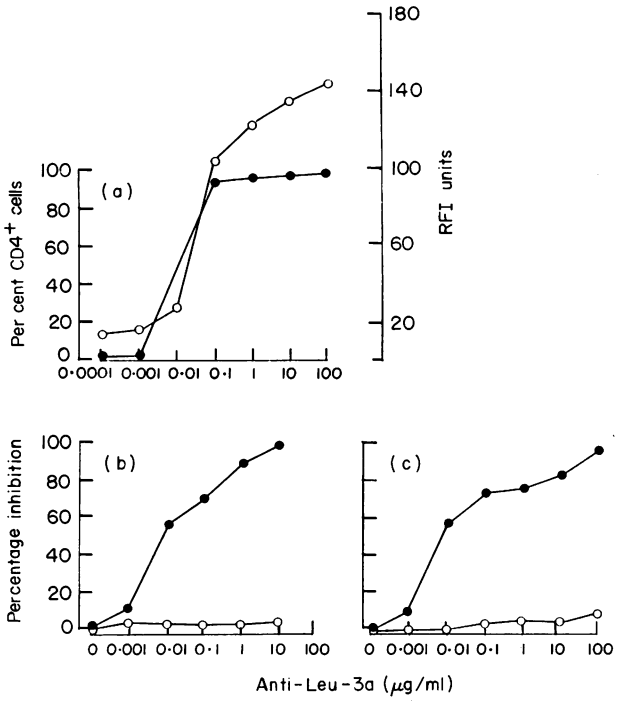

Fig. 4. Binding and inhibitory activity of anti-Leu-3a antibody. (a) Per cent CD4+ tetanus toxoid (TT)-specific T cells (left ordinate, ●) from the IL-2 expanded TT-specific line stained with anti-Leu-3a in an indirect immunofluorescence assay. Relative fluorescence intensity (RFI) is also shown (right ordinate, ○). T cells from the PPD-specific line exhibited an overlapping staining pattern. Cytofluorimetric analysis was performed with a Becton Dickinson FACS analyser. (b) and (c) Per cent inhibition of proliferative response to antigen or to IL-2 of the PPD- and the TT-specific T lines respectively, in the presence of graded concentrations of anti-Leu-3a antibody in culture wells, described under Materials and Methods.

Similar modes of action of anti-CD4 reactants

Similarities between the mode of action of soluble gp120 and another CD4 binding reactant (anti-Leu-3a) have been previously noted [7]. Therefore the interference with antigen-driven activation of T cells in culture by different doses of anti-Leu-3a was tested. Figure 4 shows that the inhibitory activity of anti-Leu-3a titrates with CD4 binding activity and that, as seen previously with gp120 [7] no inhibitory activity can be detected when T cells are activated by IL-2 alone in a CD4-independent pathway. The functional similarity between gp120 and anti-Leu-3a is also shown in Table 3, demonstrating that the inhibitory activity of anti-Leu-3a is blocked by pre-incubation with soluble recombinant CD4.

DISCUSSION

We have developed a co-culture system in which antigen-specific T cells from different individuals are stimulated by autologous APC in a restricted fashion, independently of each other. Therefore, in this system we could confirm the previous observation that specific T cells interacting with APC exposed in advance to the relevant antigen in the presence of HIV are functionally deleted when tested in an activation assay [7]. The advantage of this system over the one described previously is that the T cells are derived from different individuals and therefore carry different HLA markers that allow phenotypic identification. This assay lends itself to further development aimed at defining whether functional deletion is due to blockade or to physical loss of the relevant T cells, and whether actual HIV infection (from APC to T cells) is the initial step leading to the selective loss of T cells stimulated by APC exposed to HIV [7].

Selective loss of T cells stimulated by APC exposed to HIV confirms previous findings in a similar system in which the co-cultured T cells differed in antigen specificity rather than in MHC restriction elements [13] and equates with the clinical observation that the defective function of specific T cells is present well in advance of the generalized loss of CD4+ cells [2]. Our data are consistent with the hypothesis that APC cells are persistently infected with HIV which can infect antigen-specific T cells during antigen presentation. Accumulated deletions of antigen-specific cells would eventually lead to a decline in the total number of CD4 cells.

An additional consideration is that gp120 is able to interfere with T cell functions by binding to CD4, thus bringing about a functional blockade which can affect all T helper cells. This blockade is reversible and can be mimicked by anti-CD4 antibodies such as anti-Leu-3a. The similar mode of action may be relevant in the pathogenesis of non-responsiveness. In fact gp120 may also act as an immunosuppressant that favours tolerance induction, as has been described for anti-CD4 antibodies [17]. Therefore the activity of soluble gp120 as a reversible inhibitor of CD4- dependent T cell activation may be augmented by its function as an inducer of tolerance in the continued presence of antigen.

Cytopathic killing of specific T cells by HIV is the logical deduction to account for antigen-specific MHC restricted deletion. However, no cytopathic effects were evident in our cultures and no evidence of infection as detected by p24 antigen assay was observed. It is therefore probable that a functional deletion by virus depends upon the interaction between the T cell receptor and the whole virus envelope gp120/gp41 and that such restrictions lead to clonal anergy, and apoptosis. We have under investigation a mutant HIV virus able to fuse with cells but unable to undergo replication, in order to explore this possibility.

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