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Expression of MHC class II in T cells is associated with increased HIV-1 expression

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SUMMARY

HIV-1 replicates in activated T cells at significantly higher levels than in resting cells. Thus, certain molecules up-regulated during T cell activation appear to be important for HIV-1 replication. In this study, we present evidence suggesting that expression of MHC class II (class II) molecules on $CD4^+$ T cells facilitate HIV-1 replication. T cells that expressed class II supported greater virus replication than T cells lacking class II. The class II^+ cells, when either infected with HIV-1 or transfected with an env-minus HIV-1 provirus plasmid, produced 10–20-fold greater virus expression than class II^- cells. Anti-class II antibody markedly inhibited virus expression in class II^+ cells (but not class II^- cells) and also decreased the nuclear binding activity of AP-1, an inducible transcription factor important in T cell activation and HIV-1 expression. Most importantly, the induction of class II expression by transfection of the MHC class II transactivator (CIITA) stimulated HIV-1 replication in Jurkat T cells. Taken together, these data suggest that expression of MHC class II molecules and/or CIITA in T cells enhances HIV-1 transcription.

Keywords MHC class II CIITA T cell activation HIV-1 transcription

INTRODUCTION

The primary receptor for HIV-1 infection, CD4, is expressed mainly on T lymphocytes, monocytes/macrophages and dendritic cells. Although a variety of cell types can be infected by HIV-1, $CD4^+$ T cells are the predominant cell types found infected in both lymph nodes and in the peripheral blood [1–3]. In addition, most of the viral burden in blood plasma is derived from infected $CD4^+$ T cells [3–6]. HIV-1 binds to and fuses with the membrane of T cells via interactions with CD4 and chemokine co-receptor, but replicates at high levels only in T cells that have been activated [7–9].

Although undetectable on resting T cells, MHC class II molecules are expressed to a high level on activated T cells. The role of class II molecules constitutively expressed on antigen-presenting cells (APC) is well documented with regard to antigen presentation to $CD4^+$ T cells. However, the function of class II on activated $CD4^+$ T cells is unclear, although some reports suggest that under certain circumstances class II on T cells can also present antigen [10–12]. Following interaction with CD4 on the antigen receptor-bearing cells, class II molecules on B cells have been reported to transduce intracellular signals involving activation of protein kinase C (PKC), tyrosine kinases, phospholipase C, and the mobilization of intracellular calcium [13–16]. These class II-mediated signals are associated with either apoptosis or activation of cells depending on the activation and/or differentiation state of the cells [17,18]. Class II expression on T cells can also transmit intracellular signals [19–23]. Antibody cross-linking of HLA-DR has been shown to induce Ca^{2+} flux as well as tyrosine phosphorylation of several intracellular proteins in both B and T cells [21,24–27].

Several host cell proteins including MHC class I and II are physically incorporated into the HIV-1 membrane, where they remain functional [28–31]. Incorporation of class I and class II molecules has been shown to render the HIV-1 virions more infectious [32,33]. We showed that virions can transfer HLA-DR molecules from activated host cells to target cells during virus–cell fusion, enabling this protein potentially to contribute to cell activation and virus replication [34].

Class II expression is regulated by the class II transactivator (CIITA), which is recruited to the class II promoter by sequence-specific DNA binding proteins [35–38]. CIITA functions as a master switch for the activation of the class II promoter, resulting in surface expression of class II molecules in activated T cells. However, the relationship between class II expression and HIV-1 replication has not been investigated. We demonstrate here that HIV-1 expression is significantly greater in class II^+ T cells and that the class II transactivator can increase virus expression.

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MATERIALS AND METHODS

Cells and viruses

The H9 (HTB 176) and Jurkat (TIB 152) cells (American Type

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Culture Collection (ATCC), Rockville, MD), HPB-ALL [39] and CEM (T1 and T2) cells (obtained from P. Cresswell, Yale University, New Haven, CT) were grown in RPMI 1640 medium (Whittaker Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Hyclone, Logan, UT).

HIV-1 was obtained from H9 or CEM cells infected with the HIV-1_{MN} strain (NIH AIDS Research and Reference Reagent Program, Rockville, MD). Cells were acutely infected with HIV-1_{MN} and virus stocks prepared following standard procedure [40]. The amount of virus was quantified by measuring p24 antigen in the culture supernatant by ELISA (Coulter Co., Hialeah, FL).

Antibodies and expression plasmids

Mouse hybridoma HB-55 (L243) producing anti-HLA-DR and HD-245/332 (SD1744) producing anti-CD25 (anti-Tac) antibody were obtained from ATCC. Mouse hybridoma Sim.4 producing anti-human CD4 antibody was obtained from NIH AIDS Research and Reference Reagent Program. PE-conjugated antibody to HLA-DR, CD3, CD4, CD25, CD28, CD38 and isotype control IgG were obtained from Becton Dickinson (San Jose, CA), and PE-conjugated antibody to human CXCR4 was obtained from PharMingen (San Diego, CA).

The pNL4-3.Luc.R^-E^- (pNL4-3LucE^-), an env-minus HIV-1 provirus plasmid that expresses firefly luciferase, was obtained from NIH AIDS Research and Reference Reagent program (contributed by N. Landau, Aaron Diamond AIDS Research Center, New York, NY). The pRL-TKLuc construct, which expresses Renilla luciferase driven by the herpes simplex virus thymidine kinase promoter, was purchased from Promega Corp. (Madison, WI). The pcDNACIITA-6, human MHC class II transactivator plasmid was prepared as described [41].

Stable induction of class II in Jurkat cells

Jurkat T cells (10^7) were transfected with 25 μg of pcDNACII-TA-6 and 1 μg of neomycin DNA by electroporation using Gene Pulser Transfection Apparatus (BioRad, Richmond, CA) according to the manufacturer's protocol. Briefly, cells were transferred into an ice-cold Gene Pulser cuvette (0·4 cm) and subjected to a single pulse at 960 μF and 300 V. After shocking, the cuvette was incubated on ice for 10 min before cells were transferred to culture medium containing 10% FBS and 1 mg/ml G418 (Calbiochem, La Jolla, CA). After 3 weeks of culture in the presence of 1 mg/ml G418, the selected class II^+ cells were maintained at 500 μg/ml of G418. Surface expression of class II induced by CIITA was confirmed by flow cytometry.

Infection and binding of HIV

Cells (10^6) were infected for 2 h at 37°C with HIV-1_{MN} (multiplicity of infection 0·01) derived from H9 cells. In some infection experiments, cells and viruses were preincubated separately with or without anti-HLA-DR (L243), anti-CD25 (anti-Tac) or anti-CD4 (Sim.4) antibody (3 μg/ml) for 30 min at room temperature before they were mixed for further incubation at 37°C for 2 h. After infection, cells were washed and cultured in 96-well plates (Coster, Cambridge, MA) (20 000 cells/well in 200 μl) in the presence or absence of antibody (3 μg/ml). At 6 days post-infection, supernatants were treated with 0·5% Triton X-100 and analysed for p24 antigen by ELISA. Jurkat cells expressing or lacking class II were infected with HIV-1_{MN} at a MOI of 0·001. After 6 days cell supernatants were harvested and analysed as above.

To measure virus binding to cells, 2×10^6 CEM cells (T1 or T2) and HIV-1_{MN} virus (20 ng p24) were preincubated separately for 30 min on ice in the presence or absence of anti-HLA-DR (L243) or anti-CD4 (Sim.4) antibody (3 μg/ml). Virus and cells were then mixed and further incubated for 2 h on ice. Cells were washed to remove unbound virus, lysed with 1% Triton X-100 and analysed for cell-bound HIV by p24 ELISA.

Transfection and reporter gene assays

CEM-T1 and CEM-T2 cells were transiently transfected with the pNL4-3LucE^- construct using DMRIE-C reagent (Gibco BRL, Grand Island, NY) according to the manufacturer's protocol. Briefly, DNA complexes were prepared by incubating 4 μg of pNL4-3LucE^- DNA and 6 μl of DEMRIE-C reagent in 1 ml of opti-MEM medium (Gibco BRL) for 45 min at room temperature. Cells (2×10^6) were washed with serum-free medium, incubated with the DNA mixture for 3–4 h at 37°C. The transfected cells were cultured in RPMI 1640 medium supplemented with 1% FBS (2×10^5/well per ml) in 48-well plates for 72 h when cells and supernatants were harvested. HIV expression was determined in the cell lysates using the Luciferase Assay System (Promega) and by measuring p24 antigen in the supernatant by ELISA. In some experiments, cells (2×10^6) were co-transfected with 4 μg of pNL4-3LucE^- and 100 ng of pRL-TK DNA as described above and analysed using the Dual-Luciferase Reporter Assay System (Promega) to compare the viral expression and transfection efficiency between the two cell types.

In some experiments, cells were transfected with pNL4-3LucE^- DNA as above and cultured in the presence or absence of MoAb to HLA-DR or CD25 before analysis for luciferase activity.

DNA binding studies

Electrophoretic mobility shift assays (EMSA) were performed as described [42]. Briefly, CEM-T1 or CEM-T2 cells (5×10^6) were preincubated with or without L243 antibody (5 μg/ml) for 60 min at 37°C, and then nuclear protein extracts were prepared as described [43]. Protein extracts (5 μg) were incubated with 50 000 ct/min (approx. 0·1 ng) of ^{32}P end-labelled consensus NF-κB binding oligonucleotide probe (5′-AGTTGAGGG-GACTTTCCCAGGC-3′) or consensus AP-1 probe (5′-CGCTTGATGAGTCAGCCGGAA-3′) for 20–30 min at room temperature in a 10-μl reaction volume containing 12% glycerol, 12 mM HEPES-NaOH pH 7·9, 60 mM KCl, 5 mM $MgCl_2$, 4 mM Tris–HCl pH 7·9, 0·6 mM EDTA, 0·6 mM DTT, and 0·25 μg double-stranded poly (dI-dC). Protein–DNA complexes were resolved in 5% polyacrylamide gels pre-electrophoresed for 30 min at room temperature in 0·25 × TBE buffer (22·5 mM Tris-borate and 0·5 mM EDTA, pH 8·3). Gels were dried and exposed to radiographic film with an intensifying screen.

Measurement of cytokine and chemokine

CEM-T1 and CEM-T2 cells (10^6/ml) were cultured for 2 days. Cytokine and chemokine levels in cell-free supernatants were measured by immunoreactivity in a double-sandwich ELISA format using commercially available kits for IL-1β, IL-2, IL-4, IL-6, interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α) and RANTES (Biosource Int., Camarillo, CA), and for macrophage inflammatory protein (MIP)-1α and MIP-1β (R&D

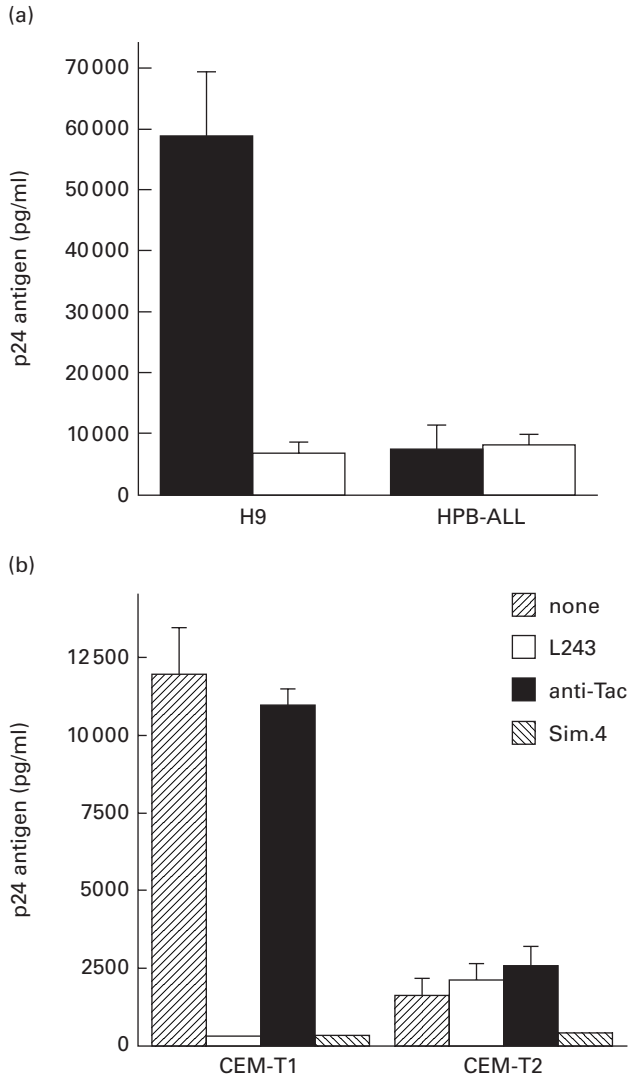

Fig. 1. Class II^+ T cells support greater HIV-1 replication and anti-class II antibody inhibits virus replication. Class II^+ H9 and class II^- HPB-ALL cells (a), and class II^+ CEM-T1 and class II^- CEM-T2 cells (b), were infected with HIV-1_{MN} in the presence or absence of anti-HLA-DR (L243), anti-Tac, or anti-CD4 (Sim.4) antibody (3 μg/ml). HIV-1 replication was assessed after 6 days by measuring p24 antigen in the culture supernatants by ELISA. The results represent the mean of three experiments + s.d. (a) ■, None; □, L243.

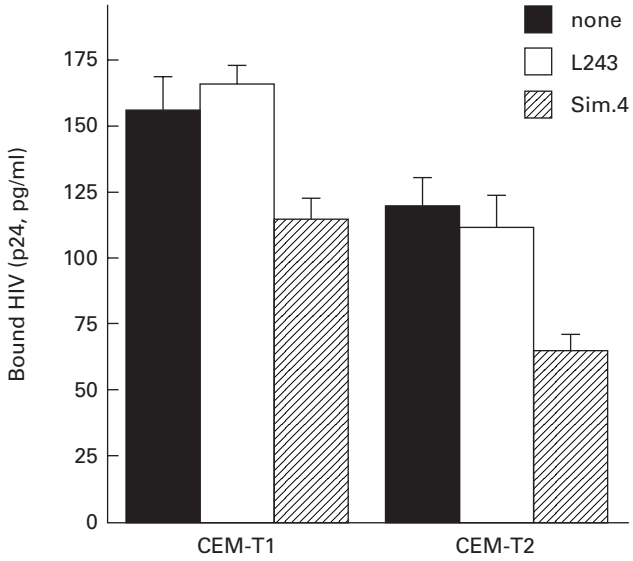

Fig. 2. Anti-class II antibody does not inhibit HIV-1 binding to cells expressing class II. CEM-T1 and CEM-T2 cells and HIV-1_{MN} virus were incubated separately with anti-HLA-DR (L243) or anti-CD4 (Sim.4) antibody for 30 min at room temperature. The virus and cells were then mixed and further incubated for 2 h on ice. The cells were washed, lysed with Triton X-100 and cell-bound virus measured by p24 antigen ELISA. The data represent the mean of three experiments + s.d.

Systems, Minneapolis, MN). All assays were performed according to manufacturers' protocols.

RESULTS

Class II^+ T cells support greater HIV-1 replication

While assessing HIV-1 infection in several cell lines we noticed that virus replicated to significantly higher levels in class II^+ T cells than in class II^- cells. For example, virus replicated to eight-fold higher levels in class II^+ H9 cells than in class II^- HPB-ALL cells (Fig. 1a).

We also analysed virus replication in CEM-T1 and CEM-T2 cells, two $CD4^+$ T cell lines derived from the same parental cell that are class II^+ and class II^-, respectively. The CEM-T1 cells supported significantly greater HIV-1 replication (7·5-fold) than the CEM-T2 cells (Fig. 1b), while the two cell lines expressed similar levels of cell surface CD4 and chemokine receptor CXCR4 (data not shown).

To determine whether soluble factors secreted by the cells were responsible for the different levels of virus replication, various cytokines and chemokines were measured in the culture supernatants. While IL-1β, IL-2, IL-4, IL-6, IFN-γ and TNF-α were undetectable in supernatants from either cell, high levels of RANTES (4000–6000 pg/ml) were secreted from both cell types. While 2000–4000 pg/ml of both MIP-1α and MIP-1β were detected in supernatants from CEM-T2 cells, both chemokines were undetectable in CEM-T1 cells. To determine the effects on HIV expression of either MIP-1 secretion or other soluble factors produced by the cells, CEM-T1 and CEM-T2 cells were infected and treated with 3%, 10% and 20% of supernatant from the other cell type. In both cases, the level of virus expression was unaffected (data not shown). These experiments indicate that the differential HIV-1 expression by CEM-T1 and CEM-T2 cells was not due to soluble factors secreted in the culture supernatants.

Anti-HLA-DR antibody inhibits HIV-1 replication

Since expression of class II was associated with higher HIV replication in these cells, we next investigated the effect of treatment of cells with antibody to HLA-DR (L243) on virus replication. As shown in Fig. 1a, L243 significantly reduced the replication of virus in H9 cells, while virus replication was unaffected in class II^- HPB-ALL cells. Treatment with anti-CD25 antibody did not have a significant effect on virus replication, while anti-CD4 antibody (Sim.4) as expected inhibited virus replication in both cell types (data not shown). The L243 antibody also markedly inhibited virus replication in CEM-T1 cells, but not

in CEM-T2 cells, while the anti-CD4 antibody inhibited virus replication in both cell lines (Fig. 1b). Anti-CD25 had no significant effect on virus replication in either CEM-T1 or CEM-T2 cells (Fig. 1b).

To determine if the L243 antibody interfered with HIV-1 binding to cells, and thereby inhibited infection, we assessed the effects of L243 on virus binding to CEM-T1 and CEM-T2 cells. As shown in Fig. 2, L243 treatment did not significantly affect binding of HIV-1 to either of the cell lines, while the anti-CD4 antibody inhibited virus binding to both cell types. The anti-CD4 antibody inhibited virus binding by only 25–45%, suggesting cell surface molecules other than CD4 can contribute to virus binding. This finding is consistent with previous reports that suggested that the interaction between virion and other cell surface proteins can be stronger than the CD4-mediated virus attachment [44]. This is also consistent with a previous report that HIV can bind HeLa cells independent of CD4 [45]. These results show that the inhibition of virus replication in class II^+ cells by L243 antibody was not due to a reduction in virus binding to the cell surface.

Another possible explanation for L243 inhibition of virus expression is decreased cell proliferation induced by the antibody. However, treatment of cells with L243 produced no significant change in ^{3}H-thymidine incorporation (data not shown), indicating that the antibody inhibition of virus replication was not due to decreased cell proliferation.

MHC class II expression is associated with increased HIV-1 provirus expression

To investigate the mechanism by which class II expression could affect HIV-1 expression, we performed a series of comparative functional studies in the CEM-T1 and CEM-T2 cell lines. We first examined whether class II expression is associated with intracellular provirus expression, by transfecting the CEM cells with pNL4-3LucE$^-$, an envelope minus HIV-1 proviral plasmid containing a firefly luciferase reporter gene. Supernatants were assessed for p24 production by ELISA, and the cell lysates analysed for luciferase activity 72 h after transfection. Since this provirus lacks the env gene, the virus produced from transfection cannot bind and infect new cells, limiting virus replication to the initial round of infection. As shown in Fig. 3, HIV-1 expression was markedly higher in class II^+ CEM-T1 cells than in class II^-CEM-T2 cells with p24 core antigen expression 10-fold higher (Fig. 3a), and provirus-mediated luciferase activity 20-fold higher (Fig. 3b). The large difference between cells in p24 production and luciferase expression was observed with either 0·5, 1·0 or 2·0 μg of transfected DNA (data not shown). These studies demonstrate that the higher HIV-1 expression in CEM-T1 cells is primarily due to an intracellular mechanism and not due to increased virus binding, entry or secondary rounds of infection.

To ensure equivalent transfection efficiencies in the two cell types, we co-transfected a second reporter plasmid expressing Renilla luciferase. As shown in Fig. 3c, Renilla luciferase activity was not significantly different between class II^+ and class II^- cells, demonstrating that the transfection efficiency of the two cell lines was similar. In addition, because the Renilla luciferase gene is driven by the herpesvirus thymidine kinase promoter, these data indicate that the low virus expression in the class II^- cell line was not due to a general transcriptional defect. Thus, it appears that the marked increase in virus expression in class II^+ cells is mediated at the level of HIV transcription.

We next examined the effect of the L243 antibody on provirus expression in the two cell types. As shown in Fig. 4, L243 inhibited provirus-mediated luciferase activity in a dose-dependent manner in CEM-T1 cells. In contrast, the antibody had little or no effect on provirus expression in CEM-T2 cells. The L243 antibody had no effect on Renilla luciferase expression in either CEM-T1 or CEM-T2 cells (data not shown). The antibody effect was specific, since an anti-CD25 antibody had no effect on provirus expression (Fig. 4). These results show that the L243 antibody inhibits virus transcription and suggests either that HLA-DR on $CD4^+$ T cells transmits an intracellular activation signal important for efficient virus expression that is blocked by L243, or that binding of L243 induces a negative signal for virus expression.

HIV-1 expression is dependent on host-cell transcription factors interacting with cis-elements in the promoter region of the viral LTR [46]. Two inducible transcription factors, AP-1 and NF-κB, have been demonstrated to play important roles in both T cell activation and HIV-1 replication [42,47–49]. To determine whether L243 antibody treatment affects the availability of these transcription factors, the two CEM cell lines were treated with L243 for 60 min and nuclear protein extracts were assessed for DNA binding activity by gel mobility shift analysis. As shown in Fig. 5, the class II^+ and class II^- cells showed marked differences in the DNA binding activity of these two transcription factors. In CEM-T1 cells, the AP-1 binding was approximately 90% greater than in CEM-T2 cells. Similarly, NF-κB binding activity was also consistently greater in CEM-T1 than in CEM-T2 cells. Importantly, L243 had a marked inhibitory effect on AP-1 binding activity in CEM-T1 cells. In contrast, the antibody had little or no effect on the NF-κB binding activity in either cell type or the AP-1 binding activity in CEM-T2 cells. These data show that class II^+ and class II^- cells have different amounts of nuclear transcription factors available for T cell activation and that treatment with antibody to class II can affect the availability of transcription factor.

Expression of CIITA increases HIV-1 expression in Jurkat T cells

To assess more directly whether class II expression increases HIV-1 expression, we created a cell line that constitutively expresses class II by stably transfecting Jurkat cells with the MHC class II transactivator, CIITA. While untransfected Jurkat cells are HLA-DR$^-$, a high level of HLA-DR (99% positive) was induced in the CIITA transfected Jurkat cells (Jurkat-DR). The expression of other surface proteins, including CD3, CD4, CD25, CD28, CD38 and CXCR4, remained essentially unchanged after transfection (data not shown).

To determine the effect of CIITA transfection on virus replication, the Jurkat and Jurkat-DR cell lines were infected with HIV-1_{MN}. As shown in Fig. 6, HIV-1 replicated five-fold higher in Jurkat-DR cells than in Jurkat cells. This result demonstrates that the induction of class II expression by CIITA in Jurkat T cells is associated with an activation of HIV-1 expression.

DISCUSSION

In this study, we provide the first evidence that class II expression on $CD4^+$ T cells contributes to HIV-1 replication. We found that the level of HIV-1 replication in class II^+ T cells was significantly greater than in class II^- cells, irrespective of whether the virus was introduced into the cells by infection or by transfection of a provirus construct. The differential virus expression observed in

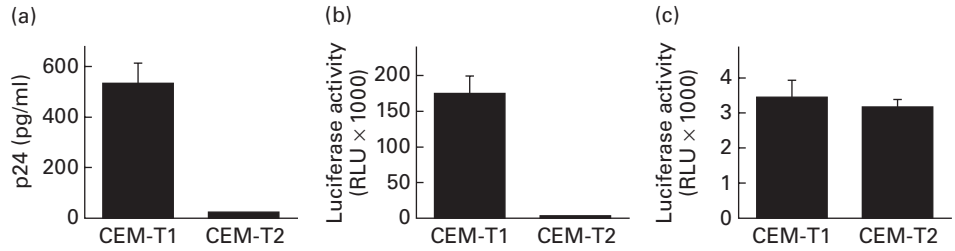

Fig. 3. Cells expressing class II support greater HIV-1 provirus expression. CEM-T1 cells expressing class II and CEM-T2 lacking class II were co-transfected with the plasmid pNL4-3LucE$^-$ and pTK Renilla-Luc. Cells were cultured for 72 h when cell supernatants and cell lysates were analysed for viral expression by measuring p24 antigen (a) and firefly luciferase activity (b), respectively. Transfection efficiency was also assessed in the same cell lysate by measuring Renilla luciferase activity using Stop and Glo reagent (c). The data represent the mean of three experiments + s.d.

class II$^+$ CEM-T1 and class II$^-$ CEM-T2 cells (which are derived from the same parental cells) suggested that the event of class II expression in T cells is critically involved in the activation of HIV-1 expression. This conclusion is directly supported by the association of increased HIV-1 expression in Jurkat cells in which class II expression was induced by the stable introduction of a CIITA expression plasmid.

HIV-1 does not replicate efficiently in primary resting T cells, and although there is no true resting T cell line available, the markedly different viral expression displayed in these two pairs of T cell lines (CEM and Jurkat) as well as in H9 and HPB-ALL cells probably reflects differences in cellular activation states. Moreover, Jurkat cells produced approximately five-fold more virus when class II was induced on their surface, indicating class II expression in Jurkat cells provided an activation signal to drive increased virus replication. A recent report showed that both resting memory and naive T cells contain virus *in vivo*, since replication-competent virions can be obtained from both cell types upon *in vitro* stimulation [50]. Although that study did not assess virus replication in memory or naive cells, the results of our studies suggest that class II expression induced in either cell type could increase virus replication.

Immune activation plays an important role in HIV-1 replication in T cells [51–54], and the level of expression of HLA-DR on CD4$^+$ T cells directly correlates with cell cycling and disease severity in HIV infection [55–57], suggesting that class II expression in T cells plays a significant role in HIV-1 replication. The hypothesis that the state of cellular activation as marked by class II expression leads to increased virus expression in T cells is supported by the fact that class II molecules are known to transduce activation signals in both B and T cells [18,19,22,23], and that anti-HLA-DR antibodies can block these activation signals [58–60]. Consistent with previous reports, class II$^+$ CEM-T1 cells showed increased nuclear binding activity of the cellular transcription factors AP-1 and NF-κB, both of which are known to be important for T cell activation and HIV-1 expression [47–49,61]. Moreover, AP-1 binding was down-regulated in CEM-T1 cells by the anti-HLA-DR antibody, suggesting AP-1 can transmit

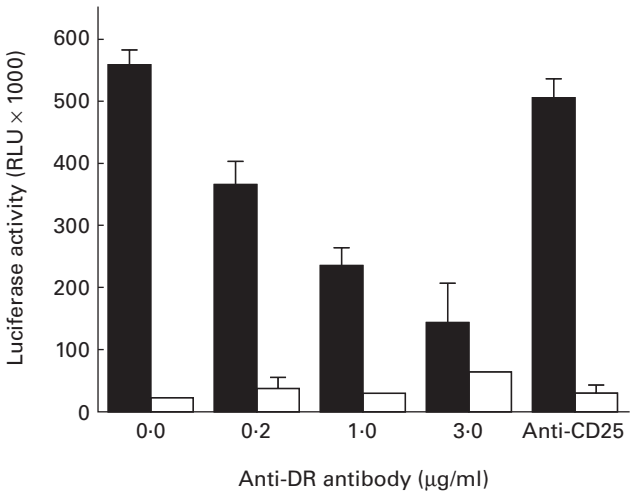

Fig. 4. Anti-class II antibody reduces HIV-1 provirus expression in class II$^+$ cells. CEM-T1 (■) and CEM-T2 cells (□) were transfected with the HIV-1 plasmid pNL4-3LucE$^-$. Transfected cells were cultured for 48 h with increasing concentrations of L243 antibody (0–3 μg/ml). Cells were lysed and provirus expression was determined by measuring luciferase activity. Anti-CD25 antibody (3 μg/ml) was used as an isotype control. The data represent the mean of three experiments + s.d.

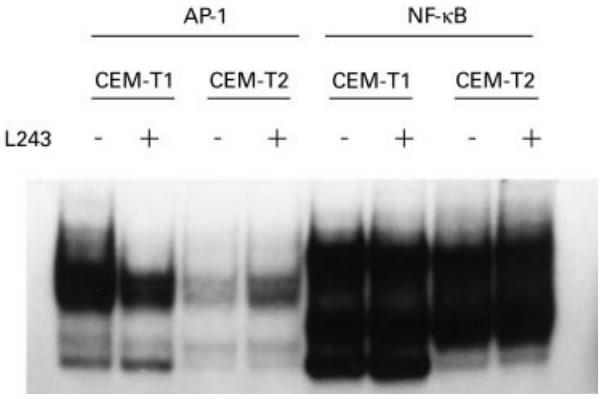

Fig. 5. Anti-class II antibody inhibits AP-1 but not the NF-κB binding activity in class II$^+$ cells. Nuclear protein extracts from CEM-T1 and CEM-T2 cells which were pretreated for 60 min with or without L243 antibody (5 μg/ml) were assessed for AP-1 and NF-κB binding activity by gel-shift assay as described in Materials and Methods. A representative autoradiogram is shown. The free probe, which migrates to the bottom of the gel, is not shown. Note that the AP-1 and NF-κB binding activity is greater in the class II$^+$ CEM-T1 cells and the L243 antibody markedly inhibits the AP-1 but not the NF-κB binding activity. The data represent one of three similar experiments.

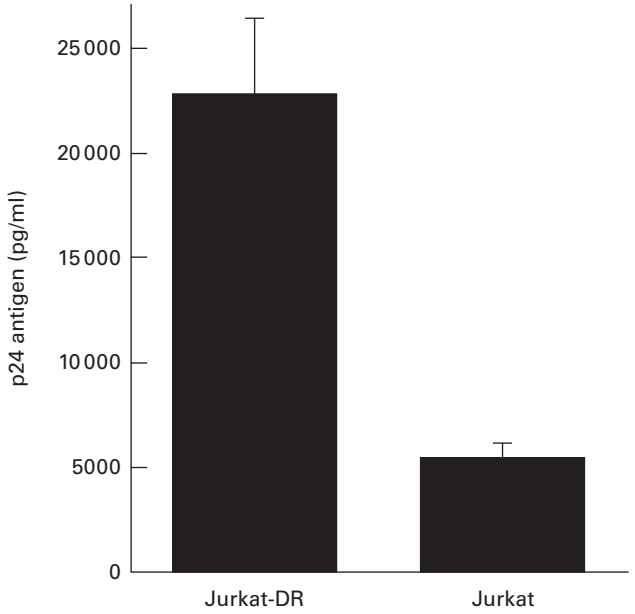

Fig. 6. Effects of class II on HIV-1 expression in Jurkat cells. Jurkat cells and Jurkat cells expressing class II (Jurkat-DR) were infected with HIV-1_{MN} (0·001 MOI) and cultured for 6 days. Virus replication was determined by measuring p24 antigen in culture supernatants. The data represent the mean of three experiments + s.d.

class II-mediated activation signals to increase provirus expression. Although it is not known whether class II expression directly activates the provirus through AP-1, we and others have shown that AP-1 binding sites in the 5′-untranslated leader region can contribute to HIV-1 expression in T cells and monocyte/macrophages [46,49]. Additional studies will be required to identify the viral elements and factors that are targeted by class II activation signals.

It has been demonstrated that HIV-1 particles contain an abundance of cellular HLA-DR that can enhance virus replication in target cells [28,32,62]. Increased virus replication may be due to enhanced virus binding, since HLA-DR can physically interact with CD4 independent of antigen processing and presentation [63–65]. In addition to the effect of virion-associated HLA-DR on virus infectivity, our results strongly suggest that class II molecules on the surface of host cells also play an important role in HIV replication by affecting transcription. Furthermore, HLA-DR antibody could inhibit virus expression in class II^+ cells, but not in class II^- cells. The effect of anti-class II antibody in HIV infection is consistent with previous studies [66,67]. Interestingly, a state of immune activation in HIV-infected individuals is further supported by the up-regulation of HLA-DR expression on $CD4^+$ T cells [68]. In addition, some *in vitro* studies also suggest that HIV-1 infection may involve binding to or modulation of HLA-DR expression on host cells [67,69]. Taken together, these results indicate that MHC class II molecules play an important role in HIV-1 replication whether they are on the virion or on the host cell.

MHC class II expression on activated T cells has long been suggested to contribute to immunoregulation and AIDS pathogenesis [66,70–73]. In this regard, several mechanisms have been proposed, such as presentation of endogenously synthesized HIV-1 envelope antigen by T cells, clonal inactivation of virus-specific T cells due to cell–cell interaction, and molecular mimicry between HIV-1 envelope and class II molecules. Moreover, a recent report suggested that HIV-1 infection causes down-regulation of MHC class II expression on target cells [74].

The data here provide the first evidence that the events leading to class II expression on $CD4^+$ T cells induce HIV-1 expression. However, the precise mechanisms of HIV-1 induction are not clear and require further investigation. We are currently exploring two possible mechanisms. The first involves the role of CIITA on the activation of the HIV-1 LTR, and the second concerns the class II signalling of HIV-1. These mechanisms are not mutually exclusive and it is possible that both may contribute to the enhancement of HIV-1 expression in activated T cells. We recently demonstrated that CIITA can directly activate the HIV-1 LTR via a transcriptional mechanism similar to that used by CIITA to activate class II gene expression [75]. Understanding class II-mediated HIV-1 expression may provide new targets or strategies for the development of an effective therapy against HIV disease.

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