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Activation of the signal transducer and activator of transcription 1 signaling pathway in thymocytes from HIV-1-infected human thymus

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Abstract

Objective—To identify HIV-induced host factors in the severe combined immunodeficient (SCID)-hu Thy/Liv mouse that may contribute to HIV pathogenesis in the thymus.

Design—To identify genes specifically altered by HIV-1 infection using the cDNA microarray assay, SCID-hu Thy/Liv organs derived from the same donors were used. Therefore, no genetic variations existed between HIV and mock-infected samples. In addition, the 12–14 day post-infection timepoint was chosen because no significant thymocyte depletion was detected in HIV-infected Thy/Liv organs, so mRNA from the same cell types could be compared.

Methods—Using SCID-hu Thy/Liv mice constructed from the same donor tissues, we analysed the expression of 9183 host genes in response to HIV infection with cDNA microarrays. Expression of selected genes with more than threefold induction was confirmed by measuring RNA (reverse transcriptase–polymerase chain reaction; RT–PCR) and proteins.

Results—HIV-1 (JD or NL4-3) infection of the SCID-hu Thy/Liv mouse led to more than threefold induction of 19 genes, 12 of which were IFN-inducible and six were unknown EST clones. We confirmed induction by RT–PCR and protein blots. Both signal transducer and activator of transcription (STAT)1 and STAT2 proteins were induced, and STAT1 was also activated by phosphorylation at the Tyr701 and Ser727 sites in human thymus infected with HIV-JD or NL4-3. Treatment of human fetal thymus organ culture or human thymocytes with recombinant HIV-1 gp120 proteins also led to induction or activation of STAT1.

Conclusion—HIV-1 infection of the thymus led to activation of the STAT1 signaling pathway in thymocytes, which may contribute to HIV-1 pathogenesis in the thymus.

Keywords

HIV/AIDS; human fetal thymus organ culture; microarray; severe combined immunodeficient-hu Thy/Liv; signal transducer and activator of transcription 1; thymus

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Introduction

The thymus is an early site of HIV-1 replication [1], and thymic organs from HIV-1-infected patients show profound parenchymal damage and involution [2]. Moreover, HIV-1-induced thymus dysfunction has been correlated with faster AIDS progression in pediatric patients [3]. As the thymus organ is difficult to study in humans, a small animal model for the analysis of human thymopoiesis [severe combined immunodeficient (SCID)-hu Thy/Liv mouse] has been constructed by engrafting fragments of human fetal liver and thymus into the immunodeficient C.B-17 scid/scid (SCID) mouse [4]. After inoculation of the SCID-hu Thy/Liv mouse with HIV-1, replication of pathogenic HIV-1 isolates reaches high levels at 2 weeks post-infection, followed by depletion of CD4 thymocytes between 3 and 6 weeks post-infection [5–8]. A higher rate of replication and thymocyte depletion is observed with rapidly replicating, syncytium-inducing virus isolated from AIDS patients than with slowly replicating, non-syncytium-inducing virus isolated from the same patients before the development of AIDS, or from long-term non-progressor patients [9]. Unlike in tissue cultures, mutations in HIV accessory genes significantly attenuated the replication and pathogenesis of HIV-1 in SCID-hu Thy/Liv mice [10,11]. The SCID-hu Thy/Liv mouse thus provides a relevant *in vivo* model to evaluate HIV-1 replication and pathogenicity in the human thymus.

Both indirect and direct mechanisms of thymocyte depletion have been implicated in HIV-1-infected thymus organs [5,8,12]. High levels of MHC class I are induced on all immature thymocytes, but only a small fraction of them are directly infected by HIV-1 [13]. Apoptosis has been associated with HIV-1-induced T cell death both *in vitro* and in vivo [14,15]. In the Thy/Liv organ, thymocytes with condensed nuclei were detected in HIV-1-infected Thy/Liv organs by thin section light microscopy and by electron microscopy [5]. Biochemically, partial chromosomal loss [5] and DNA strand breaks [8] are associated with HIV-1-induced thymocyte depletion. Consistently, it has recently been reported that indirect mechanisms of thymocyte depletion are primarily involved in the thymus of SIV-infected monkeys [16]. In support of the direct lytic infection mechanism, thymocyte depletion may be achieved by a number of HIV-1-encoded factors with cytotoxic or cytostatic activities, as demonstrated in T cells in vitro. High viral loads during the early stages of HIV-1-induced thymocyte depletion in the SCID-hu Thy/Liv mouse have been proposed to lead to direct cytolytic infection and thymocyte depletion [12]. In addition, the intrathymic T progenitor cells can be directly infected and depleted to lead to thymocyte depletion by blocking T cell development [8].

The thymus microenvironment is essential for T cell development. Destruction of thymic epithelial cells and the induction of various cytokines have been reported in the human thymus and in the SCID-hu Thy/Liv mouse after HIV-1 infection [2,7,13,17]. This may impair T cell development and result in thymocyte depletion. Indeed, two recent reports [18,19] showed that hematopoietic progenitor cells in HIV-1-infected Thy/Liv organs are preferentially depleted or suppressed by indirect mechanisms, before thymocyte depletion. Destruction of the thymus organ by HIV-1 infection is probably caused by the induced expression of viral and host pathogenic factors. Identification of the host genes will help our understanding of HIV pathogenesis in the thymus. The complementary DNA microarray

assay [20,21] has recently been developed to analyse differential gene expression profiles at the genome level. Using the cDNA microarray assay and confirmed by reverse transcriptase–polymerase chain reaction (RT–PCR) or Western blot assays, we showed that HIV-1 infection of the thymus organ led to enhanced expression of a number of genes involved in the Stat1 signaling pathway. We demonstrated that HIV-1 infection led to activation of the signal transducer and activator of transcription (STAT)1 proteins. We further showed that incubation of human fetal thymus organ culture (HF-TOC) or thymocytes with recombinant HIV-1 gp120 led to the induction or activation of the STAT1 protein.

Materials and methods

Reagents

Monoclonal antibodies reactive with human CD4 and CD8 cells were purchased from Becton Dickinson (San Jose, CA, USA). Anti-MHCI monoclonal antibody (mAb) W6/32 was from Biodesign (Kennebunk, ME, USA). Polyclonal anti-STAT1, anti-P-STAT1-Ser (or Tyr) and anti-STAT2 antibodies were purchased from New England Biolabs (Beverly, MA, USA). The monoclonal anti-p91 (STAT1) antibody was from Santa Cruz Biotech (Santa Cruz, CA, USA). The HIV-1 isolates (JD and NL4-3) used in this study have been described previously [8,22]. JD used both CXCR4 and CCR5 and NL4-3 used exclusively CXCR4 as co-receptors for entry *in vitro*. SDF1α and IFNγ (R&D Systems, Minneapolis, MN, USA) were used as recommended. Recombinant gp120 proteins (from X4-tropic IIIB or R5-tropic BaL isolates) were kindly provided by W. Chen (GlaxoSmithKline, Research Triangle Park, NC, USA).

Infection of severe combined immunodeficient-hu Thy/Liv mice

Animal transplantation and HIV infection procedures of SCID-hu Thy/Liv mice have been described previously [8,22]. Briefly, SCID-hu Thy/Liv mice were infected with supernatant collected from phytohemaglutinin-activated peripheral blood mononuclear cells (PBMC) containing no HIV-1 (mock) or 4×10^4 to 10^5 IU/ml of HIV-1 (JD or NL4-3). The Thy/Liv organs were harvested at the times indicated and thymocyte sub-populations were analysed by FACS (CD4-PE, CD8-TC, MHCI-FITC), and cell-associated p24 production (pg/10^6 thymocytes) was measured using a p24 enzymelinked immunosorbent assay kit [13].

HIV infection or treatment of human fetal thymus organ culture or thymocytes with recombinant HIV-1 gp120

The HF-TOC procedures were performed as described previously [8,22] with HIV-1 (JD) or 100 ng/ml of recombinant HIV-1 gp120 (IIIB). The medium was changed daily with fresh gp120 proteins. After 5 days, IFNγ (500 IU/ml) was added to the HF-TOC for 2 days as positive controls. Thymocytes were harvested at 7 days post-infection for analyses. Human fetal thymocytes were incubated with recombinant HIV-1 gp120 (IIIB or BaL), at 5 or 50 ng/ml for various times and harvested for Western blot analyses. IFNγ (200 IU/ml) or SDF1α (1 ng/ml) were used as controls.

Western blot analysis

Western blot analysis was performed with total thymocyte cell extracts as described [13]. The anti-p91 (STAT1) mAb, anti-P-STAT1-Tyr, anti-P-STAT1-Ser and anti-STAT2 polyclonal antibodies were used. Anti-tubulin mAb was used to monitor protein levels. Hela or 293T cells treated with IFNα or IFNγ (200 IU/ml) were used as positive controls for the antiphospho-Stat1 antibodies.

Reverse transcriptase polymerase chain reaction analysis

RT–PCR analysis was performed using the Ambion RT–PCR kit (Ambion, Inc., Austin, TX, USA). The following oligonucleotides were used: human Mx1, cagcttcagaaggccatgctgcagctcctg (up primer) and ggggag agctgcaaggtggagcgattctg (down primer); human Mx2, ggcgcgacacgcactctgtcaattctccagc (up) and ctgggatctgaaa gggaccggctcccagtcac (down); IP-10, ggaccacacagaggctg cctctcccatcac (up) and gggccccttgggaggatggcagtggaagtcc (down); and actin, atcatgtttgagaccttcaa (up) and catctc ttgctcgaagtcca (down). PCR reactions were run for 30 cycles of 94°C for 20 s, 60°C for 1 min, and 72°C for 2 min. Similar results were observed with JD or NL4-3 infected samples.

Microarray assay

Total RNA from mock and HIV-infected (JD or NL4-3) Thy/Liv organs (12–14 dpi) was prepared using the TriReagent (Sigma, St Louis, MO, USA). Fluorescence-activated cell sorter analysis showed no significant thymocyte depletion in HIV-infected Thy/Liv organs in comparison with mock-infected samples, and HIV-infected Thy/Liv organs accumulated high levels of HIV-1 (p24). PolyA+ messenger RNA was isolated using the Fast-Track 2 kit (InvitroGene, Carlsbad, CA, USA). The cDNA microarray assay was carried as reported [20,21,23]. Isolated mRNA was reverse-transcribed with 5′ Cy3 (HIV) or Cy5 (mock) labeled random 9-mers (Operon Technologies, Inc., Alameda, CA, USA). Reactions were incubated for 2 h at 37°C with 200 ng polyA RNA, 200 units M-MLV reverse transcriptase (Life Technologies, Gaithersburg, MD, USA), 4 mM dithiothreitol, 1 unit RNase inhibitor (Ambion, Austin, TX, USA), 0.5 mM dinucleotide triphosphates, and 2 μg labeled 9-mers in 25 μl volume with enzyme buffer supplied by the manufacturer. The reaction was terminated by incubation at 85°C for 5 min. The probe was hybridized to the array, which was scanned in both Cy3 and Cy5 channels using Axon GenePix scanners (Foster City, CA, USA) with a 10 μm resolution. The signal was converted into 16 bits-per-pixel resolution, yielding a 65 536 count dynamic range. Incyte GEMtools software (Incyte Pharmaceuticals, Inc., Palo Alto, CA, USA) was used for image analysis. The element signals were determined using a gridding and region detection algorithm. The area surrounding each element image was used to calculate a local background and was subtracted from the total element signal. Background subtracted element signals were used to calculate Cy3 : Cy5 (if Cy3 > Cy5, thus positive) or Cy5 : Cy3 (if Cy5 > Cy3, thus negative) ratios. The average of the resulting total Cy3 and Cy5 signal gives a ratio that is used to balance or normalize the signals. In three independent experiments (two with JD and one with NL4-3), HIV-infected Thy/Liv organs were compared with mock-infected Thy/Liv organs.

Results

Although high levels of HIV-1 were present at 2 weeks post-infection with HIV-1 (JD or NL4-3), no significant thymocyte depletion was detected (eight and data not shown). In addition to the induction of MHC I [13], HIV-1 infection of the Thy/Liv organ at 2 weeks post-infection leads to the profound suppression of the hematopoietic stem-progenitor cell activity in the thymus organ via indirect mechanisms [18,19]. We reasoned that HIV infection in the thymus led to the induction of host factors or signaling pathways that contribute to HIV replication and pathogenesis. To identify such 'pathogenic' host factors or pathways, we employed the cDNA microarray assay [20,21] to analyse the expression of 9183 host genes that were induced or suppressed by HIV infection in the thymus.

We designed our experiments as follows: to identify genes specifically induced or suppressed by HIV-1 infection with the cDNA microarray assay. First, the SCID-hu Thy/Liv model provided multiple Thy/Liv organs derived from the same donors in inbred mouse strains. No genetic variations thus existed between HIV and mock-infected samples in each experiment. Second, the 12 days post-infection timepoint was chosen because no significant thymocyte depletion was detected in HIV-infected Thy/Liv organs (eight and data not shown), so mRNA from the same cell types could be compared.

As a sensitivity control, known amounts of mRNA (2–2000 pg) from yeast-specific genes were mixed in the reactions with 200 ng sample RNA, and were hybridized with yeast cDNA on the array slide; 2 pg RNA were reproducibly detected. A linear increase in signals was detected from 2 to 2000 pg RNA. In addition, no signal spill-over was detected in wells adjacent to wells with high Cy3 or Cy5 signals (data not shown).

To demonstrate that different ratios of known RNA can be accurately quantitated, mRNA from different yeast-specific genes were added at indicated ratios to sample mRNA, labeled with Cy3 or Cy5, and hybridized on the cDNA arrays (Fig. 1a). A threefold difference (–threefold or +threefold) was consistently detected, and a linear correlation was observed from –25-fold (singlefold mRNA labeled with Cy3 and 25-fold mRNA with Cy5) to 25-fold (25-fold mRNA labeled with Cy3 and singlefold mRNA with Cy5) differences.

To monitor the quality of the HIV and mock mRNA samples, three human housekeeping genes (ribosomal S9, 23KDHBP, and α-tubulin) and three sets of human genes that are expressed in multiple tissues (complex targets) were arrayed in quadruplets (Fig. 1b). When the Cy3 (HIV)/Cy5 (mock) signals were analysed, the ribosomal S9 gene showed a –1.02 (± 0.09) ratio, demonstrating 1.02-fold (almost identical levels) of S9 mRNA in mock-infected samples. The second control gene, 23KDHBP, showed a –1.1 (± 0.00) ratio. The α-tubulin gene showed a slight reduction in HIV-infected samples with a ratio of –1.75 (± 0.19). Three different sets of human genes (complex targets) showed – 1.3 to –1.5 (± 0.2) ratios. In addition, the common thymocyte marker genes such as Thy-1, CD8 and TCRβ showed similar ratios (–1.1, –1.2 and –1.1, respectively) between the two samples. The results indicated that mock and HIV-infected samples had generated high-quality RNA, and the major housekeeping genes were not altered by HIV infection. The microarray assay thus showed reproducible detection of 2 pg RNA and threefold or higher differential (induction

or suppression) expression. In three independent microarray assays with different donor tissues (two infected with JD and one with NL4-3), a similar number of genes were shown to be induced over 10-fold (0.04%), fivefold (0.08%) or 2.5-fold (0.37%, Fig. 1c) in HIV-infected samples compared with either mock-infected samples.

When the data were analysed, 919 genes showed significant (> 1.5-fold) induction and 386 genes showed greater than 1.5-fold suppression by HIV-1 infection (data not shown). When the genes with greater than threefold induction were analysed, 19 cDNA showed (threefold to 15.7-fold) induction by HIV infection (Table 1). Interestingly, 12 of the 19 induced genes encoded products involved in the IFN-Stat1 signaling pathway, similar to the IFN-regulated gene cluster recently reported [21]. For example, Mx1 and Mx2 genes, both IFN inducible, were induced 15.7-fold and fivefold, respectively. STAT1, IP-10, and MHCI (HLA) genes, which are implicated in or regulated by the IFN signaling pathway [24], were all induced by HIV infection. We confirmed the expression profiles of some of the induced genes by RT–PCR (Fig. 2a, Table 1 and data not shown).

Consistent with the microarray assay, the MHC class I mRNA and proteins have previously been shown to be induced by HIV-1 infection [1,13]. As the activation of STAT1 could lead to the induction of the IFN-regulated gene cluster [24], we performed Western blot assays to analyse STAT1 expression. Both the STAT1 and STAT2 proteins were induced by HIV infection (Fig. 2b). Stat1 requires phosphorylation to be active [24]. To analyde the activation state of Stat1, antibodies specific for the active form of STAT1 (P-Ser727 or P-Tyr701) were used to detect phosphorylated STAT1 proteins in HIV-1-infected samples (Fig. 3). Both P-Ser727 and P-Tyr701 forms of Stat1 proteins were detected, suggesting that the STAT1 protein was also activated by HIV infection of the thymus.

Signaling through the HIV-1 co-receptor CXCR4 has recently been reported to activate STAT1 [25]. In addition, HIV-1 gp120 has been reported to induce CXCL10/IP-10 expression by astrocytes *in vivo* and *in vitro*, independent of IFN [26]. It is thus possible that the HIV-1 env protein gp120, which interacts with CD4 cells and co-receptors CXCR4 or CCR5 [27], may contribute to the induction of STAT1 and the IFN-regulated gene cluster. To test this possibility, we treated HF-TOC with recombinant HIV-1 gp120 (IIIB) proteins (Fig. 4a). As in the SCID-hu Thy/Liv mouse, HIV-1 infection led to the induction and activation of STAT1. Treatment of HF-TOC with HIV-1 gp120 proteins also led to the induction and activation of STAT1. We also incubated human fetal thymocytes with the recombinant gp120 proteins (IIIB and BaL). Expression of total STAT1 proteins was induced in cultured thymocytes with or without recombinant gp120 (Fig. 4b). Nonetheless, the activation of STAT1 proteins by phosphorylation at Tyr701 was only detected in thymocytes cultured with gp120 (IIIB or BaL), IFNγ or SDF1. Therefore, HIV-1 gp120, via its interaction with its receptor (CD4) and co-receptors (CXCR4 or CCR5), may directly or indirectly contribute to the activation of the STAT1 signaling pathway.

Discussion

Destruction of the thymus organ by HIV-1 infection was probably caused by the induced expression of the host, as well as viral, pathogenic factors. Identification of the host factors

or pathways involved will help our understanding of HIV pathogenesis in the thymus. Using the cDNA microarray assay (20, 21 and Fig. 1) and confirmed by RT–PCR or Western blot assays (Fig. 2), we showed that HIV-1 infection of the thymus organ led to the induction of a number of genes involved in the Stat1 signaling pathway (Table 1). We demonstrated that HIV-1 infection led to the induction and activation of the STAT1 protein (Fig. 3). We further showed that the incubation of HF-TOC or thymocytes with recombinant HIV-1 gp120 led to the induction and activation of the STAT1 protein (Fig. 4).

A recent microarray study with PBMC infected with HIV-1 [28] revealed the dysregulation of a number of human genes in response to HIV infection. However, no genes from the IFN-regulated cluster were induced. This suggests that alteration of gene expression by HIV-1 in the thymus *in vivo* is different from that in activated PBMC *in vitro*. It has recently been reported that PBMC from HIV-infected patients or PBMC infected with HIV *in vitro* show increased activation of STAT1 and STAT5, although the level of total STAT proteins in PBMC is the same between HIV-positive and HIV-negative samples [29]. However, a different report [30] suggested that HIV-1 infection of PBMC *in vitro* or *in vivo* may reduce the expression of STAT5 and STAT1. Differences in sample treatment and experimental procedures may result in contradictory findings. Similar to PBMC isolated from HIV-positive patients, the majority of thymocytes in the Thy/Liv organ at 2 weeks post-infection are not directly infected by HIV-1 [8,13], suggesting that indirect viral or host mediators are involved. The mechanism of HIV-1-induced STAT activation in the thymus and in PBMC *in vivo* may be similar, although thymocytes *in vivo* express very low levels of STAT1 (Fig. 2, Fig. 3, Fig. 4).

The mechanism and significance of HIV-1-induced activation of STAT1 and the IFN-regulated gene cluster in the thymus are not clear. When levels of cell-associated major IFNα, β or γ proteins in the Thy/Liv organ were analysed by ELISA, no significant induction was detected in HIV-infected SCID-hu Thy/Liv thymocytes (data not shown). And no supernatants were available in the Thy/Liv organ model. Using a human fetal thymus organ culture model, it was recently reported that IFNα production was induced, predominantly from type 2 predendritic cells in HIV-infected thymus organs [31]. It is possible, therefore, that HIV infection or gp120 activated type 2 predendritic cells to produce IFNα, which may activate the Stat1 pathway in thymocytes. The mRNA of IL-10, which has been reported to be induced in HIV-infected thymocytes and may contribute to MHC class I induction [13], was not significantly induced by the microarray assay (data not shown). As the expression of many cytokines is regulated at post-transcriptional levels, production of IL-10 protein may be regulated in HIV-infected thymus organs.

IFN-independent induction of 'IFN-responsive' RNA after human cytomegalovirus infection has previously been reported [32]. It is of interest that signaling through the HIV-1 co-receptor CXCR4 by SDF1 leads to the activation of STAT1 [25]. In addition, the expression of gp120 in astrocytes *in vivo* and *in vitro* leads to IFN-independent induction of IP-10 [26]. It is not clear whether signaling via CCR5 can also activate Stat1. As a co-receptor for HIV-1 entry, the interaction of CXCR4 or CCR5 with HIV-1 gp120 [27,33] may contribute to activation of the STAT1 pathway in human thymocytes (Fig. 4). In addition, peptides derived from HIV gp41 proteins have been shown to be structurally and

functionally similar to type I IFN [34]. It is thus possible that HIV-encoded proteins such as gp120 and gp41 may be involved in the induction of STAT1 and the IFN-regulated gene cluster.

The effect of Stat1 induction and activation in thymocytes on HIV replication is not clear. Interestingly, a recent report [35] indicated that Stat1 activation was involved in the suppression of HIV-1 gene expression mediated by CD8 T cell antiviral factors. The induction and activation of Stat1 may thus be a host cell response to resist infection. Activated STAT1 can also transduce signals affecting cell survival [36]. Thymocytes with elevated and activated STAT1 may become hypersensitive to IFN-like stimulation and to HIV-1 pathogenesis. In combination with other viral or host factors, Stat1 activation may contribute to thymocyte depletion. Further studies of HIV-1-induced activation of the STAT1 pathway in the thymus will help elucidate the mechanism of HIV-induced thymus destruction and define a novel target for modulating HIV pathogenesis.

Acknowledgments

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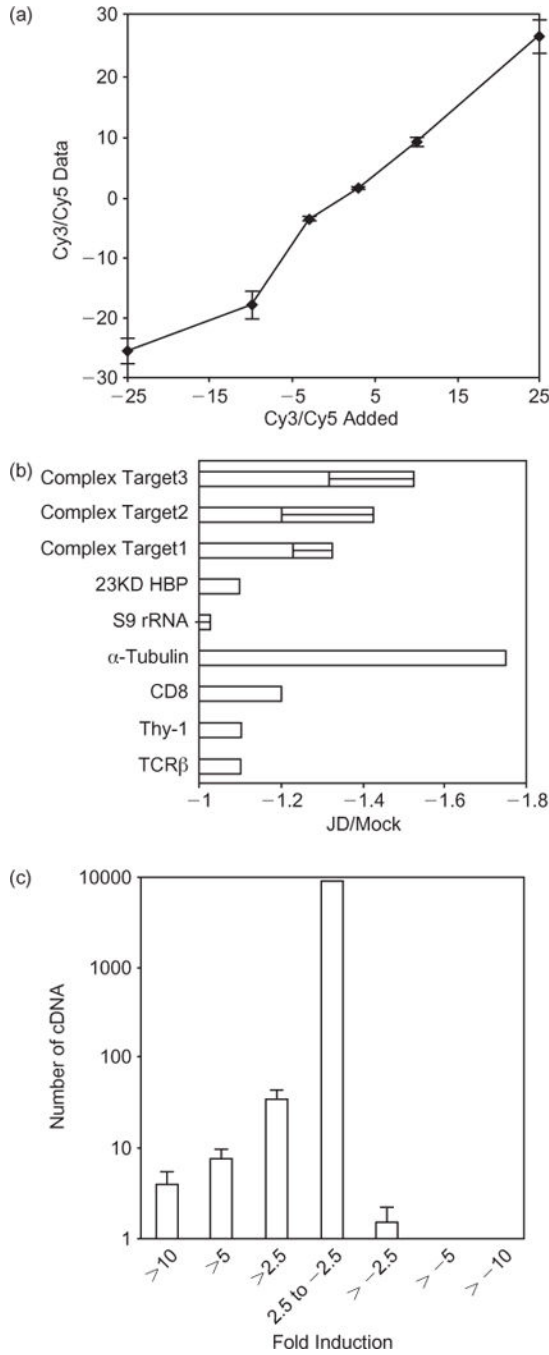
Fig. 1. Microarray assay of gene expression in HIV-1-infected Thy/Liv organs

(a) Quantitative measurement of differential gene expression. RNA from six different yeast-specific genes was mixed with the HIV or mock-infected RNA samples in the microarray assay. RNA from each yeast gene was added at a known ratio (3, 10 or 25) in the Cy3 (HIV) or Cy5 (mock) sample (Cy3/Cy5 added). The six yeast genes were arrayed on the slides in quadruplets. Positive values indicate threefold, 10-fold or 25-fold more RNA were used in the Cy3 (HIV) sample. Negative values (−3, −10 or −25) indicate that threefold, 10-fold or 25-fold more RNA were included in the Cy5 (mock) sample. An equal amount of RNA in

Cy3 and Cy5 samples is indicated as 1 (+1 or −1). The X axis shows the added ratios and the Y axis indicates Cy3/Cy5 readout data from the assay. Error bars indicate standard deviations. (b) RNA sample and reaction control. RNA samples from HIV_{JD} (Cy3 labeled) or mock (Cy5 labeled) infected severe combined immunodeficient (SCID)-hu Thy/Liv mice were analysed using the microarray assay. Relative expression levels of 23 000 M_r highly basic protein (HBP), ribosomal subunit S9 (S9 rRNA) and α-tubulin were quantified in quadruplets. Three sets of human genes known to express in multiple tissues (complex target 1–3) were also included as controls. Positive values (Cy3 signal/Cy5 signal) indicate more RNA in the Cy3 (HIV) sample, thus induction of expression by HIV. Negative values (Cy5 signal/Cy3 signal) indicate more RNA in the Cy5 (mock) sample, thus suppressed expression by HIV. Error bars indicate standard deviations from the quadruplet arrays. The relative expression of three T cell markers (TCRβ, CD8 and Thy-1) from the microarray assay was also shown. Comparison of HIV (JD or NL4-3) and mock infected samples from different donor tissues showed similar results. (c) Alteration of gene expression in SCID-hu Thy/Liv mice after HIV-1 infection. A small number (< 0.2%) of genes were induced (> threefold) in HIV-1-infected Thy/Liv organs over mock infected samples. The majority of genes were in the +2.5 to −2.5 range (99.5%). Error bars indicate standard deviations from three experiments (two with JD and one with NL4-3) with different donor tissues.
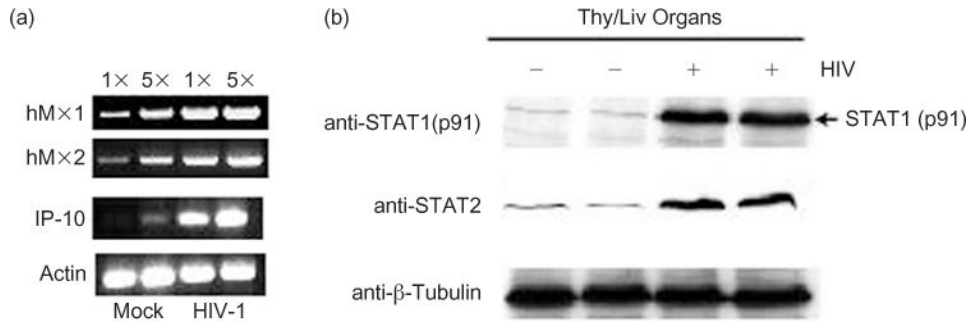
Fig. 2. Induction of the IFN-induced gene cluster and of the signal transducer and activator of transcription 1 protein in HIV-1-infected Thy/Liv organs

(a) Confirmation of gene induction by reverse trancriptase-polymerase chain reaction (RT-PCR). Severe combined immunodeficient (SCID)-hu Thy/Liv mice infected with mock (−) or with HIV-JD (+) were harvested at 2 weeks post-infection. Total thymocyte RNA from mock or HIV-infected Thy/Liv organs were analysed by RT–PCR with primers specific for the selected genes. 1 × and 5 × indicate 25 ng and 125 ng of total RNA used in the RT–PCR reaction. Expression of actin was used to monitor cellular RNA. No significant thymocyte depletion was detected in mock or HIV-infected samples. Two independent experiments with different donor tissues were performed with similar results. (b) Induction of signal transducer and activator of transcription (STAT)1 and STAT2. SCID-hu Thy/Liv mice infected with mock (−) or with HIV-JD (+) were harvested at 2 weeks post-infection. Total thymocyte proteins (50 μg) from two representative mock or two HIV-infected Thy/Liv organs were analysed by Western blot with the anti-p91 (STAT1) monoclonal antibody or anti-STAT2 polyclonal antibody. Anti-β-tubulin antibody was used to monitor cellular proteins. No significant thymocyte depletion was detected in mock or HIV-infected samples. Four independent experiments (infected with JD or NL4-3) with different donor tissues were performed with similar results.
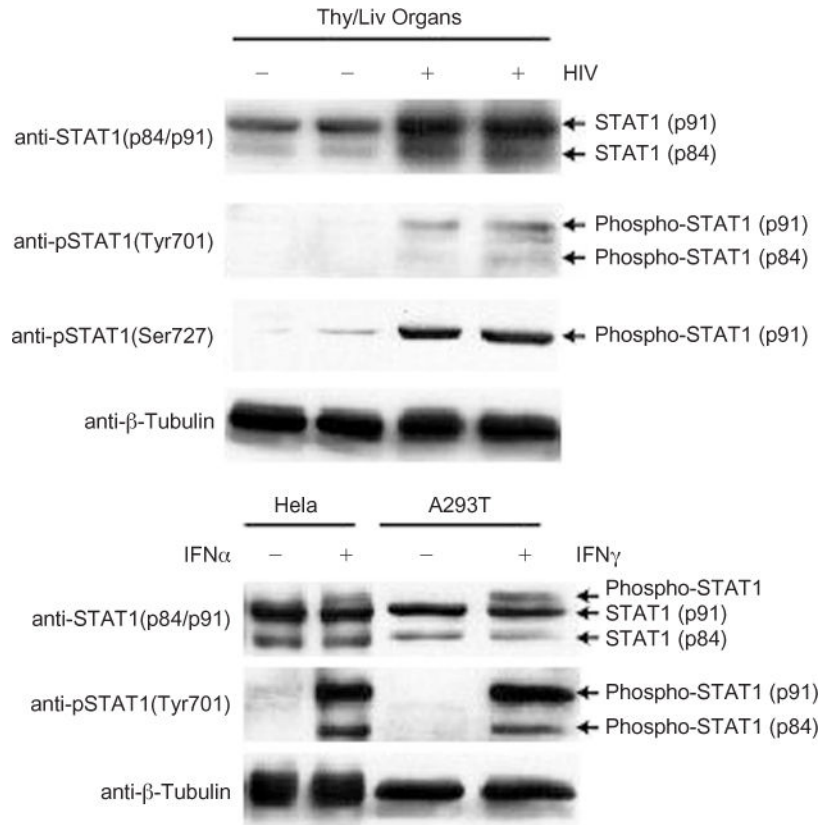
Fig. 3. Activation of the signal transducer and activator of transcription 1 protein in HIV-1-infected Thy/Liv organs

Total thymocyte proteins were blotted with polyclonal anti-signal transducer and activator of transcription (STAT)1 (p84/p91) antibody or with antibodies specifically reactive with STAT1 phosphorylated at Ser727 (pSTAT1-Ser) or Tyr701 (pSTAT1-Tyr). Anti-pSTAT1 (Y701) detects both forms of phospho-STAT1 (p91 and p84) and anti-pSTAT1 (Ser727) is specific to phospho-STAT1-p91. Two representative mock or HIV-(JD)-infected Thy/Liv samples were presented. Proteins from IFNα–treated Hela or IFNγ–treated 293T cells were used as positive control for the anti-P-STAT1 (Y701) antibody. Three independent experiments (infected with JD or NL4-3) with different donor tissues were performed with similar results.
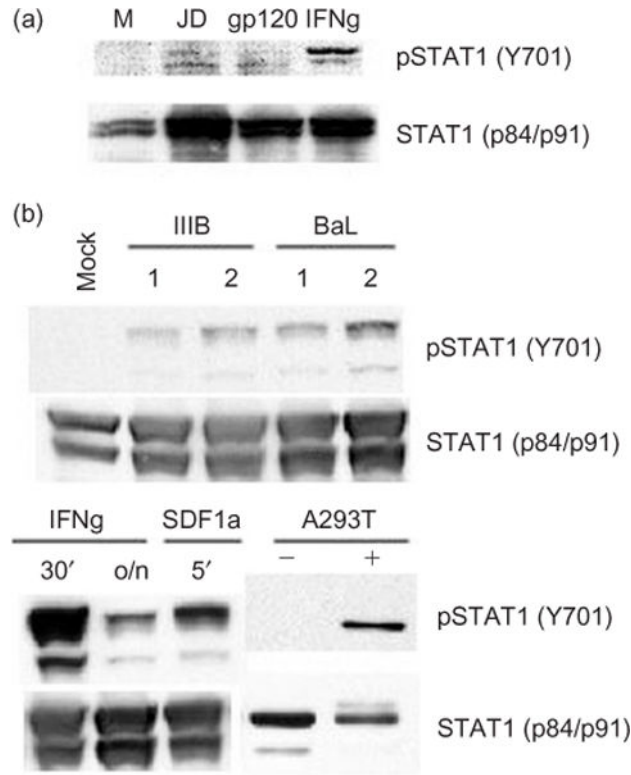
Fig. 4. Activation of signal transducer and activator of transcription 1 in human fetal thymus organ culture or thymocytes by HIV infection or recombinant HIV-1 gp120

(a) Induction and activation of signal transducer and activator of transcription (STAT)1 in human fetal thymus organ culture (HF-TOC) by HIV-1 infection and recombinant gp120. HF-TOC infected with mock (M) or HIV-JD, or treated with recombinant gp120 (IIIB, 100 ng/ml) or IFNγ were analysed for STAT1 protein expression (STAT1 p84/p91) and activation (pSTAT1Y701) as described above. NL4-3 infection also led to STAT1 activation (not shown). (b) Activation of STAT1 in human thymocytes by recombinant gp120. Human fetal thymocytes were cultured with medium (mock), with 5 ng/ml (lanes 1) or 50 ng/ml (lanes 2) of recombinant HIV-1 gp120 (IIIB or BaL) preparation. Thymocytes were harvested at 12 h (o/n) post culture and analysed for total STAT1 proteins (p84/p91) and Tyr701 phosphorylated STAT1 (Y701). Human thymocytes treated with IFNγ or SDF1α were used as controls.

Table 1

Human genes induced in severe combined immunodeficient-hu Thy/Liv mice by HIV-1 infection.

Gene name	Fold induction[a]	HIV signal (Cy3)[b]	Accession no.[c]	RT–PCR[d]	IFN induction
Myxovirus resistance 1 (Mx1, IFN-inducible protein p78)	15.7	2828	M30817	Yes	Yes
Human mRNA for 56000 M$_r$ protein induced by IFN	14.8	1021	X03557	Yes	Yes
EST1	11.9	557	AA497115		?
EST2	8.9	497	AI352370		?
IFN-induced protein 54	7.4	228	M14660		Yes
EST3	6.6	318	AI274746		?
STAT1	5.5	1028	AA478534	Yes	Yes
IFN-induced protein 17	5.2	1734	AA428847		Yes
Myxovirus resistance 2 (Mx2)	5	646	M33883	Yes	Yes
IFN, alpha-inducible protein 27	4.6	223	AA302123		Yes
Small inducible cytokine subfamily B (Cys–X–Cys), member 10 (IP-10)	4.6	123	NM_001565	Yes	Yes
EST4	4.1	1429	R13843		?
Guanylate-binding protein 1, IFN-inducible, 67000 M$_r$	4	497	M55542		Yes
29′–5′ oligoadenylate synthetase 2	3.9	2022	M87284	Yes	Yes
Homo sapiens mRNA expressed in osteoblast	3.8	3087	F12860		Yes
EST6	3.6	1352	AI093091		?
Dihydropyrimidinase-like 3	3.5	306	D78014		?
Human RNA for HLA (MCHI) antigen	3.1	8358	U04245		Yes
EST7	3.1	1770	R13843		?

RT–PCR, Reverse transcriptase–polymerase chain reaction; STAT, signal transducer and activator of transcription.

[a] Relative ratios of Cy3 (HIV)/Cy5 (mock) signals.

[b] Relative signal of Cy3-labeled (HIV) samples. Backgound signal is 28 (± 3). The average signal from 2 pg mRNA positive controls is 125 (± 14).

[c] Genebank accession numbers for detailed description of the genes and references.

[d] Confirmed by semi-quantitative PCR with mRNA isolated from mock or HIV-infected Thy/Liv organs.