Functional Genomic Analysis of the Response of HIV-1–Infected Lymphatic Tissue to Antiretroviral Therapy

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(See the editorial by Pomerantz, on pages 567–71.)

Highly active antiretroviral therapy (HAART) curtails human immunodeficiency virus type 1 (HIV-1) replication in lymphatic tissues and partially reverses the pathological damage associated with infection, but the genes that mediate these pathological and reparative processes remain largely unknown. To identify these genes, we used microarrays to profile gene expression in serial lymph node biopsy specimens obtained before and after treatment. We discovered ~200 treatment-responsive genes, many of them known mediators and moderators of immune activation and defenses, particularly innate defense genes, which, surprisingly, were expressed at all stages of HIV-1 infection. Most of the rest of the treatment-responsive genes we categorized as mediators of trafficking, reformation of active follicles, and tissue repair. We propose a model in which nearly counterbalanced functions of mediators and moderators of immune activation and defenses account for the slow dynamics of HIV-1 infection before treatment. This model suggests that there could be a role for anti-inflammatory agents, alone or in combination with HAART, in treating HIV-1 infection by tipping this balance to mitigate pathology.

In this article, we report the results of a functional genomic analysis of the changes in gene expression in HIV-1–infected lymphatic tissues (LTs) in response to highly active antiretroviral therapy (HAART). We chose to analyze LTs because, as we describe below in more detail, HIV-1 is largely produced and stored in LTs, host defenses struggle to contain infection in LTs, and the main pathological consequences of infection are manifest in LTs [1].

Virus is produced in LTs mainly by activated CD4+ T cells [2] that generate >10^9 virions/day [1, 3] and is stored in massive quantities in LTs as immune complexes bound to follicular dendritic cells (FDCs) [1, 3]. Although large numbers of virions are produced, the fraction of susceptible CD4+ T cells in LTs (10^10 cells) that are productively infected at any time (on average, 10^7 to 10^8 cells) [4] is actually relatively small (0.01–0.1), and the number of copies of viral RNA in productively infected cells in LTs is also much smaller (<100 copies/cell) than that in productively infected cells in culture (>1000 copies/cell) [3, 5]. These reduced numbers of infected cells and viral production within infected cells are thought to reflect the effects of an ongoing cellular immune response [6] and other host defense mechanisms that exert some control over HIV-1 replication in LTs.

The continued replication and deposition of HIV-1 onto the FDC network maintain the immune system in a chronic state of activation [7]. This activation is initially associated with follicular hyperplasia, but the follicles and T cell zone (TZ) are subsequently destroyed and replaced by collagen [8]. The destruction of LT...
architecture and milieu essential to maintaining immune cell populations [9], chronic immune activation, and inflammation and associated increased turnover (proliferation and apoptosis) of CD4+ T cells [4] and the death of infected CD4+ T cells collectively contribute to the depletion of CD4+ T cells characteristic of HIV-1 infection.

HAART can partially reverse these pathological changes in LTs. Within days after initiation of treatment, the population of productively infected activated CD4+ T cells in LTs decreases ∼10-fold [2], and within weeks, the pool of FDC-associated virus is similarly reduced ∼10-fold [5]. In the first and ensuing months of treatment, the frequency of proliferating CD4+ T cells and apoptotic CD4+ T cells in LTs decreases to normal levels, and the population of CD4+ T cells in LTs increases [4]. Follicles and the FDC network reform [10], immune activation and inflammation subside, and virus-specific immune defenses (e.g., HIV-1–specific CD8+ T cells [11]) decline.

Thus, it is known that (1) without HAART, HIV-1 infection chronically activates the immune system to maintain defenses that partially control infection, but the chronic activation and viral replication and storage have pathological consequences; and (2) with HAART, viral replication and immune activation and defenses decrease with concomitant reversal of the pathological changes. However, the genes involved in these processes are largely unknown. We therefore sought to discover, by profiling expression, specific genes involved in vivo in host defenses and LT pathology before treatment and in LT repair and immune system reconstitution after treatment.

In this article, we show, in a comparison of expression profiles of treated patients with untreated patients who deferred treatment, that there is a set of HAART-responsive genes. By using contextual information from examination of the tissues and an extensive literature search, we found that we could functionally categorize these treatment-responsive genes. We show that the treatment-responsive genes fit in functional categories that are clearly relevant to HIV-1 pathogenesis and reparative processes, propose a model of how the genes could account for the slow dynamics of HIV-1 infection, and, on the basis of the model, speculate on the potential therapeutic benefit of anti-inflammatory agents.

SUBJECTS, MATERIALS, AND METHODS

Lymph node biopsy specimens. Nine individuals participated in this University of Minnesota institutional review board–approved functional genomic analysis of HIV-1–infected LTs. After written informed consent was obtained, serial inguinal lymph node biopsy specimens were obtained by means of standard surgical techniques done while patients were under local anesthesia, at baseline and again 3 days and 1 month later from patients who initiated HAART after the baseline biopsy or who chose to defer treatment (the untreated group) for these studies, respectively. The portion of the biopsy specimen to be used for microarray analysis was immediately placed into a tube containing 5 mL of TRIzol (Gibco BRL Life Technologies) and taken to a biosafety level 3 facility for extraction of RNA (see below). The portion to be used for tissue analysis was placed in 4% paraformaldehyde for 3–4 h and then transferred to 70% ethanol and paraffin-embedded for in situ hybridization (ISH), immunohistochemical staining (IHCS), and histological examination.

ISH and IHCS. Tissue sections of 8 μm were cut, adhered to slides, deparaffinized, and subsequently pretreated as described elsewhere to detect HIV RNA by ISH [3] or to detect Ki67+ cells by IHCS [2]. In brief, for ISH, sections were pretreated with HCl, digitonin, and proteinase K to enhance diffusion of probes and then acetylated to reduce nonspecific binding of probes. After hybridizing a collection of 35S-labeled HIV-specific riboprobes, sections were washed, treated with ribonuclease, dehydrated, coated with nuclear track emulsion 2, and developed and stained after radio-autographic exposure of 4 days. For IHCS, after blocking to reduce nonspecific binding, sections were reacted sequentially with primary antibody, biotinylated secondary antibody, and ABC reagent and then stained with diaminobenzidine and counterstained with hematoxylin.

Isolation of RNA and preparation of microarray probes. The portion of the lymph node biopsy specimen in TRIzol was homogenized with a power homogenizer (Heat Systems Ultrasonic) and total RNA was isolated following the manufacturer’s protocols. Double-stranded cDNA was synthesized from total RNA with T7–(dT)24 primer (GENSET) using the Superscript Choice System, following the manufacturer’s protocol, and subsequently purified with phase-lock gel (Eppendorf) phenol/chloroform extraction. Biotin-labeled cRNA was synthesized using a BioArray HighYield RNA Transcipt Labeling Kit (T7; Enzo Diagnostics) and purified with an RNeasy Mini Kit (Qiagen).

These procedures generated high-quality RNA and cRNA probes, as shown by agarose gel electrophoresis (ratio of 28S to 18S RNA, broad range of sizes of cRNA probes) and the approximately equal ratio of 5′ and 3′ ends of arrayed genes detected by the cRNA probes (see below).

Microarray analysis. Fifteen micrograms of fragmented cRNA was hybridized to an Affymetrix Human Genomic U95A chip array of ~12,000 human genes, most of which have known functions, after the probe was hybridized to a test chip to assess the representation of the 5′ and 3′ ends of arrayed genes in the cRNA probes. All of the probes had approximately equal representation of the 5′ and 3′ ends, which attests to the high quality of RNA isolated from the lymph node biopsy specimens. After hybridization to the first or U95A chips, the arrays were washed, stained with streptavidin-phycocerythrin, and scanned according
to Affymetrix protocols (http://www.affymetrix.com/support/downloads/manuals/fs400-manual.pdf). Data were analyzed with the Affymetrix Microarray Analysis Suite 4.0 and Affymetrix Data Mining Tool 2.0. Fluorescent signal data in the microarray image files were scaled to a target intensity of 1000 for absolute and comparative analysis.

Replicates and reverse-transcription polymerase chain reaction (PCR) verification. A replicate cRNA probe was prepared from each RNA sample and hybridized to a second U95A chip. More than 90% of genes were scored as present or absent, and fold changes were correlated in the replicate analysis (results of the replicate analyses are shown in e-figure 1, which is available in the online version of this article). Genes were not further analyzed if the results of the replicate analyses were not in close agreement.

Altered expression of genes detected in the microarray analysis was confirmed by LightCycler real-time PCR. Gene-specific oligonucleotide probes were designed with Roche LightCycler Probe Design Software (Roche Diagnostics). Total RNA was digested with an RNase-Free Digestion Set Kit (Qiagen). cRNA was synthesized from 2 μg of total RNA with Superscript II reverse transcriptase and oligodeoxyribonucleotide (dT 12–18) primer. Serially diluted cDNA templates were amplified with a LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics), according to the manufacturer’s instructions. PCR products were shown to be specific by sizing the products by agarose gel electrophoresis. The relative number of copies was determined with LightCycler software from a plot of the log concentration of diluted cDNAs versus cycle number at which products were detectable (fluorescence intensity >1).

### RESULTS

Patient Characteristics and Response of HIV-1–Infected LTs to HAART

We analyzed inguinal lymph node biopsy specimens from 9 individuals, 5 who began receiving HAART after the first biopsy was performed and 4 (the untreated group) who wished to defer treatment. Biopsy specimens were obtained from all 9 at baseline, and satisfactory samples were obtained from 8 of the 9 patients 3 days and 1 month later. The stages of infection in the treated individuals ranged from acute through early to presymptomatic to AIDS (table 1).

All of the treated individuals responded to HAART with significant reductions in plasma virus load by 1 month (table 1) and with decreases in FDC-associated HIV-1 and productively infected cells in the lymph nodes (figure 1A and 1C). Before HAART, we found, using Ki67 as a marker of activated and proliferating cells, increased numbers of Ki67+ cells, which is indicative of immune activation in the TZ. By contrast, in the B cell zone, there were fewer Ki67+ B cells than in normal active secondary follicles, where there are large numbers of Ki67+ cells undergoing selection for high-affinity antigen receptors. There were also pathological changes—destruction and disorganization of lymphoid follicles and lymph architecture (figure 1B)—as well as the decrease in the numbers of CD4+ T cells that has been documented elsewhere [4]. After treatment, the numbers of Ki67+ T cells in the TZ decreased, whereas the number of Ki67+ B cells increased in secondary lymphoid follicles that had reformed after 1 month of HAART (figure 1D). Decreased immune activation, repopulation of the lymph nodes, and reformation of active secondary follicles are the expected

<table>
<thead>
<tr>
<th>Patient</th>
<th>Months HIV positive</th>
<th>Disease stage</th>
<th>Treatment regimen</th>
<th>CD4+ T cell count, cells/mm³</th>
<th>Plasma HIV RNA level, copies/mL</th>
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<td>1329</td>
<td>1</td>
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<td>32,173 702</td>
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**NOTE.** ABV, abacavir; EFV, efavirenz; IDV, indinavir; preSX, presymptomatic; RTV, ritonavir; 3TC, lamivudine.

* Patients who tested positive for HIV-1 RNA in plasma but negative for HIV antibody were considered to be in the acute stage of infection. Patients who were within 6 months of known (or observed) seroconversion were considered to be in the early stage of infection. Patients who had been infected for at least 6 months (based on the date on which they first tested positive for HIV-1 and their study entry date), who had CD4+ T cell counts >200 cells/mm³, and who had no AIDS-defining illness were considered to be preSX. Patients with CD4+ T cell counts <200 cells/mm³ or AIDS-defining illness were considered to have AIDS.

### Table 1. Demographic characteristics of HIV-1–infected patients and changes in plasma HIV-1 RNA and peripheral CD4+ T cell count in response to highly active antiretroviral therapy.
Figure 1. Histological and virological changes in lymphatic tissues from an HIV-1–infected patient receiving highly active antiretroviral therapy (HAART).

A, The diffuse pattern of bright dots in the circled germinal center (GC) is the signal after in situ hybridization from HIV-1 RNA in virions bound to the follicular dendritic cell (FDC) network. The arrow points to a bright, productively infected cell in which HIV-1 RNA has been found by in situ hybridization. (Original magnification, ×200.) B, Before the initiation of treatment, the no. of brown (immunohistochemically stained) activated proliferating Ki67+ T cells is high in the T cell zone (TZ) of a section of a lymph node biopsy specimen from a patient with AIDS. The no. of Ki67+ B cells on the GC is low in an involuted follicle. (Original magnification, ×200.) C, After 1 month of HAART, HIV-1 RNA–positive cells are no longer or only rarely detectable by in situ hybridization, and the signal from HIV-1 RNA in virions bound to the FDC network is greatly reduced. (Original magnification, ×40.) D, The no. of Ki67+ T cells is reduced. Secondary follicles have reformed with normal nos. of Ki67+ B cells. (Wright’s stain; original magnification, ×40.)

Comparison of Gene Expression Profiles in HIV-1–Infected Lymph Nodes from Patients with and without Treatment and Identification of a Set of Treatment-Responsive Genes

Comparing gene expression profiles from the serial lymph node biopsy specimens from treated or untreated patients provided an opportunity to determine whether a set of treatment-responsive genes existed. We found, in samples from the treated patients, that expression of ~200 genes of the ~12,000 represented in the U95A chip decreased or increased by a statistically significant amount (>1.7 times) [12] after treatment, and we verified by real-time PCR that there were changes of at least this magnitude or greater for 5 of the genes (signal transducer, activator of transcription 1 [STAT1], RANTES, X-linked inhibitor of apoptosis protein, and interferon [IFN]–γ) and a gene encoding a hypothetical protein (results of the real-time PCR are shown in e-figure 2, which is available in the online version of this article). The ~200 genes in the treated patients were largely unaltered in expression in the untreated patients, which justifies their designation as treatment responsive. In e-figures 3 and 4, which are available in the online version of this article, we provide information on the magnitude of the change in gene expression for each gene. Generally, this was in the range of 2 to ~10 times, with such notable exceptions as, at day 3, changes of 10 to >100 times in levels of macrophage inflammatory protein (MIP)–1β, IFN–γ, and collagen type 1. Although these larger changes may be particularly significant, we included all of the genes that met the “1.7 times” criterion...
in our functional analysis, because we think the smaller changes could have a combined effect that is just as important.

**Functional Categorization of HAART-Responsive Genes**

The comparison of treated with untreated patients reduced the data set from ~12,000 to ~200 genes—a more manageable but still complex and challenging set to analyze. We began by clustering the treatment-responsive genes according to their similarity in expression level. Figure 2 shows individual treated and untreated patients and the genes, with color depicting changes. Green indicates decreased expression; red, increased expression; and color saturation, the magnitude of the fold change. Black indicates insignificant change. The rows are ordered by the clustering results shown in the dendrogram. Looking vertically, the expression levels in individual treated patients contrast markedly with those in the untreated patients—evidence that there is a set of HAART-responsive genes. Looking horizontally at the annotations for the genes in the clusters of similar changes in expression, some categories were immediately evident. Notably, in the exploded segment in figure 2, genes such as granzymes, IFN-γ, STAT1, and CIQB are recognizable components of innate and adaptive immune system defenses. However, these and other components of the immune system were not functionally clustered together in the dendrogram of similar changes in levels of expression. Moreover, functions of many of the genes were not obvious from the limited annotations provided, and, in general, we found that this initial clustering method did not give us a very clear picture of how the genes might function in HIV-1 pathogenesis and LT reparative processes. To develop relevant functional categories, we undertook an extensive literature search and synthesized what we found, along with other contextual information, such as the histological changes illustrated in figure 1, to define the categories and assign genes to each category. These categories and genes are shown in figure 3.

**General Functional Categories and Increased or Decreased Expression of Genes after Initiation of HAART**

Overall, we found in the annotations, searched literature, and histological changes grounds to assign >80% of the treatment-responsive genes to generic categories relevant to pathogenesis and reparative processes. These included immune activation and defenses, inflammation, trafficking, repopulation, follicle reformation, and wound healing (figure 3). Below, we discuss in greater detail the major categories and assignments in figure 3.

**Immune defenses: prominent role for innate immunity in chronic infection.** We found that there were many treatment-responsive genes in the category of immune defenses. Expression of nearly all of the genes in this category decreased, which was consistent with the decrease in immune defenses seen after initiation of treatment. For example, the decreased expression of cytotoxic T lymphocyte–related genes (CD3, CD8, T cell receptor–γ, and BY55) correlates with the known decrease after treatment in HIV-1–specific CD8+ T cells [11].

However, the most striking finding in this category was the change in expression, extending beyond the early into the late stages of infection, of genes predominantly related to innate immunity: NK cell genes; complement (C') system genes; and 26 genes related to IFNs and to the STAT1 signal transduction pathway [13, 14], including IFN-γ, associated chemokines IFN-α–inducible protein (IP)–10 and monokine induced by INF-γ (MIG), STAT1, and IFN regulatory factors that activate IFN-α genes [15, 16], all known mediators of IFN antiviral effects [13], and stimulated trans-acting factor (50 kDa) (Staf50) and known repressor of HIV-1 gene expression [17].

**Immune activation.** There were nearly as many genes in the category of immune activation as in that of immune defenses. Again, expression generally decreased, which is consistent with the decrease in immune activation after treatment illustrated in figure 1. Within this category are genes encoding proinflammatory cytokines and early activation markers for NK, T, and B cells and genes encoding for signal transduction pathways that generate transcriptional regulators of immune cell activation, such as NF-kB and nuclear factor of activated T cells. There was also indirect evidence of NO production in the decrease of the rate-controlling enzyme for the synthesis of tetrahydrobiopterin, GTP cyclohydrolase, which is an essential cofactor for NO synthesis [18].

There was a parallel decrease in all but 2 genes that encode inhibitors and moderators of immune activation and inflammation, such as (1) known negative regulators of T and B cell activation and proliferation, lymphocyte activation gene 3 [19], CD163 [20], and indolamine dioxygenase; (2) negative regulators of the C' system and hypoxia-protectant intestinal trefoil factor [21] and other antioxidants and protectants against vascular injury; (3) protease inhibitors [22]; and (4) a phospholipase inhibitor of the potent inflammatory mediator platelet activating factor [23]. The increased expression of lectin galectoside binding soluble 1 (LGALS1) and glutathione peroxidase may indicate that these genes play a role in the resolution phase of immune activation.

**Tissue repair and remodeling.** We found that expression generally increased for genes that mediate LT repair. This category included genes encoding structural components of cells and extracellular matrix (β-tubulin, collagens, fibronectin, laminin, and proteoglycans), genes related to myofibroblasts that mediate wound healing [24], and transcriptional regulators of wound healing, cell proliferation, and differentiation, such as platelet-derived growth factor (receptor), insulin-like growth factor binding proteins, and osteoblast-specific transcription factor 2, a transcriptional activator of stromal cells that supports growth of hematopoietic cells [25].
Figure 2. Hierarchical clustering of treatment-related changes in lymphatic tissues from HIV-1–infected patients at 1 month after initiation of highly active antiretroviral therapy. A hierarchical clustering algorithm from DecisionSite (Spotfire) was used to create dendrograms of similar fold changes in gene expression. Black indicates that there were no significant changes in expression. Green indicates decreased expression, and red indicates increased expression, compared with baseline. The treatment status and stage of infection of individual patients are indicated at the top and bottom, respectively. Some of the genes for which expression decreased after treatment are identified in an exploded segment to the right. CRTAM, cytotoxic and regulatory T cell molecule; IFN, interferon; IP, IFN-γ–inducible protein; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; NKG7, NK cell group 7 sequence; OAS, oligoadenylate synthetase; preSX, presymptomatic; SECTM1, secreted and transmembrane 1; Staf50, stimulated trans-acting factor (50 kDa); STAT1, signal transducer, activator of transcription 1; TCR, T cell receptor.
Although expression of genes related to immune activation and inflammation generally decreased after treatment, expression of some of these genes increased. We interpreted these increases as evidence of an active alternative inflammatory pathway involved in tissue repair [26] and remodeling. This category includes macrophage activation–associated CC chemokine–1 and macrophage migration inhibitory factor, a proinflammatory cytokine, both of which are known to be involved in wound repair [27, 28], and genes encoding proteases that stimulate collagen synthesis (tryptase) [29] and metalloproteinases and their inhibitors, which mediate tissue remodeling.

**Trafficking, repopulation, reformation of lymphoid follicles, and apoptosis.** There were with HAART mixed increases and decreases in expression of genes encoding chemokine receptors and ligands, mediators of vascular adhesion, B cell proliferation and differentiation, and apoptosis. We categorized these genes by referring to known changes in trafficking, repopulation and apoptosis in LTs after HAART [4, 30, 31] and histological evidence of reformation of active follicles (figure 1).

Because we know that HAART alters trafficking between infected LTs and peripheral blood [4, 30, 31], we interpreted the decreased expression of chemokine and chemokine receptor genes (CCR1, CCR5, CXCR3, MIG, MIP-1β, RANTES, IP-9, IP-10, and lymphotactin) as evidence that these genes mediate reentry of T cells into the circulation after treatment. Conversely, we interpreted the increased expression of thymus- and activation-regulated chemokine, a ligand for CCR4 and a major trafficking receptor for memory T cells [32], as evidence that this gene could be one of the mediators of the repopulation of LTs by memory cells within the first few weeks after initiation of HAART [4]. We also know that there are fewer apoptotic CD4+ T cells in LTs after HAART [4], which reflects decreased expression of apoptotic genes, such as Fas ligand and phospholipid scramblase.

We interpreted the increased expression of (1) genes encoding a B cell homing receptor (Burkitt lymphoma regulator 1) that promotes B cell migration into follicles [33]; (2) several B cell genes related to maturation of high-affinity B cell receptors, such as transcriptional regulators (mybmyeloblastosis viral oncogene homolog 2 and inhibitor of DNA binding family members [34, 35]); and (3) immunoglobulin isotype switch recombination genes (SWAP and histone family member X [36]) as evidence that these genes were mediators of the reformation of active secondary follicles such as the one shown in figure 1D. Similarly, increased expression of apoptosis-promoting BCL-2 antagonist of cell death and BCL-2 interacting killer and of T cell leukemia/lymphoma 1A (the putative function of which is to protect naive preactivated B cells in the mantle zone from apoptosis [37]) could be related to modulated programmed cell death of B cells in the evolution of high-affinity receptors.

**DISCUSSION**

**Innate immunity and partial of control of HIV-1 infection in LTs.** The prominence of innate immunity genes in patients in the early to late stages of HIV-1 infection supports the conclusion that innate immunity may contribute to partial control of HIV-1 infection in LTs over the long course of the disease. This might be especially important in the late stages, as adaptive immune defenses wane. Ironson et al. [38] similarly concluded, on the basis of the preservation of NK cells and NK cell cytotoxicity in otherwise healthy HIV-infected individuals with low CD4+ T cell counts, that the innate arm of immunity may be a factor in this relatively rare group of patients who remain asymptomatic despite low CD4+ T cell counts.

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Figure 3. General functional categories and subcategories with lists of specific genes in those categories revealed by functional genomics analysis of the response of lymphatic tissue from HIV-1–infected individuals to highly active antiretroviral therapy (HAART). The general categories are indicated by labels over the boxes connected to the pie-sector diagram in the middle of the figure. The size of the sector is proportional to the nos. of genes in each category (in parentheses) whose expression was altered by HAART. Green letters indicate decreased expression after treatment; red letters indicate an increase in expression of ≥1.7 times the level at baseline. The genes shown in the figure increased or decreased in expression at 3 days or 1 month after the initiation of treatment or at both times (see e-figures 3 and 4, which are available in the online version of this article, for separate listings of changes at these time points). BAD, BCL-2 antagonist of cell death; BCL7A, B cell CLL/lymphoma 7A; BLR, Burkitt lymphoma receptor; BIK, BCL-2 interacting killer; CARD, caspase recruitment domain protein; CRTAM, cytotoxic and regulatory T cell molecule; DSPI, Δ sleep-inducing peptide immunoreceptor; FEN, flap structure–specific endonuclease; HSP, heat shock protein; ID, inhibitor of DNA binding; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IFTM, interferon-induced transmembrane; IL, interleukin; IP, IFN-γ–inducible protein; ITP, IFN-induced protein; IRF, IFN regulatory factor; ITF, intestinal trefoil factor; KLRG1, killer cell lectin-like receptor subfamily G, member 1; LAG, lymphocyte activation gene; LAMP, lysosomal-associated membrane protein; LGALS, lectin galactoside binding soluble; LRMP, lymphoid-restricted membrane protein; MIF, macrophage migration inhibitory factor; MIG, monokine induced by IFN-γ; MIP, macrophage inflammatory protein; MMP, matrix-like growth factor binding protein; MYB, myeloblastosis viral oncogene homolog; MYD, myeloid differentiation primary response gene; NKG7, NK cell group 7 sequence; NMI, N-myc (and STAT) interactor; OAS, oligoadenylate synthetase; OPN, osteopontin; OSF, osteoblast-specific factor; PSME2, proteosome activator subunit 2; RARRES, retinoic acid receptor responder; RGS, regulator of G-protein signaling; SERTM, secreted and transmembrane; STAT5, stimulated transacting factor (50 kDa); STAT1, signal transducer, activator of transcription 1; TCLA, T cell leukemia/lymphoma 1A; TCR, T cell receptor; TIMP, tissue inhibitor of metalloproteinase; XIAP, X-linked inhibitor of apoptosis protein.
Figure 4. Two balancing acts and the slow dynamics of HIV-1 infection in vivo without treatment. In this hypothetical model, it is postulated that 2 mechanisms account for the overall slowness (years) of the dynamics of infection in vivo, compared with the rapid progress (days) of infection in vitro, where replication is unchecked by immune defenses. A, In vitro, infection rapidly spreads through the culture, and the cells die from the cytopathic effects of replication. In vivo, immune defenses reduce the number of productively infected cells and virus production per cell to slow propagation. However, the transcriptional activators generated by immune activation enhance viral gene expression (indicated by the positive effects on the HIV-1 long terminal repeat) to enable continued replication. B, Inflammation associated with the chronic state of immune activation required to maintain immune defenses damages lymphatic tissues and depletes CD4+ T cells. The rate at which these pathological changes accumulate is slowed by moderators of inflammation. Both balances are tilted toward continued replication and pathology consistent with the development of AIDS without treatment. NFAT, nuclear factor of activated cells; PMBCs, peripheral blood mononuclear cells.
The functional genomic analysis of innate immunity suggests a way that NK cells might counter HIV-1 defense mechanisms. HIV-1 is thought to avoid detection by cytotoxic T lymphocytes and yet escape NK cell attack through the action of Nef in selectively down-regulating HLA-A and HLA-B but not HLA-C and HLA-E [39]. This leaves intact potential interaction with the inhibitory CD94/KLRC1 (killer cell lectin-like receptor subfamily C, member 1) receptor. However, the treatment-responsive NK receptor gene, CD94/NKG2E, is a putative activating receptor [40] that could enable NK cells to kill HIV-1–infected cells despite Nef expression.

The functional genomic analysis provides evidence that the type 1 and 2 IFN systems, as a whole, are engaged in the response to HIV-1 infection from the early to late stages. We documented expression of a large number of genes in the type 1 and 2 systems downstream of the STAT1 signal transduction pathway [13, 14], including (1) IFN-γ and its associated chemokines IP-10 and MIG; (2) STAT1 itself and its amplifiers N-myristoylated (and STAT) interactor and proteosome activator subunit 2 (PSME2); (3) IFN regulatory factors that activate IFN-α genes [15, 16]; (4) all known mediators of IFN antiviral effects [13]; and (5) Staf50, a known repressor of HIV-1 gene expression [17]. Thus, this analysis greatly expands the number of IFN and IFN-related genes known to be involved in the innate immune response to HIV-1 infection beyond the previously documented higher levels of IFN-γ in HIV-1–infected LTs (high compared with levels in enlarged lymph nodes from HIV-uninfected individuals). We speculate here, on the basis of the many IFN system genes revealed by microarray analysis, that these genes might act in concert to suppress viral replication. Furthermore, they may turn out to be CD8+ T cell–associated factors—plural rather than singular—that suppress HIV-1 replication. This speculative hypothesis is supported by the demonstration by Chang et al. [41] that CD8+ T cells inhibit HIV-1 replication by secreting a factor or factors that activate STAT1 and downstream expression of IFN system genes.

Two balancing acts and the slow dynamics of HIV-1 infection without treatment. By looking broadly at the functions of the genes listed in figure 3 under “Immune Activation” and “Immune Defenses,” we can envision mechanisms by which these genes might work together to account for the overall slowness of the dynamics of HIV-1 infection without treatment [42]. We propose a model (figure 4), based on functional genomic analysis, in which we attribute the slowness of the dynamics to 2 mechanisms: (1) slow propagation of infection in vivo and (2) slow accumulation of tissue damage from chronic immune activation and inflammation. In this model, chemokines with antiviral activity and innate and adaptive defenses reduce the size of the population of productively infected cells and decrease viral gene expression and production within infected cells, compared with unchecked replication in infected cultures (figure 4A). At the same time, these constraints are counterbalanced by the proinflammatory cytokines and immune activation required to maintain immune defenses. These enhance HIV-1 gene expression [7] and thereby enable continued propagation of infection. The pathological effects of chronic immune activation and inflammation are also counterbalanced by moderators in infected LTs discovered in our microarray analysis (figure 4B). In this model, it is hypothesized that they slow the accumulation of tissue damage and immune depletion. Both balances are slightly tilted toward continued replication and tissue damage, to be consistent with the continued production of virus and tissue damage without treatment.

Prospectus and concluding comments on gene profiling, HIV pathogenesis, and treatment. This global view of the gene expression changes that occur in LTs in response to HAART points, we think, to many promising avenues for future research: (1) profiling changes in response to HAART in depth in more patients at different stages of infection, (2) localizing changes in expression to specific cell types and anatomic sites and structures, and (3) distinguishing changes in expression per cell from changes that result from changes in the size of a population of a particular cell type.

There are also potential therapeutic implications of the counterbalancing of immune defenses and activation of mediators and moderators of inflammation (figure 4). The addition of anti-inflammatory agents that can be used safely for long periods might tip the balance, improve control of infection, and reduce the inflammatory pathology and collagen deposition that we have shown to compromise the ability of the LT niche to support immune cell homeostasis [8]. This might make it possible to prudently delay initiation of HAART and thus limit the expense and side effects of treatment. The balance between mediators and moderators of inflammation might also vary between HIV-1–infected individuals, to account for the variable rates of progression to AIDS that reflect different rates of accumulation of inflammation-associated tissue damage. If this proves to be the case, histopathological assessment and measurements of collagen in LN biopsy specimens, analogous to the management of cancer, could be used to assign stages to HIV-1 disease pathology. Such an assessment might make it possible to rationally decide on the optimal time to initiate HAART and to identify patients who would benefit, in addition to HAART, from cyclooxygenase-2 inhibitors and other agents that have been shown to reduce fibrosis [43] and scarring.

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