IL-22 Participates in an Innate Anti-HIV-1 Host-Resistance Network through Acute-Phase Protein Induction

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IL-22 Participates in an Innate Anti-HIV-1 Host-Resistance Network through Acute-Phase Protein Induction

Dorothée Misse,† Hans Yssel,‡ Daria Trabattoni,‡ Christelle Oblet,* Sergio Lo Caputo,§ Francesco Mazzotta,§ Jérôme Pène,† Jean-Paul Gonzalez,* Mario Clerici,‖ and Francisco Veas³*

Certain individuals are resistant to HIV-1 infection, despite repeated exposure to the virus. Although protection against HIV-1 infection in a small proportion of Caucasian individuals is associated with mutant alleles of the CCR5 HIV-1 coreceptor, the molecular mechanism underlying resistance in repeatedly HIV-1-exposed, uninfected individuals (EU) is unclear. In this study, we performed complementary transcriptome and proteome analyses on peripheral blood T cells, and plasma or serum from EU, their HIV-1-infected sexual partners, and healthy controls, all expressing wild-type CCR5. We report that activated T cells from EU overproduce several proteins involved in the innate immunity response, principally those including high levels of peroxiredoxin II, a NK-enhancing factor possessing strong anti-HIV activity, and IL-22, a cytokine involved in the production of acute-phase proteins such as the acute-phase serum amyloid A (A-SAA). Cell supernatants and serum levels of these proteins were up-regulated in EU. Moreover, a specific biomarker for EU detected in plasma was identified as an 8.6-kDa A-SAA cleavage product. Incubation of in vitro-generated myeloid immature dendritic cells with A-SAA resulted in CCR5 phosphorylation, down-regulation of CCR5 expression, and strongly decreased susceptibility of these cells to in vitro infection with a primary HIV-1 isolate. Taken together, these results suggest new correlates of EU protection and identify a cascade involving IL-22 and the acute phase protein pathway that is associated with innate host resistance to HIV infection. The Journal of Immunology, 2007, 178: 407–415.

The course of HIV-1 infection is known to vary widely among individuals. In addition to long-term nonprogressors, who are asymptomatic and have normal CD4+ T cell counts despite a long history of untreated HIV-1 infection, certain individuals, defined as persistently exposed but uninfected (EU), are resistant to HIV infection despite repeated exposure via sexual or systemic routes (1, 2). Certain rare polymorphisms in the gene encoding CCR5, the principal coreceptor for primary strains or HIV-1, have been shown to confer a high degree of protection against HIV-1 infection to a very small proportion of the Caucasian population who are homozygous for these gene variants (3–5). However, this particular genetic variation cannot account for resistance to HIV-1 infection in all EU who carry the wild-type CCR5 gene, indicating that other mechanisms of protection against viral infection must exist. Additional resistance to HIV-1 infection is reportedly associated with the activity of HIV-1-specific helper and CTL (2, 6, 7) and with enhanced local mucosal IgA responses (8). Moreover, an association between resistance to HIV-1 infection and enhanced NK activity (9), as well as increased production of IFN-γ produced by CD4 T cells (10), CD8 antiviral factors (11), and defensins (12) has been described. More recently, the frequencies of CD4+ and CD8+ CD45RA−CCR7+ central memory cells, as well as terminally differentiated CD8+ CD45RA−CCR7− and CD27−CD28− T lymphocytes, were reported to be increased in EU, compared with HIV-1-infected patients (13), suggesting a role for the latter cells in preventing actual infection. However, the overall understanding of the molecular mechanisms underlying resistance to infection of EU with HIV-1 remains ill-defined.

In the present study, we performed comparative transcriptome and proteome analyses on peripheral blood T cells and plasma or serum from EU, their HIV-1-infected sexual partners (HIV+), and healthy control subjects (HC) to investigate whether the differential expression of certain gene products by T cells from EU could be associated with long-lasting resistance to HIV-1 infection.

Materials and Methods

Patients and healthy controls

Twenty-one heterosexual couples discordant for HIV-1 serostatus and 21 HC were enrolled in the study, all Caucasians from the Tuscany region with no HIV-1 coreceptor polymorphism (13). In each case, the EU was the sexual partner of an HIV+ and in each couple a prolonged history of penetrative sexual intercourse without condom, but no other known risk factors, was reported. The female partner was HIV-1-infected in 10 couples, whereas the male partner was HIV-1-infected in the remaining 11 couples.
The inclusion criteria were a history of multiple unprotected sexual episodes for ≥5 years with at least three episodes of at-risk intercourse within 4 mo before the study point. Self-administered questionnaires showed that the couples reported an average of 34 unprotected sexual episodes/year (range 12 to >50) in the 5 years previous to the study. Vaginal intercourse was the rule and anal sex was not reported by any of the participants in the study. The HIV-1 status of all the EU individuals enrolled in this longitudinal protocol, assessed at regular scheduled visits (every 6 mo), was consistently negative using ELISA, Western blotting, and RT-PCR techniques. In all infected individuals, the diagnosis of HIV-1 infection was made during the chronic phase of infection and thus unprotected sexual intercourses had been initiated long before the diagnosis. Mean CD4+ T cell counts of the infected partners at the time of this study was 401 (range 16–1220). All EU, HIV+, and HC had been longitudinally followed for at least 5 years before the study period by the Department of Infectious Diseases and Virology of the Santa Maria Annunziata Hospital, Florence, Italy. This information allowed us to exclude EU and HC from the study in whom sexually transmitted diseases or other pathology had been reported during that time period. Moreover, during the regular scheduled examinations of this follow-up, none of the individuals from the EU group presented any particular clinical symptom that could be considered as a pathological sign. The research protocol was approved by the ethics committee of the above hospital. Written informed consent was obtained from all enrollees and clinical samples, rendered anonymous, were analyzed in a blinded fashion.

Cells

PBMC of the individuals of each group studied were isolated by centrifugation over Ficoll/Hypaque. T cells were generated following stimulation of the PBMC (2 µg/ml immobilized anti-CD3 mAb (SPV-T3b; Beckman Coulter) and 1 µg/ml anti-CD28 mAb (L293; BD Biosciences). Myeloid immature CD1a+, CD14+, CD83+, CD86+ dendritic cells (iDC; purity >97%) were generated following culture of purified peripheral blood monocytes for 4 days with 100 ng/ml rGM-CSF and 10 ng/ml rIL-4 (BruCells). T cells and iDC were cultured in Yssel’s medium (16) supplemented with 1% human serum and 10% FCS (Invitrogen Life Technologies), respectively.

Western blotting analysis

Surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) Protein chip arrays (Ciphergen) were used according to the manufacturer’s recommendations. Briefly, serum samples were diluted 10-fold in a binding buffer (250 mM NaCl and 50 mM HEPES (pH 7.5)) and applied to a strong anion exchanger (SAX2) chip. After incubation for 1 h at room temperature, unbound proteins were removed by three successive washes with 20 µl each with a buffer containing 1 mM EDTA and 50 mM HEPES (pH 7.5). Chip-captured proteins were air-dried and covered with a matrix (3,5-dimethoxy-4-hydroxycinnamic acid in 99.9% acetonitrile and 0.1% trifluoroacetic acid), used as an absorbent for laser energy. The ionized and desorbed proteins were detected and their molecular masses were determined on the proteogame peaks were determined using SELDI-TOF-MS analysis with the Protein-Chip Biology System II software (Ciphergen) and Ciphergen Peaks software. Depletion of the 8.6-kDa protein from EU plasma was achieved as follows: 25 µl of magnetic beads (Dynal) were washed three times with PBS, added to 25 µg of a rat anti-acute-phase serum amyloid A (A-SAA) or an isotype-matched control mAb (BioSource International) and incubated for 1 h at 4°C with orbital shaking. After rigorous washing of the mAb-coated magnetic beads with PBS, they were incubated with a pool of five EU plasma samples at 4°C for 3 h with orbital shaking. After removal of the beads by a magnetic device, 5 µl of EU plasma was analyzed by SELDI-TOF under the conditions described above.

Measurements of soluble A-SAA, IL-22, and peroxiredoxin II (PRDX2) levels

Myeloid iDC were stimulated with rA-SAAB (PeproTech) or MIP-1β (a gift of F. Baleux, Pasteur Institute, Paris, France). Cells were plated on ice for 20 min and lysed under intermittent with lysis buffer, consisting of 1% Triton X-100, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 15% glycerol, 5 mM EDTA, supplemented with 1 mM phenylsulfonyl fluoride, 5 µg/ml aprotinin, 5 µg/ml leupeptin, 1 mM sodium orthovanadate, and 1 mM EGTA. Phosphorylated proteins were precipitated and analyzed by Western blotting using a polyclonal anti-phosphoserine Ab (BD Biosciences) and antibodies previously described (20).

Phosphorylation of CCR5

A-SAA levels in serum were analyzed by chemiluminescence (SearchLight proteome analysis; Perbio). Soluble IL-22 levels from anti-CD3 and anti-CD28 mAb-activated freshly isolated PBMC and PRDX2 serum levels were assessed, respectively, using an ELISA from R&D Systems and an ELISA previously described (21).
In vitro HIV-1 infection of iDC

In vitro-generated myeloid DC were preincubated for 30 min with different concentrations of rA-SAA at 37°C, followed by incubation with the R5/X4 dual tropic primary isolate HIV-1 4757 (a gift from P. Lusso, Istituto San Raffaele, Milan, Italy), at a multiplicity of infection of 0.1. After an incubation for 2 h, the cells were both extensively washed and cultured for 4 additional days. HIV-1 p24 levels in cell supernatants were determined using a commercial ELISA (Beckman Coulter). In some experiments, immediately after viral incubation for 2 h, the cells were extensively washed, and viral load was assessed in cells by a real-time RT-PCR assay. Briefly, total RNA was isolated from 1/1100310⁵ cells by using the RNAble reagent according to the manufacturer’s specifications (Eurogentec). Twenty microliters of total RNA was used as the template for real-time PCR with the LightCycler RNA Master Hybprobe (Roche Diagnostics). The respective sequences of the forward and reverse primers from HIV-1/long terminal repeat region (22) were 5'-GCCTCAATAAAGCTTGCCTTGA-3' and 5'-GGCGCCACTGCTAGAGATTTT-3'. The real-time detection was performed using a 5' exonuclease assay with a FAM/TAMRA, dually labeled, internal long terminal repeat probe. Subsequent viral quantification was obtained using Roche LightCycler 3 software.

Statistical analysis

Quantitative data were analyzed using ANOVA followed by the Tukey-Kramer multiple comparison test with the GraphPad PRISM program (GraphPad Software). Data are expressed as mean ± SD.

Results

Activated T cells from EU overexpress transcripts of IL-22 and other proteins of the innate immune response

Comparative SAGE analysis on 20,000 gene tags per group was conducted on pooled libraries prepared from activated T cells with the aim of identifying gene products associated with resistance to HIV-1 infection. To target genes with different kinetics of expression, the three pooled libraries were prepared from cells stimulated for 2, 6, and 24 h via CD3 and CD28 and subjected to SAGE. Among the genes whose expression was up-regulated in EU figured STAT1, as well as the suppressor of cytokine signaling-1 (SOCS1) (Fig. 1). Indeed, results from Western blotting analyses confirmed that STAT and SOCS proteins were up-regulated and/or

FIGURE 1. Differentially expressed genes in activated T cells from HIV-1-exposed uninfected individuals, their HIV-1-infected sexual partners, and HCs. Transcription profiles of T cells, activated via CD3 and CD28 mAbs for 2, 6, and 24 h were subsequently pooled before analysis, generated from the three groups of EU, HIV⁺, and HC (n = 21) were analyzed by SAGE. Differences in relative levels of gene expression are indicated in color, where red indicates up-regulation and green indicates down-regulation.

FIGURE 2. Expression and activation of STAT/SOCS axis elements observed in HIV-1-exposed uninfected individuals, their HIV-1-infected sexual partners and HCs. Activated T cells from five randomly chosen individuals from each group were pooled and lysed, and protein status was analyzed by Western blotting STAT1 (A), STAT3 (B), SOCS1 (C). The relative equivalent signal per lane was corrected by stripping and reprobing the blot with an anti-α-tubulin Ab (lower panel) and detected using the ECL method. Data are representative results of two independent experiments.

FIGURE 3. PRDX2 serum levels from HIV-1-exposed uninfected individuals, their HIV-1-infected sexual partners, and HC individuals. Pooled serum samples taken from two sets of five randomly chosen individuals from each group were assessed for their content in PRDX2 by ELISA. Results are expressed as mean ± SD.
activated in EU (Fig. 2, A–C). Furthermore, these results also evidenced an increased expression of genes reported to be involved in both host defense activities such as granzyme B and antiapoptotic genes including TNFR superfamily 4 (TNFRSF4) and defender against apoptotic death 1 (DADI) (Fig. 1).

Taking into account genes with a strongly enhanced expression in EU as compared with HC and HIV\(^+\) T cells, two particular gene products, PRDX2 and IL-22, were identified (Fig. 1). In line with the results of the transcriptional analysis, the PRDX2 protein was also found to be up-regulated in EU sera from two subsets of randomly chosen individuals, as compared with sera from HC and HIV\(^+\) (Fig. 3). Whereas PRDX2 is a NK-enhancing factor (NKEFB) that is reported to exhibit a strong direct anti-HIV-1 activity (21), IL-22 is a cytokine with no known direct anti-HIV-1 activity. To individually validate the results of SAGE that showed the overexpression of IL-22 transcripts in EU compared with HC and HIV\(^+\), a comparative RT-PCR analysis was conducted on RNA from fresh and CD3/CD28-activated PBMC from two sets of five randomly chosen individuals of each group. Given the fact that preliminary experiments with T cells from healthy donors indicated that expression of IL-22 transcripts are induced in T cells as early as 2 h following activation increases over time, reaching maximal levels after 24 h, subsequent experiments were therefore conducted at the latter time point. Thus, using a minimal number of PCR-amplification rounds, differential expression of transcripts 24 h after activation was readily detected in EU, to a lesser extent in those from HIV\(^+\), and was absent in HC (Fig. 4A). To determine whether the enhanced mRNA expression correlated with increased secretion of IL-22 protein, freshly isolated PBMC from four randomly chosen individuals of each cohort were stimulated via CD3 and CD28, and IL-22 production was measured in the culture supernatants by cytokine-specific ELISA. PBMC from all individuals produced IL-22, and production levels of this cytokine did not differ between HC and HIV\(^+\). However, IL-22 production was enhanced in a statistically significant manner by T cells from EU, compared with those from HC and HIV\(^+\) (Fig. 4B). Moreover, results from RT-PCR analysis conducted on purified CD4\(^+\) and CD8\(^+\) T

<table>
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<tr>
<th>Table I. Immunovirologic and epidemiological characteristics of EU and HIV(^+)</th>
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<td>EU Individuals</td>
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cells from each cohort confirmed that PRDX2 was mainly expressed in CD8\(^+\) T cell subset and that the expression of IL-22 transcripts was exclusively observed in the CD4\(^+\) T cell subset (results not shown). These results are in line with the literature data reporting that IL-22 is produced by activated CD4\(^+\)CD45RO\(^+\) T lymphocyte subset and, to a certain extent, NK cells (23) and that PRDX2 is mainly produced by CD8 (21). In PBMC, as expected, the CD4-CD8 ratio from all HIV\(^+\) was inversed, as compared with that from the HC and EU (Tables I and II), whereas the population of memory CD4\(^+\)/CD45RO\(^+\) T cells from EU was proportionally increased (data not shown), as had already been demonstrated before for this particular cohort of individuals (13).

**Plasma from EU contains a specific 8.6-kDa cleavage product of A-SAA**

Results from a SELDI-TOF mass spectrometry analysis, conducted in parallel on plasma samples from all subjects of each group, revealed the presence of a soluble protein with a molecular mass of 8.6 kDa in plasma from EU that was absent in all plasma samples from the other groups (Fig. 5A). A search in the Swiss-Prot data bank (www.expasy.org) based on the exact mass of this peak showed that it matched, among eight possible proteins, a cleavage product of 76 N-terminal residues of a member of the

<table>
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<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD4-CD8 Ratio</th>
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<tbody>
<tr>
<td>EU</td>
<td>49.6 ± 9.6</td>
<td>27.8 ± 8.2</td>
<td>1.78 ± 0.14</td>
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<tr>
<td>HIV(^+)</td>
<td>21.2 ± 6.3</td>
<td>53.4 ± 7.8</td>
<td>0.62 ± 0.21</td>
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<tr>
<td>HC</td>
<td>54.2 ± 7.4</td>
<td>26.6 ± 6.3</td>
<td>1.66 ± 0.19</td>
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\(^a\) Mean ± SD.

FIGURE 5. Plasma from HIV-1-exposed uninfected individuals contains an 8.6-kDa A-SAA-cleaved fragment and serum contains elevated A-SAA levels. A, Protein profiles of serum samples from HC, EU, and HIV\(^+\) were determined by SELDI-TOF-MS, using strong anion exchanger (SAX2) protein chips. A protein peak at \(\sim 8.6\) kDa was specifically detected in all 21 EU plasma samples tested (five samples from each cohort are shown) but was absent in all samples from the other cohorts. B, A pool of EU plasma samples (\(n = 5\)) was incubated either with an mAb, specific for the 13.5-kDa unprocessed precursor of A-SAA, or with an isotype-matched control mAb coupled to magnetic beads and after removal of the beads reanalyzed by SELDI-TOF-MS. C, Pooled serum samples taken from two sets of five randomly chosen individuals from each cohort of EU, HIV\(^+\), and HC were analyzed for the presence of A-SAA by SearchLight proteome analysis. Data are expressed as mean ± SD of a typical experiment and similar results were observed in two separate experiments (\(*\ast, p < 0.01\) EU vs controls, NS = \(p > 0.5\) HC vs HIV\(^+\)).
13.5-kDa unprocessed precursor A-SAA family (UniProtKB/Swiss-Prot entry P02738), corresponding to amyloid A protein. The identification of the protein corresponding to the 8.6-kDa peak was confirmed by the use of an mAb specific for the A-SAA precursor that cross-reacts with the proteolytic products of this family. Incubation of five pooled EU plasma samples with this mAb, bound to magnetic beads, followed by the depletion of the mAb-protein complex, resulted in complete removal of the 8.6-kDa peak from the mass spectrometry protein profile (Fig. 5B), confirming that the protein is indeed derived from the A-SAA family. These results indicate that this cleavage product of A-SAA, present in the plasma of all EU tested, might serve as a specific biomarker for this particular group of individuals.

Serum levels of A-SAA are enhanced in EU

To correlate the presence of the 8.6 kDa cleavage product of A-SAA in EU plasma with enhanced production of their A-SAA levels, serum samples of the individuals from all three groups were analyzed for the presence of A-SAA by SearchLight proteome analysis. Basal serum levels of A-SAA in EU were strongly enhanced, compared with those of HC and HIV−, and thus mirrored the enhanced production of IL-22 by activated T cells of these individuals (Fig. 5C).

A-SAA inhibits in vitro HIV-1 infection in iDC

A-SAA is one of many agonists of a group of FPR that belong to the seven-transmembrane G-protein-coupled receptor family. Activation of FPR has been shown to modulate the expression and function of certain G-protein-coupled receptors such as the HIV-1 coreceptors CXCR4 and CCR5 by heterologous receptor desensitization, which is correlated with enhanced phosphorylation of serine residues present in the relevant receptor components (24). The functional effects of A-SAA were therefore analyzed on in vitro-generated myeloid iDC that, like their monocyte precursor cells, express FPR at their surface (results not shown) and that are susceptible to infection with HIV-1 via CCR5. rA-SAA induced CCR5 phosphorylation in myeloid iDC cells (Fig. 6A), whereas it also significantly down modulated CCR5 expression on the latter cells (Fig. 6B). Importantly, culture of myeloid iDC with the HIV-1 X4/R5 dual tropic primary isolate 4757, in the presence of increasing concentrations of rA-SAA, resulted in a decreased infection rate in a dose-dependent manner, compared with that of cells infected in the absence of this protein (Fig. 6C). As the effect of A-SAA appears to be due to FPR activation and HIV-1 coreceptor down-regulation, it would be expected that HIV-1 infection is restricted at viral entry. Indeed, as shown in Table III, viral load

Table III. Viral load after a 2-h exposure to HIV-1 following A-SAA cell pretreatment

<table>
<thead>
<tr>
<th>A-SAA Concentrations</th>
<th>Control† (0 μg/ml)</th>
<th>With A-SAA Preincubation† (0.5 μg/ml)</th>
<th>(50 μg/ml)</th>
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<tr>
<td>HIV-1 copies/10⁶ cells</td>
<td>261,500 ± 31,820</td>
<td>275,500 ± 10,607</td>
<td>3,867 ± 100</td>
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† Mean ± SD.

FIGURE 6. A-SAA decreases HIV-1 infection via CCR5. In vitro-generated immature myeloid dendritic cells were stimulated with rA-SAA. A. Serine phosphorylation of CCR5 was analyzed by Western blotting following stimulation of iDC for 1 min and 5 min with A-SAA; MIP-1β was used as a positive control. B, Cell surface expression of CCR5 was analyzed by flow cytometry: cells incubated with an isotype matched control mAb (a), cells preincubated with 10 μg/ml rA-SAA (b), or preincubated with culture medium alone (c) were stained with an FITC-conjugated anti-CCR5 mAb. Results are representative of three independent experiments. C, In vitro-generated myeloid dendritic cells were preincubated with rA-SAA for 1 h before infection with the R5/X4, dual tropic, primary isolate HIV-1 4757, and HIV-1 p24 levels were determined by ELISA after 4 days of culture. Results are expressed as mean ± SD.
was reduced by ~100-fold in iDC preincubated with A-SAA before infection with HIV-1, as compared with cells preincubated in the absence of A-SAA.

Discussion

Susceptibility to HIV-1 infection seems to vary significantly in the human population and a particular group of individuals has been identified who, despite frequent exposure via sexual or systemic routes, are resistant to infection with this virus (1, 2, 25, 26). In the present study, we investigated whether the differential expression of certain gene products by T cells from these frequently exposed, uninfected individuals could be associated with their long-lasting resistance to HIV-1 infection, that, for many of the individuals included in the study, surpasses more than a decade.

The results from the SAGE indicated that genes involved in host defense and in the innate immune response are either up-regulated in EU compared with HC and HIV+ groups or show a positive balance in favor of EU compared with HIV+. This is the situation for granzyme B, which is involved in host defense activities (27), and the antiapoptotic genes, including TNFRSF4 and DADI (28). Also this situation was found for some genes involved in the innate immune response that show a positive balance in favor of EU as compared with HIV+. Their protein up-regulation needed to be ascertained in clinical samples such as IFN-γ, that could confirm previously published results (29). We could confirm the up-regulation of both STAT1 and SOCS1. It can be noted that the STAT/SOCS axis participates in the regulation of inflammatory responses, controlling IFN-γ (30), and playing a key role in the control of infection with intracellular pathogens (31).

Results from SAGE of activated T lymphocyte also revealed two gene products, PRDX2 and IL-22, as genes having potential functional effects on protection against HIV-1 infection, with a strongly enhanced expression in EU, compared with HC and HIV+ T cells. The presence of PRDX2 has been reported in long-term nonprogressors, where it exhibits a strong, direct anti-HIV-1 activity in vitro (21). In contrast, IL-22 acts as an innate immune inducer; and because of its target cells, the only possible anti-HIV-1 activity of this cytokine is indirectly exerted by its downstream acute phase products. Independently, IL-22 and PRDX2 are capable of exerting, respectively, indirect and direct anti-HIV-1 activities. The results from the present study confirm that IL-22 mRNA is expressed by CD4+ and not by CD8+ T cells; however, transcripts for PRDX2 were detected in CD8 and to a lesser extent in CD4+ T cells (data not shown), indicating that both the CD4+ and CD8+ T cell subsets were targeted in this analysis.

Independent of sex, age, and HIV-1-exposure conditions (Table I), the observation of enhanced expression of IL-22 transcripts in T cells from EU was confirmed in freshly isolated PBMC. No differences in the kinetics of expression of IL-22 transcripts were found between T cells from the three cohorts: IL-22 mRNA was induced as early as 2 h following activation, increased over time, and reached maximal levels after 24 h of stimulation, thereby confirming a previous report in the literature (23). Moreover, increased transcription of the IL-22 gene correlated with the production of this cytokine, which was strongly enhanced in a statistically significant manner by T cells from EU, compared with those from HC and HIV+. In comparing EU and HIV+ with HC, memory CD4+CD45RO+ T cells were proportionally increased (13). Because IL-22 is produced by these cells, this observation could explain, at least in part, why HIV+ patients exhibit a level of IL-22 secretion comparable to the basal level produced by HC. This is also reflected at the transcriptional level where the relative proportion of IL-22 mRNA in HIV+ was found to be slightly increased compared with HC. It is therefore likely that HIV-1 infection will induce the production of IL-22, but that production levels will diminish, due to a progressive inversion of the CD4+/CD8+ ratio in individuals who develop AIDS, while being maintained in HC. Whether this increased production of IL-22 mRNA expression and protein production is linked to a genetic polymorphism in the regulatory sequences of the promoter region of the gene encoding this cytokine remains to be established, but this is a likely possibility, given the differences in IL-22 production between HC and EU following polyclonal stimulation of their T cells.

The IL-22R is expressed on epithelial cells of various tissues, including skin, colon, liver, lung, and pancreas (32). In hepatocytes and epithelial cells, IL-22 strongly up-regulates the production of acute-phase serum proteins such as A-SAA, β-defensins 2 and 3, α-1 antichymotrypsin, and haptoglobin (33–35). Considering that the expression of IL-22R in keratinocytes was up-regulated by IFN-γ (32) and that a positive balance of the expression of IFN-γ in EU compared with HIV+ and IL-22 increased precisely in EU as shown in this work, it could be suggested that IL-22 might exert a more efficient local action on its target cells.

Increased production of IL-22 by EU T cells was reflected in a specific increase in secretion levels of the A-SAA protein in these individuals, confirming an association between the biological functions of IL-22 and that of A-SAA. It is of note that the secretion of A-SAA by hepatocytes and epithelial cells is not only induced by IL-22 but also by several other proinflammatory cytokines such IL-1β, IL-6, and TNF-α (36). However, as the SAGE results did not reveal high transcription levels of these latter cytokines, it is likely that the observed A-SAA levels result principally from the action of IL-22. In addition to a specific increase in A-SAA serum levels in EU, plasma of these individuals also contained a soluble protein of 8.6 kDa, derived from the A-SAA family that was absent in all plasma samples from the other groups, thereby constituting a highly specific biomarker for EU individuals. A-SAA is the product of two hyperinducible, highly conserved genes, SAA1 and SAA2, which share 95% overall nucleotide identity in their exon, intron, and promoter regions (35). The expression of the two isoforms A-SAA1 and A-SAA2 is strongly induced during the acute phase of inflammation, playing a beneficial role in host innate defense, and both proteins are coordinately released from the liver into the circulation. However, prolonged high expression levels of A-SAA in the circulation may have deleterious effects, notably, secondary amyloidosis, characterized by fibril deposits that consist of amyloid A fragments and that affect multiple tissues and organs. In fact, A-SAA is degraded in the various tissues by a large number of cell-associated and serum proteases, which generate its major metabolic cleavage products, A1 and A2, the A-SAA2 protein of 8.6 kDa, derived from the A-SAA family that was absent in all plasma samples from the other groups, thereby constituting a highly specific biomarker for EU individuals. A-SAA is the product of two hyperinducible, highly conserved genes, SAA1 and SAA2, which share 95% overall nucleotide identity in their exon, intron, and promoter regions (35). The expression of the two isoforms A-SAA1 and A-SAA2 is strongly induced during the acute phase of inflammation, playing a beneficial role in host innate defense, and both proteins are coordinately released from the liver into the circulation. However, prolonged high expression levels of A-SAA in the circulation may have deleterious effects, notably, secondary amyloidosis, characterized by fibril deposits that consist of amyloid A fragments and that affect multiple tissues and organs. In fact, A-SAA is degraded in the various tissues by a large number of cell-associated and serum proteases, which generate its major metabolic cleavage products, A1 and A2. The A-SAA2 precursor seems to have little or no amyloid, fibril-forming potential in vitro, as compared with A-SAA1, supporting the observation that there is a predominant deposition of A1 in human amyloidosis (37). Our in-progress studies should permit discrimination between the two isoforms on the basis of sequence analysis of the 8.6-kDa protein. Moreover, exhaustive clinical examination showed that none of the EU presented any clinical signs of secondary amyloidosis and, moreover, did not have elevated levels of other inflammatory markers such as C-reactive protein. Consequently, it seems that the serum concentrations of A-SAA in EU, which are much lower (between 20- and 100-fold) than those detected in acute inflammation, do not have detectable pathological effects.

A-SAA is one of many agonists of a group of FPR that belongs to the seven-transmembrane G protein-coupled receptor family. FPR and its variant FPR-like 1 are highly expressed on neutrophils and monocytes and are involved, via their interaction with...
N-formyl peptides, in the trafficking of phagocytes to sites of bacterial invasion or tissue damage (38). However, the function of FPR is more complex, as signaling events mediated by these receptors via their interaction with a wide variety of ligands are associated with different diseases, including amyloidosis, Alzheimer’s, and prion diseases. Furthermore, activation of FPR modulates the expression and function of certain GPCR-protein-coupled receptors, such as the HIV-1 coreceptors CXCR4 and CCR5 and consequently reduces HIV-1 infection, in iDC, through heterologous receptor desensitization (24). The latter process correlates with the degree of phosphorylation of serine residues present in the relevant receptor components. The present study shows that A-SAA induces CCR5 phosphorylation in myeloid iDC, which results in a strongly decreased infection rate of iDC that express, like their monocyte precursors, FPR at their surface. These latter indirect effects on CCR5 could be compared with direct effects of the natural CCR5 ligands, MIP-1β and RANTES, on both CCR5 cell surface expression down-regulation and decreased signaling capacity in response to both ligands (39).

It can be noted that polymorphisms in the CCR5 gene were shown to confer a high degree of resistance to infection with HIV-1 (3–5). However, all EU included in the present study were homozygous for the wild-type form of CCR5 (data not shown), indicating that the possible protective effect of IL-22 to HIV-1 infection acts via a mechanism independent of CCR5 gene polymorphism. Recently, a significant association was reported between genotypes on chromosome 22q12-13 and a putative dominant locus conferring anti-HIV-1 immune responses in several of the Italian EU included in the present study. In contrast, distributions of linkage disequilibrium across chromosome 22 also were found to differ among EU and HIV + and HC (40). As the IL-22 gene is located on chromosome 12q15 in humans (41), the genetic factor(s) possibly underlying the overproduction of IL-22 in EU could be unrelated to these genotypes. Enhanced IL-22 production by activated T cells of these individuals is thus likely to contribute to their resistance to HIV-1 infection, although the possible genetic nature underlying this protective effect, orchestrated by IL-22, remains to be determined.

Our complementary analyses from cell transcripts, serum, and plasma revealed the presence of up-regulated innate immune proteins that could be considered as HIV protection correlates in EU individuals. These complementary analyses provide clear evidence, for the first time, that IL-22, an innate immunity inducing produced by CD4 memory T cells, was the most importantly up-regulated gene and secreted protein in EU compared with both HIV + and HC. A-SAA, an IL-22 downstream acute-phase protein having desensitization and down-modulation activities on HIV-1 coreceptors and consequently having anti-HIV-1 activity (24), was also up-regulated in EU serum compared with HIV + and HC. Additional evidence for the involvement of IL-22 in innate host responses to HIV-1 infection is provided by the observation that this cytokine also induced a 3- and 10-fold up-regulation of expression of the transcripts of β-defensins 2 and 3 transcripts, respectively, in cervical epithelial cells, whereas β-defensin 1 was not induced (data not shown), confirming the results obtained with cells from other origins (32). These acute-phase proteins are reported to have strong anti-inflammatory activity on HIV-1/X4-tropic isolates (42). In conclusion, our data suggest that the IL-22-induced cascade, together with the other innate immune proteins revealed by these complementary studies, including PRDX2, IFN-γ, and the proinflammatory regulatory STAT/STAT6 axis, could contribute to a concerted and enhanced local immune response, defining, at least in part, a complex network that protects EU against HIV-1 infection.

IL-22-MEDIATED RESISTANCE TO HIV-1 INFECTION

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