Release of Anti-HIV Mediators after Administration of Leukotriene B₄ to Humans

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Background. CD8⁺ T cells can control human immunodeficiency virus (HIV) through the lysis of infected cells and the release of soluble mediators, such as macrophage inflammatory protein (MIP)–1β, which prevent entry of HIV and/or inhibit HIV replication. Because neutrophils represent a major source of α-defensins and, to a lesser extent, MIP-1β, we determined whether leukotriene B₄ (LTB₄), a potent neutrophil agonist, would trigger the release of these 2 anti-HIV peptides.

Methods. Plasma samples from HIV-uninfected subjects receiving intravenous bolus of LTB₄ were analyzed for α-defensins and MIP-1β levels by use of enzyme-linked immunosorbent assay. Furthermore, in vitro analysis of intracellular and secreted levels of α-defensins of resting and LTB₄-activated neutrophils from HIV-uninfected and HIV-infected subjects were determined. LTB₄ modulation of CD63 and CD66b markers associated with degranulation were studied by use of flow cytometry. Chemotaxis of neutrophils from HIV-uninfected and HIV-infected subjects toward LTB₄ or interleukin (IL)–8 was determined by use of migration assays.

Results. Administration of LTB₄ to humans caused a dose-dependent plasmatic increase in α-defensins and MIP-1β proteins, with peak levels observed 2 h after administration of LTB₄. Neutrophils isolated from HIV-infected and HIV-uninfected subjects contained similar levels of stored α-defensins that were effectively secreted in vitro, in response to LTB₄ activation. Chemotaxis of neutrophils toward LTB₄ or IL-8 was identical among the groups of subjects.

Conclusion. LTB₄ induced the secretion α-defensins and MIP-1β. Neutrophils from HIV-infected subjects were fully responsive to LTB₄, which highlights a potential usefulness of this lipid mediator in the management of HIV infection.
tomegalovirus, and vesicular stomatitis virus), including that against HIV, have been recognized for years [5–9]. In most instances, damages to the bacterial cell membrane and viral envelope appear to be responsible for the antimicrobial activities of α-defensins. However, in the case of HIV, the precise inhibitory mechanisms of α-defensins are not fully understood. A recent report suggested that inhibition of HIV-1 by α-defensins occurs at a step subsequent to viral entry [10]. The major cellular producers of such peptides are neutrophils, which are the most prevalent circulating leukocyte type. α-Defensins are stored within cytoplasmic granules and are released in the extracellular milieu after appropriate stimulation through a process known as “degranulation.” Neutrophil granules are defined as primary granules, which house α-defensins, and secondary granules, whose contents include lysosomal enzymes. Because of the potent antimicrobial activity of the compounds that comprise these granules, the ability of neutrophils to release the content of these granules is essential in the fight against microbes. α-Defensins are the most abundantly expressed neutrophilic proteins, making up to 5% of the total cellular protein content. In addition to α-defensins, neutrophils are known to secrete various other immunomodulatory cytokines, including other anti-HIV mediators, such as macrophage inflammatory protein (MIP)–1β [11–13].

Neutrophils isolated from HIV-infected subjects are reported to have functional defects, such as reduced or impaired chemotactic responses [14–16], diminished phagocytic activities [17, 18], and decreased biosynthesis of leukotriene B4 (LTB4) [19–21]. These functional defects are suspected to account, at least in part, for the development of bacterial and fungal opportunistic infections observed during AIDS. Administration of granulocyte colony-stimulating factor (G-CSF) to HIV-infected subjects partially restores potential neutrophil biosynthesis of LTB4 and antimicrobial activity of LTB4, the latter being strictly dependent on the former [20]. LTB4 is a 20-carbon, tetraunsaturated fatty acid derived from arachidonic acid and produced mostly by activated macrophages and neutrophils; biosynthesis of LTB4 also has been reported to occur in basophils, mast cells, and B lymphocytes (as well as in other a few cell types) [22, 23]. LTB4 activates leukocytes through specific interactions with at least 2 distinct cell-surface receptors (leukotriene B4 receptor 1 and 2 [BLTR1 and BLTR2]) to which it binds with high affinity [24–26]. The presence of LTB4 receptors has been clearly demonstrated in several human hematopoietic cells, including monocytes, granulocytes, and lymphocytes, and in several hematopoietic cell lines [24–26]. LTB4 stimulates a number of phagocyte functional responses that are important in host defense, including secretion of lysosomal enzymes, activation of NADPH oxidase activity, formation of nitric oxide, and phagocytosis. LTB4 also has been shown to stimulate the expression of the β2-integrin (CD11b/CD18), an effect that is probably related to the ability of LTB4 to stimulate migration and phagocytosis of leukocytes [27, 28]. LTB4 also is reported to increase the cytotoxic activity of human NK cells [29, 30]. LTB4 activates proliferation of B lymphocytes and stimulates formation of antibodies in vitro [31, 32]. Finally, a beneficial role of leukotrienes in host defense against viral infections, other than HIV, also is also reported [33], which suggests that these lipidic mediators probably are important for an effective immune antiviral response.

In light of the recent findings that described a potentially important role for α-defensins in the host defense against HIV and because neutrophils are, by far, the major source of such peptides, our objectives in the present study were as follows: (1) to determine, in the context of a phase 1 clinical trial, whether administration of a potent neutrophil activator, such as LTB4, to HIV-uninfected subjects would trigger the release of anti-HIV peptides (α-defensins and MIP-1β); (2) to determine whether neutrophils isolated from HIV-infected subjects have normal levels of stored α-defensins; and (3) to assess the LTB4-responsiveness of neutrophils isolated from HIV-infected subjects by measuring the in vitro release of α-defensins, the modulation of cell-surface markers (CD63 and CD66b), and the chemotactic activity after exposure to this bioactive lipid.

MATERIALS AND METHODS

In vivo release of α-defensins and MIP-1β after administration of LTB4 to HIV-uninfected subjects. Plasma samples were obtained from 19 HIV-uninfected human subjects taking part in a phase 1 clinical study on the safety and tolerability of an intravenously (iv) administered bolus of LTB4. In brief, 3 subjects received saline (placebo), and 16 subjects (4 subjects/group) received LTB4 in doses ranging from 0.05 μg/kg/day to 50 μg/kg/day for 7 consecutive days. Venous blood samples were obtained twice before injection (5 and 2 min before injection) to establish baseline values. After iv administration of saline or LTB4, blood samples were obtained at 0.5, 1, 2, 4, 6, and 24 h after injection into blood-collection tubes, using EDTA as an anticoagulant. Immediately after blood collection, the samples were put on ice until processed. All plasma isolation procedures were performed at 4°C. Plasma samples were stored at −80°C until assayed by use of ELISA for α-defensins (HyCult Biotechnology bv) and MIP-1β (Amersham Pharmacia). The present study complied with relevant guidelines for human experimentation, and all subjects provided informed consent.

Isolation of polymorphonuclear leukocytes (PMNLs). Venous blood samples (30 mL) were obtained from HIV-uninfected and HIV-infected subjects and were obtained in heparinized collection tubes. PMNLs were isolated as described elsewhere [34], and suspensions contained >98% neutrophils.

Detection of intracellular α-defensins. For detection of
intracellular α-defensins, PMNLs from HIV-uninfected and HIV-infected subjects were fixed with 2% paraformaldehyde and were permeabilized in 2 mL of cold 80% ethanol solution. After an overnight incubation at −20°C, cells were washed with 0.1% Triton X-100 in PBS and were resuspended in 0.1 mL of wash buffer, followed by the addition of 0.5 μg of biotinylated-α-defensin monoclonal antibody (MAb; HyCult Biotechnology bv) or with a biotinylated isotype-matched control antibody. Cells were incubated for 45 min on ice, washed, and resuspended in 0.1 mL of Hank’s balanced salt solution (HBSS) containing 2.5 μg/mL phycoerythrin (PE)–labeled streptavidin (Roche Pharmaceuticals). After 40 min on ice, cells were washed with 0.1% Triton X-100 and were resuspended in PBS containing 1% paraformaldehyde. PMNLs (1 × 10⁶ cells/mL) were analyzed by use of flow cytometry.

**In vitro release of α-defensins.** Aliquots of cell suspensions (0.5 mL; HBSS with Ca²⁺ and 20% fetal bovine serum [FBS]) containing 3 × 10⁶ PMNLs from HIV-uninfected and HIV-infected subjects were distributed in a 48-well plate. The cells were allowed to sediment and rest at 37°C for 1 h. Next, 60% of the volume was replaced with fresh medium supplemented with cytochalasin B (final concentration, 10 μmol/L), and the cells were incubated at 37°C for an additional 30 min. PMNLs were stimulated in triplicate for each condition by adding 0, 10⁻⁷, and 10⁻⁶ mol/L final concentrations of LTB₄. Cell suspensions were collected after 30 min and centrifuged at 4°C, and cell-free supernatants were frozen at −80°C until assayed for α-defensins.

**Cell-surface expression of CD63 and CD66b on of PMNLs.** PMNLs (10⁶ PMNLs/tube) for HIV-uninfected and HIV-infected subjects were primed with cytochalasin B (final concentration, 10 μmol/L) at 37°C for 30 min, which was followed by stimulations with 0 (mock) or 10⁻⁶ mol/L LTB₄. After 30 min at 37°C, 6 mL of ice-cold PBS was added to each tube. PMNLs were centrifuged and resuspended in 0.1 mL of PBS containing PE-labeled anti-CD63 and fluorescein isothiocyanate (FITC)–labeled anti-CD66b or PE- and FITC-labeled isotype-matched control antibodies. Cells were incubated on ice for 30 min and then were washed and fixed with 0.5 mL of PBS containing 1% paraformaldehyde. PMNLs (1 × 10⁴ cells/mL) were analyzed for expression of CD63 and CD66b by use of flow cytometry, using the isotype-matched antibodies as controls.

**Chemotaxis assays.** PMNL migration assay was performed by use of a disposable 96-well chemotaxis chamber with a 3-μm pore-size polycarbonate filter (NeuroProbes). Freshly isolated PMNLs (3 × 10⁶ cells/mL) from HIV-uninfected and HIV-infected subjects were resuspended in RPMI 1640 medium containing 10% FBS (complete medium), were loaded with 5 μg/mL of the fluorescent Calcein-AM probe, and were incubated at 37°C for 30 min. Loaded PMNLs were resuspended in complete medium (3 × 10⁶ cells/mL). The wells of the 96-well chemotaxis plate were filled in duplicate with a control solution (PBS) or with LTB₄ or IL-8 (10⁻¹⁸–10⁻⁸ mol/L). The polycarbonate membrane was placed over the wells, and 75,000 PMNLs/well were added on top of the membrane. Cells were allowed to migrate for 60 min. Cells that did not migrate through the filter were removed gently by scraping. The filter was analyzed by use of a fluorescence reader (Bio-Tek Instruments), using excitation and emission wavelengths of 485 and 530 nm, respectively. Fluorescence intensities were proportional to the number of cells that have crossed the filter.

**Statistical analysis.** Statistical analyses were performed by use of analysis of variance.

**RESULTS**

**Release of α-defensins and MIP-1β after administration of LTB₄ to HIV-uninfected subjects.** Zhang et al. [2] recently identified α-defensins as components of the anti-HIV factors secreted by activated CD8⁺ T cells. Because neutrophils represent a major cellular source of α-defensins, plasma samples from HIV-uninfected subjects participating in a phase 1 clinical study on the safety and tolerability of iv administration of LTB₄ were examined for α-defensins levels. Subjects were randomized into 5 groups: 1 placebo group (n = 3) and 4 LTB₄ groups, depending on the dosage (0.05 μg/kg [n = 4], 0.5 μg/kg [n = 4], 5 μg/kg [n = 4], and 50 μg/kg [n = 4]). LTB₄ was administered once daily for 7 consecutive days, and plasma samples (obtained 0–24 h after administration of LTB₄) from all 19 subjects were tested for the presence of α-defensins by use of a commercial ELISA. The mean ± SE level of α-defensin (nanograms per milliliter of plasma) for each group are presented in figure 1, with statistical analysis performed by use of the placebo control group. Plasma samples obtained before administration of either placebo or LTB₄ indicated that there were no significant differences in the baseline levels of α-defensins among the groups. In striking contrast to the placebo group, in vivo administration of LTB₄ triggered a dose-dependent release of α-defensins in the plasma of subjects. The lowest dose of LTB₄ (0.05 μg/kg) did not cause release of α-defensins. The second lowest dose of LTB₄ (0.5 μg/kg) caused a measurable, but not significant, increase in plasma levels of α-defensins, with maximum detection at 2 h after administration of LTB₄. The 5-μg/kg and 50-μg/kg doses of LTB₄ triggered a significant release of α-defensins at 30 min after administration of LTB₄. Levels of α-defensins peaked (5–7-fold over the placebo group) at 2 h (P < .001) and remained above the control levels (P < .05) for up to 6 h after LTB₄ administration. Levels of α-defensins returned to the predadministration levels when assayed 24 h after administration of LTB₄ (data not shown).

Because soluble anti-HIV factors include β-chemokines and because neutrophils are capable of producing MIP-1β, we mea-
Figure 1. Dose-dependent release of α-defensins after administration of leukotriene B4 (LTB4) to HIV-uninfected subjects. HIV-uninfected subjects received a placebo (n = 3 subjects) or varying doses of LTB4 (0.05–50 μg/kg; n = 4 subjects/group). Plasma samples were obtained at various time points before and after LTB4 injections, and α-defensin levels were determined by ELISA. Data are the mean ± SE of α-defensins (ng/mL of plasma) of all subjects within each group. *P < .001; **P < .05.

sured the levels of this β-chemokine in plasma samples obtained from subjects who received a placebo or LTB4 (same cohorts as mentioned above). The data, expressed as the mean ± SE of the plasma MIP-1β (picograms per milliliter) levels, are presented in figure 2. Our data indicate that plasma MIP-1β levels determined before administration of placebo or LTB4 were identical. In contrast to the placebo group, subjects who received LTB4 (5–50 μg/kg) showed an elevation in their plasma MIP-1β levels, beginning at 1 h and reaching maximum levels at 2 h after administration of LTB4. At 6 h after administration of LTB4, plasma MIP-1β had returned to original levels. Although the kinetics of MIP-1β production and accumulation were very similar for both groups, statistical significance was observed only for those who received the highest dose of LTB4, a likely consequence of the small number of subjects (n = 4) in each group. Plasma samples obtained from subjects who received the 2 lowest doses of LTB4 were not tested for MIP-1β.

Intracellular levels of α-defensins and their release by neutrophils of HIV-uninfected and HIV-infected subjects. The results presented in figure 1 show that LTB4 is very effective at promoting the release of α-defensins in the plasma of HIV-uninfected subjects. However, cells from HIV-infected subjects often have functional defects that prevent the extrapolation of our in vivo results from HIV-uninfected to HIV-infected subjects. Therefore, we determined and compared the levels of α-defensins released from LTB4-activated neutrophils isolated from HIV-uninfected and HIV-infected subjects. Venous blood samples obtained from 42 subjects (13 HIV-uninfected and 29 HIV-infected subjects) were used in the present study. All but 3 HIV-infected subjects were receiving highly active antiretroviral therapy (HAART) at the time of sampling. The HIV-infected subjects were divided into 2 groups on the basis of whether their CD4+ cell counts were <200 or ≥200 cells/mm³. For subjects with a CD4+ cell count <200 CD4+ cells/mm³ (n = 18), the median CD4+ cell count in blood was 109 cells/mm³, and the median HIV load in plasma was 18,870 HIV RNA copies/mL. Only 3 of these subjects maintained a virus load below the detection limit (50 HIV RNA copies/mL of plasma). For subjects with blood CD4+ cell counts ≥200 CD4+ cells/mm³ (n = 11), the median CD4+ cell count was 280 cells/mm³, and the median HIV load was <50 HIV RNA copies/mL.

We first performed the intracellular detection of α-defensins in permeabilized neutrophils by use of a biotin-labeled Mab specific for α-defensins 1–3 and PE-labeled streptavidin. Cells were analyzed by use of flow cytometry, using a biotinylated isotype-matched antibody as control. Representative results of 1 donor from each group are presented in figure 3. The anti-α-defensins MAb reacted strongly with the neutrophils of subjects from all 3 groups, which suggests that the stored intracellular levels of α-defensins were similar for all subjects and were independent of their HIV status.

Having determined that the intracellular levels of α-defensins were similar for all groups investigated, we next compared the
LTB₄ Induces Release of α-Defensins

Figure 3. Intracellular α-defensins levels in neutrophils from HIV-uninfected (HIV⁻) and HIV-infected (HIV⁺) subjects. Neutrophils were isolated from HIV⁻ subjects (top), HIV⁺ subjects with CD4⁺ cell counts <200 cells/mm³ (middle), and HIV⁺ subjects with CD4⁺ cell counts >200 cells/mm³ (bottom); permeabilized; and stained by use of anti-α-defensins (black curve) or isotype-matched control antibodies (white curve). A representative result from 1 subject belonging to each group is presented. PE, phycoerythrin.

Figure 4. In vitro triggered release of α-defensins from neutrophils by leukotriene B₄ (LTB₄). Isolated neutrophils from HIV-uninfected (HIV⁻) subjects (n = 11), HIV-infected (HIV⁺) subjects with CD4⁺ cell counts <200 cells/mm³ (n = 15), and HIV⁺ subjects with CD4⁺ cell counts >200 cells/mm³ (n = 8) were stimulated with 0 (mock), 10⁻⁷, or 10⁻⁶ mol/L LTB₄ for 30 min, and then secreted α-defensins levels were determined. Data are mean ± absolute error mean. *P<.007, vs. mock control value.

ability of neutrophils from HIV-uninfected and HIV-infected subjects to release α-defensins after exposure to LTB₄. Isolated neutrophils from HIV-uninfected and HIV-infected subjects (>98% pure) were incubated in the absence or in the presence of LTB₄ (10⁻³–10⁻⁷ mol/L) for 30 min, and the release of α-defensins was determined, as described above. The summary of results, expressed as mean ± absolute error mean, is presented in figure 4. The results obtained indicate that stimulation of neutrophils with 10⁻⁷–10⁻⁵ mol/L LTB₄ triggers a significant dose-dependent release of α-defensins. Neutrophils from all groups responded equally to stimulation with LTB₄, with no statistically significant differences (P > .01) in the quantity of α-defensins released. The only statistically significant (P = .006) difference among the groups was obtained when we analyzed the spontaneous release levels of α-defensins, which is higher for the HIV-infected subjects with CD4⁺ cell counts ≥ 200 cells/mm³ (51.7 ± 12.8 ng/mL) than for the HIV-uninfected subjects (28.6 ± 12.5 ng/mL). Lower concentrations of LTB₄ (10⁻⁷–10⁻⁵ mol/L) proved to be ineffective in promoting α-defensins release by neutrophils. Furthermore, in the absence of cytochalasin B priming, neutrophils secreted marginal amounts of α-defensins in response to LTB₄ (10⁻⁷–10⁻⁶ mol/L; data not shown), highlighting major differences between the in vivo and in vitro requirements needed for proper neutrophil activation.

Primary and secondary granule release after neutrophil stimulation with LTB₄. As mentioned above, neutrophils release their intracellular granules content when appropriately
activated. Such release can be determined by quantifying the expression levels of specific primary (CD63) and secondary (CD66b) granule markers on neutrophil surface. Isolated neutrophils from HIV-uninfected and HIV-infected subjects were incubated in the absence or in the presence of $10^{-6}$ mol/L LTB$_4$ for 30 min and were analyzed for expression of CD63 and CD66b. Data (mean ± absolute error of the mean) from 13 HIV-uninfected subjects, 12 HIV-infected with CD4$^+$ cell count <200 cells/mm$^3$, and 10 HIV-infected subjects with CD4$^+$ cell counts $\geq$200 cells/mm$^3$ are presented in figure 5, top. On average, for all 3 groups of subjects, the percentage of neutrophils expressing the CD63 antigen under resting conditions varied between 5% and 7%. After administration of LTB$_4$, a significant ($P \leq .0153$) increase in the percentage (mean, 30%) of CD63-expressing neutrophils was observed. This increase in CD63, which reflects the release of primary granules, is in agreement with our $\alpha$-defensin results presented in figure 4. There were no differences ($P > .55$) in expression of CD63 (spontaneous or induced) between neutrophils isolated from HIV-uninfected and those obtained from HIV-infected subjects.

Neutrophils from the same subjects were tested for expression of CD66b. Because expression of CD66b is constitutive, the mean fluorescence intensity (MFI) of CD66b was used for comparative purposes. Isolated neutrophils expressed similar levels of CD66b, with no statistical differences among the 3 groups ($P > .42$; figure 5, bottom). After administration of LTB$_4$ ($10^{-6}$ mol/L), statistically significant ($P < .05$) cell-surface increases in expression of CD66b were detected on neutrophils isolated from subjects belonging to each group, regardless of their HIV status. No difference ($P > .41$) in CD66b MFI was noted among the groups.

Neutrophil chemotactic responses toward LTB$_4$ and IL-8.
Chemotaxis experiments were performed to determine whether notable differences in the motility of neutrophils from HIV-uninfected and HIV-infected subjects toward chemoattractants could be observed. For these studies, neutrophils from HIV-uninfected and HIV-infected blood donors were tested against 2 known chemoattractants, LTB$_4$ and IL-8. The data, expressed as the mean chemotactic response of neutrophils for each group of subjects against varying doses of chemoattractants ($10^{-10}$–$10^{-6}$ mol/L), are presented in figure 6. The results obtained clearly demonstrate that neutrophils from HIV-uninfected and HIV-infected subjects have similar chemotactic responses toward LTB$_4$ (figure 6, top) and IL-8 (figure 6, bottom) with no apparent functional defects in motility or responsiveness.

**DISCUSSION**
In the present study, we have provided evidence that iv administration of LTB$_4$ to HIV-uninfected subjects causes significant plasma accumulation of $\alpha$-defensins and MIP-1$\beta$, which are 2 peptides known for their anti-HIV activities. Of importance, using different functional assays, we have reported that neutrophils from HIV-infected subjects have in vitro biological responsiveness toward LTB$_4$ similar to that of HIV-uninfected subjects. First, we showed that the neutrophil intracellular levels of $\alpha$-defensins are identical among the 3 groups of subjects investigated. Next, we provided evidence that neutrophil’s primary and secondary granule release after LTB$_4$ activation, as measured by cell-surface expression of CD63 and CD66b and secretion of $\alpha$-defensins, were identical for all groups. Finally, the migratory responses of neutrophils toward chemoattractants (LTB$_4$ and IL-8) were indistinguishable among the groups.
Neutrophils are short-lived phagocytes and, together with resident macrophages, they play a key role in the mounting of an effective immune response during the initial phase of infections. The role of neutrophils in viral infections has been often overlooked, with the pretext that neutrophils are mostly involved in antibacterial and antifungal clearance. However, phagocytosis of viral particles [35] and the release of antiviral molecules (e.g., α-defensins, activated oxygen species, and lysosomal enzymes) are factors that probably contribute to viral clearance. In a retrospective analysis of children undergoing hematopoietic stem cell transplantation, the probability of developing a viral disease (mostly HSVs) was 62%, with 1 major contributing factor being the duration of pre-engraftment neutropenia [36]. Experimentally, the in vivo role of neutrophils in the clearance of influenza virus, HSV-1, and HSV-2 infections were reported, highlighting the importance of these cells in the fight against these infectious agents [37–40]. Therefore, the roles of neutrophils in viral infections are probably underestimated. Of potential importance, HIV-infected subjects are reported to have impaired neutrophil functions [14–18]. These neutrophil defects are probably associated with the increased incidence in opportunistic infections (OIs), both bacterial and fungal, observed in these subjects. However, the results presented in the present study suggest that neutrophil degranulation and chemotaxis are not different between HIV-uninfected and HIV-infected subjects. Although the precise reasons as to why our results differ from those cited above are not known, it is worth noting that all the above-cited studies were performed prior to the introduction of HAART or used blood samples from HIV-infected subjects who have not received anti-HIV medication. Of interest and in support of this putative explanation, Mastroianni et al. [41] indicated that HAART restores neutrophil chemotactic responses to normal levels in 72% of HIV-infected subjects tested. The fact that the vast majority of our subjects were receiving HAART or had previously received HAART may explain the differences between our results and those aforementioned.

The observation that neutrophils from HIV-infected subjects receiving HAART regained normal functions may explain, at least in part, the lower incidence in OIs in these subjects [42–45]. However, because HAART is noncurative, associated with significant side effects, and highly costly, it is now general practice to delay HAART initiation until CD4+ T cell counts decrease to <200 cells/mm³. Therefore, HIV-infected subjects must rely on their own immune system to control HIV. Therefore, it is anticipated that drug-development programs that focus on immune restoration and reconstitution and regulators of immune functions will emerge. In fact, new therapeutics based on the use of chemokine-receptor antagonists are already in clinical trials [46]. Drugs that stimulate the immune response have had some limited success for the treatment of HIV infections. With AIDS considered to be primarily a T cell–associated disease, researchers were initially very optimistic about the potential benefit of IL-2, the main T cell growth factor, for the treatment of HIV infection. For several reasons, administration of IL-2 to HIV-infected subjects yielded unsatisfactory results and is no longer considered to be a promising approach for the treatment of this disease. In light of these results, it is expected that other types of immunomodulators need to be studied and developed for the treatment of HIV infection. At the present time, it is too early to tell whether LTB₄ represents a useful therapeutic for the treatment of HIV-infected subjects. The development of lipid mediators as antimicrobial therapeutics experiences a slow growth rate, in light of the fact that some eicosanoids, including the cysteneyl-leukotrienes, are associated with undesirable inflammatory diseases. The pharmaceutical industry’s primary research interest is to
focus on the development of leukotriene biosynthesis inhibitors. However, not all eicosanoids are alike, and the utmost prudence is needed when it comes to modulate the biosynthetic pathways of these lipid mediators, considering that several studies have reported on the importance of lipoxigenase metabolites in host defense. For example, Canny et al. [47] provided evidence that a lipoxin A4 (LXA₄) analogue modulates the expression of the bactericidal/permeability-increasing protein in epithelial cells, which suggests a potential protective role for LXA₄ in mucosal immunity. Furthermore, very recent studies have identified a new and adaptive immune responses [48–50]. If it proves to be effective, treatment with LTB₄ could benefit subjects recently infected with HIV who must often wait several years for their CD₄⁺ cell counts to decrease (<300 cells/mm³) before initiating HAART. By boosting the innate immune response, favoring the release of anti-HIV mediators, and the recruitment of CD8⁺ cells at inflammatory sites, one might expect a slower decrease in CD₄⁺ cell count to occur after LTB₄ administration, ultimately postponing the onset of HAART. The use of LTB₄ in late-stage HIV-infected subjects who are already receiving anti-HIV medication also could prove beneficial to help fight against OIs, considering that this lipid mediator is a key activator of the innate immune response associated with the clearance of such pathogens.

References


2008 • JID 2004:189 (1 June) • Flamand et al.