Antituberculosis Treatment: Increasing Evidence for Drug Effects on Innate Cellular Immunity

C. T. TIEMESSEN,1,2* S. SHALEKOFF,1,2 S. MEDDOWS-TAYLOR,1,2 AND D. J. MARTIN2

AIDS Virus Research Unit, National Institute for Virology,1 and Department of Virology, Witswatersrand University,2 Johannesburg, South Africa

Over the past several years we have gathered increasing evidence suggestive of direct effects of one or more of the currently used antituberculosis (TB) drugs on various immune parameters in patients with pulmonary TB. Throughout the studies, the standard regimen for treatment of tuberculosis consisted of rifampin, isoniazid, pyrazinamide, and ethambutol given for 2 months during the intensive phase of treatment followed by isoniazid and rifampin given for 4 months. The mean duration of anti-TB treatment at the time of blood collection from patients did not differ significantly between the TB and human immunodeficiency virus-infected TB (HIV/TB) groups in any of the studies. Some of the effects on neutrophil function and receptor expression were contrary to what one might expect given that the resolution of active TB would be associated with recovery of function and normalization of receptor expression. Most of this evidence is inferred from cross-sectional studies conducted on TB patients, with only some support from longitudinal data. The primary objective in all of these studies was not the evaluation of the role of anti-TB treatment, and findings reported with respect to drug treatment were incidental to the hypothesis being tested in each individual study. Nonetheless, the findings highlight some important issues that deserve consideration.

INNATE CELLULAR IMMUNITY

The innate immune system differs from adaptive or acquired immunity in that it is not dependent on memory of a previous exposure and therefore has no specificity. Adaptive immunity, while not present at birth, develops after antigenic exposure to an organism and is boosted by repeated exposures. The adaptive immunity effector function is mediated by T lymphocytes, whereas natural killer cells and phagocytes (neutrophils, monocytes, and macrophages) constitute innate cellular immune effectors. The neutrophil is an essential component of innate cellular immunity, functioning as a first line of defence in combating bacterial and fungal infections. The functional integrity of this arm of the immune system is therefore pivotal in protection of the host against primary or secondary invasion by pathogenic microorganisms. It is in the presence of immune suppression caused by viruses such as HIV or the use of various drugs that this becomes evident, and it manifests as an increase in host susceptibility to infections with bacteria or fungi. Neutrophils in their resting state in the peripheral circulation undergo a series of events including signaling, activation, and subsequent migration to the appropriate site of infection, which culminates in effector cell function. These functions include phagocytosis of opsonized microorganisms (coated with complement and/or antibody); elicitation of the oxidative burst, which results in killing of microorganisms via the production of reactive oxygen intermediates; and degranulation, which results in nonoxidative killing via the release of potent antimicrobial enzymes.

PULMONARY TUBERCULOSIS AND DEFECTIVE NEUTROPHIL FUNCTION

*Mycobacterium tuberculosis* is currently the most common opportunistic pathogen found in HIV-1-infected individuals in sub-Saharan Africa. Morbidity and mortality in coinfection are commonly the result of bacterial superinfections. Our studies have concentrated on delineating neutrophil abnormalities that could further shed light on the pathogenesis of TB and HIV-1 infection, which leads to the susceptibility of the host to bacterial and fungal infections (for a review, see reference 20). Impairments in neutrophil functions of TB patients, including HIV-1-coinfected patients (HIV/TB group), were found to be related to both phagocytosis and oxidative burst (a measure of oxidative killing capacity) (19). On the other hand, phagocytosis was significantly increased and the oxidative burst was unimpaired in a group of HIV-1-infected patients without TB. Neutrophils from HIV-1-infected patients, however, were deficient in their ability to degranulate in response to an agonist (a measure of nonoxidative killing capacity); this was true for both HIV and HIV/TB groups (11). Reduced expression of the interleukin-8 (IL-8) receptors (CXCR1 and CXCR2) has also been associated with a reduced ability of neutrophils to respond appropriately by directed migration, calcium flux (12), and degranulation (11). It is therefore evident that the greatest impairment of neutrophil function (decreased phagocytic ability and a compromised oxidative and nonoxidative armature) was present in the HIV/TB group. This is consistent with clinical findings of an increased risk of acquiring new opportunistic infections in patients dually infected with HIV-1 and *M. tuberculosis* compared to that in persons infected with HIV-1 alone (22).

* Corresponding author. Mailing address: National Institute for Virology, Private bag X4, Sandringham 2131, South Africa. Phone: (27-11) 321-4285/00. Fax: (27-11) 882-0596. E-mail: caroline@niv.ac.za.
ANTITUBERCULOSIS TREATMENT AND EXPRESSION OF G-PROTEIN-COUPLED RECEPTORS ON NEUTROPHILS

G-protein-coupled receptors form part of a family of receptors that mediate specific functions in response to small polypeptides such as chemokines and complement fragments. The most notable of these functions is the recruitment of leukocytes from the circulation to sites of infection. Complement factor 5 (C5a), produced during activation of the complement cascade, is a classical chemoattractant that binds to the CD88 receptor and thereby mediates directed cell movement (6). In addition to CD88, a number of CXC–G-protein-coupled receptors are expressed on neutrophils and include the two IL-8 receptors, CXCR1 and CXCR2 (9, 15), and CXCR4, the HIV-1 coreceptor utilized by T-cell-tropic virus strains for entry into cells bearing the CD4 molecule (2, 5).

Results from independent studies carried out to determine how these various receptors are modulated in response to the presence of HIV-1 infection and pulmonary TB showed an overall down-regulation of all G-protein-coupled receptors studied on neutrophils in all patient groups (HIV, TB, and HIV/TB). This reduction in expression was, however, most exaggerated in HIV/TB-coinfected patients. As each infectious state alone (HIV or TB) has an effect on receptor expression in its own right, certain relationships due to drug treatment may become evident or, alternatively, be obscured in TB and/or HIV/TB patients, thus often confounding the interpretation of results. Here we discuss the main findings of receptor modulation in the context of anti-TB treatment and attempt to dissect out disease effects from drug effects.

When the two TB patient groups (TB and HIV/TB) were combined, there was no correlation between the expression of either IL-8 receptor (CXCR1 or CXCR2) on neutrophils and the duration of anti-TB drug therapy (12). Interestingly, the TB group on its own showed a significant negative correlation between the fluorescence intensity of CXCR1 (a measure of receptor density) and the duration of anti-TB treatment. It was clear that the presence of HIV-1 coinfection obscured this relationship. When patients were stratified into two groups on the basis of time of treatment, approximately half had received treatment for <2 months and the other half had received treatment for >2 months. There was no significant difference between the expression of CXCR1 (percentage of positive cells) or CXCR2 receptor (percentage of positive cells or relative receptor density) between the <2-month and >2-month treatment groups. However, the intensity of CXCR1 expression in TB patients was significantly higher in the <2-month treatment group than in the >2-month treatment group, clearly showing decreased expression despite resolution of TB, the opposite of what one might expect if the original down-modulating effect were due to TB alone. Whether the decrease in CXCR2 expression on neutrophils would be noted in drug-naive patients versus those on treatment could not be assessed since all patients recruited for this study were already receiving anti-TB treatment. It was clear, however, that the duration of anti-TB treatment had no apparent effect on CXCR2 expression.

The expression of the HIV-1 coreceptor, CXCR4, showed a similar pattern of modulation in the different disease groups to that of CXCR2, in that the most profound decreases occurred in the two TB groups (S. Shalekoff, S. Pendle, D. Johnson, D. J. Martin, and C. T. Tiemessen, submitted for publication). A significant negative correlation was found between the relative receptor density of CXCR4 receptors on neutrophils and the duration of anti-TB treatment in the HIV/TB group. Interestingly, similar patterns of down-modulation occurred in other leukocyte subsets. In the HIV/TB group, significant negative correlations were found between the proportions of CXCR4-expressing CD3+, CD4+, and CD8+ memory cells (identified by coexpression of the CD45RO marker), CD14+ cells, and NK cells and the duration of treatment. There were no correlations between CCR5 expression on various leukocytes, the other major HIV coreceptor studied in parallel, and the duration of anti-TB treatment. That these changes show a relationship to the duration of anti-TB treatment in the HIV/TB group and not in the TB group suggests that it is a combination of both the duration of anti-TB drug therapy and HIV-1 infection that results in diminished receptor expression and that different cell types also show similar changes in receptor modulation.

The expression of the classical chemoattractant C5a receptor, CD88, on neutrophils showed a reciprocal trend to that found for CXCR1 and CXCR4 with respect to duration of anti-TB treatment (13). A significant positive association was observed between the length of time a patient had received anti-TB treatment and the relative density of CD88 receptors on neutrophils in both the TB and HIV/TB patient groups, with levels approaching those found for healthy volunteer blood donors. Furthermore, quite irrespective of the presence of concomitant HIV-1 infection, resolution of TB resulted in an increase in CD88 fluorescence intensity with no evidence of drug effects on this receptor.

Although the overall patterns of modulation by the presence of disease (HIV, TB, and HIV/TB) were similar for the different receptors, anti-TB drug therapy differentially affected their expression. This provides us with some understanding of the capacity of these receptors, which have similar functions, to be modulated by different agents, either infectious or therapeutic. CD88 represented the most robust of this group of receptors in that it was not susceptible to direct drug effects in vivo. It may be significant that the tendency of any of these receptors on neutrophils to be easily modulated by a variety of cytokines in vitro seemed to be related to an equivalent ability to be modulated in vivo, whatever the cause. In particular, CD88 was least likely to be modulated by a wide variety of cytokines whereas CXCR2 and, to a greater extent, CXCR4 were easily modulated (C. Tiemessen, unpublished data). The question remaining is whether CXCR2 and CXCR4 are down-regulated only in response to administration of anti-TB treatment, with no further change as a result of its duration, or to TB itself, or to a combination of the two.
ANTITUBERCULOSIS TREATMENT AND NEUTROPHIL FUNCTION

In a study conducted to assess the integrity of phagocytic and oxidative burst capabilities of neutrophils from individuals with pulmonary TB, which were found to be diminished, no significant correlation was found between either of these abilities and the duration of anti-TB drug therapy (19). This was true whether patients had concomitant HIV-1 infection or not. When patients were stratified into two groups on the basis of time of treatment, half had received treatment for <2 months and half had received treatment for >2 months. There was no significant difference between neutrophil phagocytic capacity or ability to produce ROI between the <2-month and >2-month treatment groups.

Therefore there was a persistence of both defects in neutrophil phagocytosis and oxidative burst in in patients with TB despite successful anti-TB treatment. Again, these results show no return to normal values within the time that drug treatment was still being administered but the TB was resolved. Interestingly, the depression of either function occurred to a similar extent in the TB and HIV/TB groups, suggesting that the presence of HIV-1 was not exacerbating either of these defects in function. As mentioned previously, neutrophils from HIV-1-infected patients without TB showed enhanced phagocytosis and unaltered oxidative burst (19). Further support for a direct role in depressed neutrophil function by one or more of the current drugs in the standard regimen can be inferred from a study that showed decreased neutrophil chemotaxis, phagocytosis, and killing of Candida regardless of active or chronic disease in TB patients receiving anti-TB treatment (1). In addition, another study showed a significantly increased phagocytic ability of neutrophils from patients with active pulmonary tuberculosis prior to commencement of any treatment (16). Perhaps the greatest support in this regard comes from a recent longitudinal study conducted to assess the effect of short-course granulocyte-macrophage colony-stimulating factor administration on neutrophil function in TB patients, where phagocytic ability in particular was found to progressively decrease from baseline untreated values to 1 month. Patients treated with granulocyte-macrophage colony-stimulating factor, however, showed marked recovery of this ability to above baseline values within the same period (Tiemessen, unpublished).

Infection with HIV-1 is accompanied by activation of the immune system (7, 21), which seems more pronounced in African patients (17). This immune activation has widespread effects including stimulation of viral replication and accelerated progression of HIV-1 disease (22). Despite successful treatment of TB in coinfectcd individuals, sustained activation of the immune system has been reported (10). Such activation may play a role in the continued down-regulation of some of the G-protein-coupled receptors described as well as in diminished neutrophil function.

CONCLUSIONS AND FUTURE PERSPECTIVES

In summary, patients with HIV-1 infection together with pulmonary TB present with the greatest neutrophil impairment, as evidenced by defects in several separate effector functions. Furthermore, alterations in either the numbers of cells expressing a particular G-protein-coupled receptor or the respective cellular receptor densities are likely to affect cell function, particularly cellular trafficking in response to the specific receptor ligands. This could present as either altered numbers of cells migrating or inappropriate responses such as an altered order of specific cell types moving in response to a stimulus at the site of infection. The infecting agent and use of the current anti-TB drugs affect these functions differently, depending on the particular function or receptor or whether patients are dually infected.

We would therefore, on the basis of the above findings, raise concerns that the use of various antibacterial agents may in fact affect natural immune functions that are essential to the clearance of invading microorganisms, particularly in patients known to be at higher risk of acquiring secondary infections. However, it must be kept in mind that these findings represent biological phenomena that may reflect activities in vivo which may well not have profound clinical significance. There may be a critical phenomena that may reflect activities in vivo which may well not have profound clinical significance. There may be a critical threshold at which defective function results in the patient becoming susceptible to further infections. On the other hand, it is not unreasonable to assume that drug-induced suppression may further aggravate defects in one or more functions of neutrophils from infected patients. It is also significant that rifampin-induced suppression of lymphocyte function, which has been recognized for several decades, has been recently attributed to its ability to bind to glucocorticoid receptors on these cells (4). Whether some of the effects on neutrophils noted in our studies could be mediated through a similar mechanism remains to be elucidated.

It is therefore important that the true clinical significance of the effects of anti-TB drugs be systematically determined. Good longitudinal follow-up of large numbers of patients is essential to determine disease outcomes or altered susceptibilities to other infectious agents. Therefore, a detailed evaluation of anti-TB drug therapy in the context of direct drug effects on neutrophil functions and phenotype of TB patients (in both the TB and HIV/TB groups) is required, with patients being monitored from admission throughout treatment to completion of therapy. In vitro studies aimed at determining the specific effects of each individual drug and combinations of the drugs on cell phenotype and function require elucidation by using cells from both healthy persons and those infected with HIV-1. Once the drug or drug combinations that appear to have adverse effects on function are identified, it is important to determine if they can be replaced with equally active and cost-effective drugs that have less influence on immune functions. Adjuvant-type therapies such as the use of colony-stimulating factors could be useful in complementing anti-TB drug therapy, although cost considerations would preclude their widespread use. Overall, these findings underscore the need for routine chemotherapeutic prophylaxis of bacterial infections in patients with HIV and TB coinfection, a strategy which is increasingly being implemented as common practice (8, 23).

REFERENCES