Infection of Human Monocytes with *Mycobacterium tuberculosis* Enhances Human Immunodeficiency Virus Type 1 Replication and Transmission to T Cells

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*Mycobacterium tuberculosis* and human immunodeficiency virus type 1 (HIV-1) are virulent intracellular pathogens that invade and multiply within macrophages. The effect of *M. tuberculosis* on HIV-1 infection and replication was analyzed in vitro using human monocyte-derived macrophages (MDM) isolated from peripheral blood mononuclear cells by countercurrent centrifugal elutriation. Preinfection of MDM with *M. tuberculosis* followed by HIV-1 infection resulted in an increase in p24 release, reverse transcriptase activity, and infective virus production. In contrast, no increase in HIV-1 production was observed when MDM were infected with *Mycobacterium avium* complex or heat-killed *M. tuberculosis*. Coinfected MDM were potent stimulators of T cell proliferation, while HIV-1–infected MDM failed to present exogenous tuberculin to T cells. Furthermore, coinfecting MDM showed an increased capacity to transmit HIV-1 to activated T cells. These results suggest that *M. tuberculosis* infection can both up-regulate HIV-1 infection and replication within MDM and increase the efficiency of virus transmission from infected MDM to T cells.

Macrophages harbor human immunodeficiency virus (HIV) infection and are responsible for the spread of the virus in tissues [1]. Infection with and replication of HIV-1 are influenced by host factors and coexisting infections. In particular, *Mycobacterium tuberculosis* infection is considered a potential cofactor for AIDS progression [2].

Macrophages and other antigen-presenting cells (APC) play an important role in T cell activation, renewal, and homeostasis. It is thought that defective antigen presentation leads to T cell anergy, loss of immunologic memory, and consequently depletion of CD4 T cells [3]. On the other hand, HIV-1 replication occurs predominantly in activated CD4 cells, for example, responding to antigenic challenge [4]. Formal evidence that *M. tuberculosis*–induced activation of the immune system is responsible for HIV-1 replication has been recently provided by Goletti et al. [5], who showed there is increased plasma virus load in the course of active pulmonary tuberculosis.

Here we analyze the effect of coinfection in vitro of monocyte-derived macrophages (MDM) with the virulent strain of *M. tuberculosis* H37Rv on HIV-1 replication.

**Materials and Methods**

Purification of peripheral blood mononuclear cells (PBMC) and monocytes. PBMC were isolated from the EDTA-treated blood of healthy donors by standard Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Monocytes were isolated by countercurrent centrifugal elutriation (JE-6B Elutriation System; Beckman Instruments, Palo Alto, CA), as previously described [6]. Monocytes were 87%–96% pure and T cells >95% pure as judged by staining with appropriate antibodies (anti-CD14, anti-CD3; Becton Dickinson, San Jose, CA). T cells were purified from elutriated lymphocytes by rosetting with neuraminidase-pretreated sheep erythrocytes as previously described [6]. T cells and monocytes were adjusted to 10^6/mL and cultured in RPMI 1640 containing 10% fetal calf serum, 2 mM l-glutamine, and gentamicin (Sigma, St. Louis). Gentamicin was used because it does not affect viability and growth of *M. tuberculosis* [7]. Because long-term cultures of monocytes were used, we call these cells MDM, as they become more mature cells with decreased CD14 and increased CD68 expression.

*Mycobacterial* infection of MDM. *M. tuberculosis* strain and *Mycobacterium avium* complex (MAC) were grown in 7H9 modified medium, and the bacilli concentration was determined by densitometry at 580 nm calculated from the standard curve established by correlation of optical density with colony-forming units, as previously described [8]. MDM (10^5/well in a volume of 1 mL) were incubated in 24-well plates (Falcon 3074; Becton Dickinson, Lincoln Park, NJ) for 3 h with *M. tuberculosis* or MAC using a bacilli-to-cell ratio of 1:1, 10:1, or 50:1 in the culture medium. In some experiments, monocytes were treated with heat-killed *M. tuberculosis* (90°C, 15 min) with the same protocol. MDM were then washed twice and cultured in the medium for various time intervals. The intracellular localization of *M. tuberculosis* was checked by modified Kinyoun carbol-fuchsin staining for acid-fast bacilli as previously described [8]. More than 80% of MDM contained *M. tuberculosis* at day 4 of infection. For MDM untreated or treated with *M. tuberculosis*, surface expression of HLA-DR, CD14, CD44, CD54, and CD68 was analyzed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA) using appropriate antibodies (Becton Dickinson).

Received 12 August 1996; revised 23 January 1997.
Grant support: Istituto Superiore di Sanità, Progetto AIDS (9304-42, 1995; fellowships to R.P., S.B., M.Z.); I Progetto Tuberculosi. 80% of MDM contained *M. tuberculosis* on HIV-1 replication. forming units, as previously described [8]. MDM (10^6/well in a 24-well plate) were infected with *M. tuberculosis* H37Rv by Goletti et al. [5], who showed there is increased plasma virus load in the course of active pulmonary tuberculosis.

The Journal of Infectious Diseases 1997;175:1531–5 © 1997 by The University of Chicago. All rights reserved. 0022–1899/97/7506–0037$01.00
HIV-1 infection of MDM. The virus isolate used in this study was HIV-1Lai. The virus stock was assayed for particle-associated reverse transcriptase (RT) activity [9]. HIV-1 infection was accomplished by incubating MDM with 100 μL of HIV-1Lai (RT activity = 11,500 cpm) diluted 1:10 for 2 h at 37°C and then washing three times with warm medium. MDM were then cultured in 24-well plates. Cultures preinfected with M. tuberculosis or MAC or pretreated with heat-killed M. tuberculosis were infected with HIV-1 at day 4 of culture. MDM infected with HIV-1 only on day 0 or after 4 days of culture in medium alone were used as control. Virus production was followed by monitoring p24 antigen level by ELISA (Coulter, Miami) and RT activity in the culture supernatants at 2, 4, and 6 days after infection [9, 10]. To determine the yield of infective virus (TCID50), the C8166 T cell line assay was used [11]. The proportion of MDM infected with HIV-1 was evaluated by intracellular staining of p24 antigen using a specific monoclonal antibody (KC57; Coulter) and subsequent flow cytometry.

T lymphocyte proliferation assay. MDM and T lymphocytes were purified from the blood of tuberculin purified protein derivative (PPD)–positive healthy donors. MDM (105/well) were infected with HIV-1 alone, M. tuberculosis alone, or M. tuberculosis and HIV-1 together and then cultured in flat-bottom 96-well plates (Falcon; Becton Dickinson) with autologous T lymphocytes (105/well). PPD (Statens Seruminstitut, Copenhagen) was used as the recall antigen (25 μg/mL) in HIV-infected and uninfected cocultures. Cells were cultured for 6 days at 37°C with a 6-h terminal pulse of [3H]thymidine (1 μCi/well; Amersham Life Science, Amersham, UK).

Transmission of HIV-1 from MDM to activated T cells. MDM (105 cells/well) were infected with HIV-1 alone or coinfected with M. tuberculosis plus HIV-1 and cultured for 3 days. MDM were washed twice, and then autologous T cell blasts (T cells previously stimulated for 3 days with 10 μg/mL phytohemagglutinin [Difco, Detroit]) were added (105 cells/well). After 24 h of coculture, T cells were removed, washed twice, transferred to separate wells, and cultured for a further 4 days in the presence of interleukin-2 (20 U/mL, Sigma). The culture supernatants were then assayed for p24 release.

Statistical analysis. Statistical significance was evaluated by Student’s t test. Differences are regarded as significant when P < .05.

Results

Increased HIV-1 replication in human MDM preinfected with M. tuberculosis. MDM were coinfected with M. tuberculosis H37Rv and HIV-1 following the protocol described above. HIV-1Lai was used to establish a low-level productive infection in MDM. Figure 1A shows that preinfection of MDM with M. tuberculosis at day −4, followed by HIV-1 infection at day 0, significantly enhanced p24 antigen release, which was already apparent at 2 days after HIV-1 infection. Cytodme-try of p24 antigen intracellular staining showed an increased proportion of HIV-1–infected cells in M. tuberculosis–preinfected MDM compared with that in singly infected MDM (data not shown). Preinfection of MDM with MAC did not substantially modify p24 production. A slight but not significant increase in p24 release was observed when MDM were preinfected with heat-killed M. tuberculosis or when MDM were cultured for 4 days before HIV-1 infection. The enhanced HIV-1 replication caused by M. tuberculosis preinfection was not critically dependent on the dose of mycobacteria used, as it was observed at M. tuberculosis–to–MDM ratios of 1:1, 10:1, and 50:1. In all subsequent experiments, the ratio 10:1 was used. Simultaneous M. tuberculosis–HIV coinfection or M. tuberculosis infection of HIV-preinfected MDM did not cause up-regulation of p24 release (data not shown). It was concluded that preinfection of MDM with M. tuberculosis is a critical factor for the interaction with HIV-1 and also for the early enhanced viral replication.

The effect of preinfection of MDM with M. tuberculosis on HIV-1 replication was further investigated by measuring RT activity and production of infective virus particles in 4-day culture supernatants. Figures 1B and C show that preinfection of MDM with M. tuberculosis resulted in enhancement of RT activity and p24 release compared with that of MDM infected with HIV-1 only. Titration of the infectious virus in the culture supernatants of MDM infected with HIV-1 yielded a TCID50 of only 104.62, while coinfected cells yielded a TCID50 of 106.75 (figure 1D).

MDM-induced T cell proliferation and virus transmission. To assess the effect of coinfection on antigen presentation, MDM were cultured for 6 days with autologous T cells. PPD was used as antigen when M. tuberculosis–uninfected MDM were used as APC, while PPD was omitted when APC consisted of M. tuberculosis–infected MDM. Figure 2A shows that PPD-driven T cell proliferation was completely inhibited when HIV-1–infected MDM were used as APC. M. tuberculosis–infected MDM induced a strong proliferative response of T cells, which was significantly higher when coinfected MDM were used. Flow cytometry showed an increased expression of HLA-DR, CD14, CD44, CD54, and CD68 on M. tuberculosis–treated MDM compared with surface marker expression on untreated MDM (data not shown).

The ability of MDM to transfer HIV-1 to T cells was then analyzed by use of MDM preinfected with M. tuberculosis. Figure 2B shows that HIV-infected MDM transmitted the virus to phytohemagglutinin-induced T cell blasts, as judged by p24 production during 4 days of culture. However, a significant increase in p24 production by T cell blasts was observed when MDM preinfected with M. tuberculosis were used as the source for virus transmission.

Discussion

This study demonstrates that preinfection of MDM with virulent M. tuberculosis H37Rv enhances HIV-1 replication, evaluated as p24 antigen release, RT activity, and infective virus production. The association of increased RT activity and p24 antigen production probably reflects an efficient virus particle assembly and release in M. tuberculosis–infected MDM. Fur-
Figure 1. A, Effect of preexposure of MDM to different mycobacteria (viable and heat-killed [hk] M. tuberculosis [MTB], viable M. avium complex [MAC]) on HIV-1 replication measured as p24 release in culture supernatants at different times after virus infection. B–D, Comparison of p24 antigen release, reverse transcriptase (RT) activity, and infective virus particle titer (TCID₅₀) in supernatants of coinfected MDM 4 days after HIV-1 infection. As MDM from various donors show different production of HIV-1, data from 1 representative experiment are shown. Similar results were obtained in 4 other independent experiments. * Significant difference vs. HIV-1 only–infected cultures (P < .001).

Moreover, the yield of the infective virus in the supernatants from coinfected MDM was higher than that in singly infected cells. As enhanced production of p24 by coinfected MDM was observed as early as 2 days after infection with HIV-1, it is likely that the M. tuberculosis facilitated interactions of the virus with the cells, resulting in an increased proportion of infected cells as revealed by cytofluorimetry. M. tuberculosis–induced activation and differentiation of MDM, as shown by changes in several surface markers (CD14, CD44, CD54, CD68, HLA-DR), could facilitate HIV-1 infection; moreover, an increase of the membrane expression of β-chemokine receptors on M. tuberculosis–infected MDM cannot be excluded [12].

Recently, Zhang et al. [13] have shown increased replication of HIV-1 in the course of in vitro infection with M. tuberculosis, but in their experimental model, monocytic cell lines (which, in contrast to MDM, proliferate in vitro) and an avirulent strain of M. tuberculosis were used. That study suggested the involvement of cytokines (tumor necrosis factor-α, interleukin-1β, and interleukin-6) in facilitating HIV-1 production. However, in the present study, tumor necrosis factor-α is unlikely to be involved in the increased HIV-1 replication, since MAC-infected MDM or heat-killed M. tuberculosis–treated MDM produced high levels of tumor necrosis factor-α (data not shown) but failed to increase p24 antigen release. However, the role of other molecules, such as reactive oxygen intermediates, which are produced by M. tuberculosis–infected macrophages, in potentiating HIV-1 replication cannot be excluded.

Skin test anergy to recall microbial antigens is commonly seen in HIV-1–positive subjects, and it has been explained, at
Figure 2. Effect of M. tuberculosis (MTB)–infected MDM on antigen-specific T cell proliferation (A) and HIV-1 transmission to T cells (B). A, Responses of T lymphocytes from purified protein derivative (PPD)–positive donors cultured with MDM infected with either M. tuberculosis or HIV-1 or coinfected with both. Cultures stimulated with PPD served as positive control and unstimulated cultures as negative control (<300 cpm). Mean of [3H]thymidine incorporation of triplicate cultures is shown. B, Ability of coinfected MDM to infect syngeneic phytohemagglutinin-induced T cell blasts. T lymphocytes were cocultured for 24 h with either HIV-1– or M. tuberculosis–infected MDM. T cells were then harvested and cultured for another 4 days. Data show level of p24 antigen in culture supernatants, expressed as mean ± SD. Results of representative experiment of 4 are shown. * Statistically significant (P < .05) increase or decrease.

least in part, by the failure of HIV-infected macrophages to present antigens to T cells [14]. This is in keeping with our findings, as HIV-infected MDM failed to induce PPD-specific T cell proliferation. In contrast, MDM infected with M. tuberculosis and HIV-1 were potent APC. The capacity of coinfected MDM to present antigen to T cells may be explained by the property of M. tuberculosis to up-regulate major histocompatibility complex class II, CD44, and CD54, all molecules critical for antigen presentation and APC–T cell interactions. It is also possible that up-regulation of these molecules may be associated with enhanced transmission of HIV-1 to T cells.

The ability of M. tuberculosis–infected MDM to induce antigen-specific T cell proliferation and virus transmission is of great relevance in the pathogenesis of AIDS, as T cells specific for recall microbial antigens are deleted early in the course of HIV-1 infection [15]. Recent studies have clearly demonstrated that M. tuberculosis–specific T cell activation is a critical event in HIV-1 replication in seropositive persons. In fact, plasma virus load is increased during active pulmonary tuberculosis in vivo, and a strict correlation occurs in vitro between M. tuberculosis–induced T cell activation and HIV-1 replication [5]. Our data are in keeping with these findings, as coinfected MDM not only induced a strong proliferative response of T cells but also significantly enhanced the ability to transmit the virus to T cell blasts. It is likely that this mechanism may be operative in persons from geographic areas where tuberculosis is endemic, where infection with M. tuberculosis occurs before HIV contact, and where the average lifespan of dually infected patients is dramatically reduced. Finally, other infections caused by several opportunistic pathogens have been shown to increase HIV-1 replication in vivo [16, 17].

In conclusion, the increased HIV-1 replication in M. tuberculosis infection may be due to direct and indirect mechanisms: The first is related to the cellular events that lead to an increase in HIV-1 infection and replication in M. tuberculosis–activated MDM; the second is linked to the ability of M. tuberculosis–infected MDM to drive antigen-specific T cell proliferation and to facilitate virus transmission to T cells. Thus, both mechanisms may be important in seropositive patients with tuberculosis in whom an accelerated progression of HIV-1 infection is seen.

Acknowledgments

Special thanks are due to L. Fattorini (Istituto Superiore Di Sanità) for providing the M. tuberculosis and M. avium strains used in this study and to F. Nasella for literature research.

References

Estimation of the Annual Risk of Tuberculous Infection for White Men in the United States

Thomas M. Daniel and Sara M. Debanne

The annual risk of tuberculous infection for white men in the United States was estimated from published tuberculin surveys and found to be related to tuberculosis incidence as reflected in the annual case rate during the past 3 decades, with a constant ratio of ~150. It is currently estimated to be 0.03% per year. The technique developed for this estimation is not complex and should be applicable to other segments of the population for which suitable data are available.

Annual risk of infection (ARI) is the term widely used to refer to the annual incidence of tuberculous infection as distinguished from the incidence of tuberculosis disease. As calculated from tuberculin skin test surveys, it has become widely accepted as an index of the transmission of tuberculosis within a population and, in many countries, as a more reliable measure of the magnitude of the tuberculosis problem facing those charged with tuberculosis control than the reported incidence of newly active cases of disease [1, 2]. For those interested in modeling the transmission of disease, the ARI is a necessary input variable.

Methods for estimating the ARI from tuberculin skin test surveys have been developed and tested in both high- and low-prevalence populations [1, 3]. In the United States, it has been difficult or impossible in recent years to calculate the ARI because tuberculin skin test surveys capable of providing the necessary input data have not been carried out systematically. The largest and most carefully done American tuberculin survey was conducted with naval recruits from 1958 to 1965 (midpoint, 1962) [4]. Data on lifelong 1-county resident, white men aged 17–21 years (median, 19) were obtained and tabulated in readily accessible form.

Because Styblo [5] has demonstrated a relatively constant ratio between ARI and disease incidence in several populations, we felt it reasonable to postulate that the ARI in the United States would decrease in parallel with decreasing tuberculosis case rates. To test this hypothesis, we projected the ARI calculated from the naval recruit database in parallel with the tuberculosis case rate and compared the projected ARIs with those calculated from a number of available tuberculin skin test surveys.

Methods

Tuberculosis case rates for the United States were taken from the annual reports of the Centers for Disease Control and Preven-