Macrophage Cell Coinfected with HIV-1 and H37Ra

Erica C. Larson,¹ Chris D. Pond,¹ Matthew A. Szaniawski,² Laura J. Martins,²
Vicente Planelles,² and Louis R. Barrows¹

FIG. 1. THP-1, a macrophage cell line, coinfects with DHIV-mCherry and H37Ra *gfp*. THP-1 cells were differentiated with PMA (20 ng/ml) for 24 h before coinfection. DHIV-mCherry (*red*; $\sim 5 \times 10^6$ TU/ml, determined by flow cytometry) and H37Ra *gfp* (*green*; MOI 1:1) were coinoculated for 16 h. Cells were fixed in 1% formaldehyde and actin was stained with AbnovaTM Fluorescent Dye 405-I Phalloidin (*cyan*). The image was acquired on a Nikon A1 confocal microscope using a 60 $\times$ oil lens and processed using Fiji¹. It is an average intensity projection of 6 z-stacks, spaced 0.5 μ m apart. MOI, multiplicity of infection; PMA, phorbol-12-myristate-13-acetate. Color images available online at www.liebertpub.com/aid

Keywords: HIV/TB coinfection, HIV, tuberculosis, H37Ra, macrophage

PATIENTS COINFECTED WITH human immunodeficiency virus (HIV) and *Mycobacterium tuberculosis* (Mtb; the causative agent of TB) have dramatically increased rates of mortality and morbidity compared with singly infected patients. Evidence supporting various mechanisms of synergism has been published: HIV replication at sites of Mtb infection leads to reduced bacterial containment and facilitated HIV spread, HIV inhibits macrophage killing of Mtb, HIV reduces CD4 T cells within granulomas, thereby allowing activation of Mtb, and HIV impairs Mtb-specific T cells.²

Although macrophages are susceptible to infection by both HIV and Mtb, the concept that coinfection on the cellular level could contribute to the particularly poor prognosis of coinfecting individuals has received little consideration. To our knowledge, the existence of HIV/Mtb coinfecting cells has never been demonstrated directly. Here we report that HIV and Mtb can, in fact, productively infect the same macrophage (Fig. 1).

The monocytic leukemia macrophage cell line, THP-1, is known to phagocytose Mtb *in vitro*. We confirmed this using simple coculture of H37Ra *gfp*,³ an attenuated GFP-expressing Mtb strain, with THP-1 cells (1:1 MOI) for 4 h or longer and flow cytometry to quantify infection, showing a GFP-positive subset within the viable THP-1 cell population (LIVE/DEAD[®] Fixable Violet Stain). We showed that H37Ra *gfp* was likely phagocytosed by THP-1 cells in this culture system because the percentage infected THP-1 cells increased after phorbol-12-myristate-13-acetate (PMA) activation (not shown). Furthermore, we observed that Rab20, a marker of early-to-late stages of phagosomal/endosomal maturation, was upregulated in H37Ra *gfp*-containing THP-1 cells and that Rab20+/GFP+ populations increased, indicating internalization (not shown).

To investigate whether THP-1 macrophages could harbor HIV and Mtb concurrently, we used the mentioned system and coinoculated THP-1 overnight (16 h) with DHIV-mCherry and H37Ra *gfp*. DHIV is a “defective” HIV strain that contains a short deletion within the *env* gene, thereby preventing more than one round of infection. The strain we used expresses mCherry in substitution for the *nef* gene, which allows for quantitation of infection by flow cytometry or cell imaging when the mCherry protein is synthesized during viral replication. DHIV-mCherry was constructed by replacing the GFP gene with that for mCherry in the DHIV-

GFP construct previously described.⁴ Although the H37Ra *gfp* is probably located within the THP-1 phago/endosome, we hypothesize that the concentrated regions of mCherry apparent in the image most likely indicate the pooling of Env proteins in the trans-Golgi network during the early stages of the viral life cycle. In any case, the image indicates viral entry and progression through its life cycle occurs in the presence of intracellular H37Ra.

This is the first image to capture replication of HIV within an Mtb-infected macrophage. This productive cohabitation (producing both GFP and mCherry-Env) within the same cell *in vitro* suggests that cellular coinfection of macrophages, or we speculate T cells or syncytia, may be common in coinfecting individuals. If this observation is confirmed in patients, it is possible that coinfecting cells contribute to the remarkable pathogenic synergism observed in coinfecting individuals.

Author Disclosure Statement

No competing financial interests exist.

References

- Schindelin J, Arganda-Carreras I, Frise E, *et al.*: Fiji: An open-source platform for biological-image analysis. *Nat Methods* 2012;9:676–682.
- Diedrich CR, Flynn JL: HIV-1/*Mycobacterium tuberculosis* coinfection immunology: How does HIV-1 exacerbate tuberculosis? *Infect Immun* 2011;79:1407–1417.
- Collins LA, Torrero MN, Franzblau SG: Green fluorescent protein reporter microplate assay for high-throughput screening of compounds against mycobacterium tuberculosis. *Antimicrob Agents Chemother* 1998;42:344–347.
- Bosque A, Planelles V: Induction of HIV-1 latency and reactivation in primary memory CD4 T cells. *Blood* 2009;113:58–65.

Address correspondence to:

Louis R. Barrows

Department of Pharmacology and Toxicology

University of Utah

30 South 2000 E

Salt Lake City, UT 84112

E-mail: lbarrows@pharm.utah.edu