HLA-E–restricted CD8⁺ T Lymphocytes Efficiently Control Mycobacterium tuberculosis and HIV-1 Coinfection

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Abstract

We investigated the contribution of human leukocyte antigen A2 (HLA-A2) and HLA-E–restricted CD8⁺ T cells in patients with Mycobacterium tuberculosis and human immunodeficiency virus 1 (HIV-1) coinfection. HIV-1 downregulates HLA-A, -B, and -C molecules in infected cells, thus influencing recognition by HLA class I–restricted CD8⁺ T cells but not by HLA-E–restricted CD8⁺ T cells, owing to the inability of the virus to downmodulate their expression. Therefore, antigen-specific HLA-E–restricted CD8⁺ T cells could play a protective role in Mycobacterium tuberculosis and HIV-1 coinfection. HLA-E– and HLA-A2–restricted Mycobacterium tuberculosis–specific CD8⁺ T cells were tested in vitro for cytotoxic and microbicidal activities, and their frequencies and phenotypes were evaluated ex vivo in patients with active tuberculosis and concomitant HIV-1 infection. HIV-1 and Mycobacterium tuberculosis coinfection caused downmodulation of HLA-A2 expression in human monocyte–derived, macrophages associated with resistance to lysis by HLA-A2–restricted CD8⁺ T cells and failure to restrict the growth of intracellular Mycobacterium tuberculosis. Conversely, HLA-E surface expression and HLA-E–restricted cytolytic and microbicidal CD8 responses were not affected. HLA-E–restricted and Mycobacterium tuberculosis–specific CD8⁺ T cells were expanded in the circulation of patients with Mycobacterium tuberculosis/HIV-1 coinfection, as measured by tetramer staining, but displayed a terminally differentiated and exhausted phenotype that was rescued in vitro by anti–PD-1 (programmed cell death protein 1) monoclonal antibody. Together, these results indicate that HLA-E–restricted and Mycobacterium tuberculosis–specific CD8⁺ T cells in patients with Mycobacterium tuberculosis/HIV-1 coinfection have an exhausted phenotype and fail to expand in vitro in response to antigen stimulation, which can be restored by blocking the PD-1 pathway using the specific monoclonal antibody nivolumab.

Keywords: CD8⁺ T lymphocytes; HLA-E; Mycobacterium tuberculosis; HIV-1; tetramers; PD-1

According to the World Health Organization’s Global Tuberculosis Control Report 2018, 10.0 million people developed tuberculosis (TB) and the disease caused 1.6 million deaths in 2017, including 300,000 deaths that resulted from TB and human immunodeficiency virus 1 (HIV-1) coinfection (1). Moreover, approximately one-fourth of the global population is latently infected with Mycobacterium tuberculosis (Mt) (2). Although active TB is curable with chemotherapy, drug treatment does not
eradicate the disease and patients never become entirely free of infection (3). Also, the currently available vaccine, bacillus Calmette-Guérin, is effective in children with disseminated forms of TB but not against pulmonary TB in adults (4). Therefore, there is an urgent need for a novel and effective TB vaccine, especially given the emergence of drug-resistant Mtb strains (4).

Over recent years, it has become evident that CD8+ T cells also contribute to protection through production of IFN-γ and killing of both infected macrophages and intracellular mycobacteria (5, 6). In humans, Mtb-specific CD8+ T cells include both major histocompatibility complex class Ia (human leukocyte antigen [HLA]-A, -B, and -C)–restricted and class Ib (HLA-E, MR1, and CD1)–restricted T cells (7).

In particular, HLA-E is a highly conserved HLA class Ib molecule with rather unique properties. HLA-E is primarily involved in the prevention of lysis by natural killer (NK) cells through ligation with the NKG2/C9 complex (8, 9). Moreover, it can also present antigens to CD8+ T cells and thus plays a role in both innate and adaptive immunity (10–13). Due to its low allelic variability positions, HLA-E is an interesting candidate antigen-presenting molecule for peptide-based vaccination strategies (14–16). In contrast to class Ia molecules, HLA-E is enriched in Mtb phagosomes and accessible for loading with Mtb peptides (17, 18). Another advantage with regard to TB vaccination strategies is that, unlike HLA class Ia molecules, HLA-E is not downregulated by the HIV-1 Nef (negative regulatory factor) protein (18, 19). Moreover, p24 Gag-derived peptides of HIV-1 may even stabilize HLA-E cell-surface expression to prevent NK-mediated lysis of HIV-1–infected cells (20). This is particularly important in countries where 70% of patients with TB are coinfected with HIV-1, such as South Africa. In support of HLA-E as a promising vaccine target, a recent study demonstrated that vaccination of rhesus macaques with a cytomegalovirus simian immunodeficiency virus–gag protein elicited CD8+ T cells that were restricted by HLA-E and contributed to protection against a subsequent simian immunodeficiency virus challenge (21).

In this study, we aimed to investigate the relative contribution of HLA class Ia (HLA-A2)– and HLA class Ib (HLA-E)–restricted CD8+ T cells to the protective host response against intracellular pathogens. We took advantage of Mtb/HIV-1 coinfection because HIV-1 downregulates HLA-A, -B, and -C molecules from the infected cell surface, which in turn influences infected-cell recognition by HLA class Ia–restricted CD8+ T cells (18, 19, 22). We then addressed whether HLA class Ia– and HLA-E–restricted CD8+ T cells were equally able to recognize and kill macrophages coinfected with HIV-1 and Mtb, and to reduce the viability of both intracellular pathogens.

Methods

Human Subjects
Peripheral blood was obtained from 10 patients with TB disease (5 men and 5 women, age range 28–52 yr) from the Department of Sciences for Health Promotion and Mother-Child Care “G. D’Alessandro, Palermo University Hospital, 7 patients with active TB disease who were coinfeéccted with HIV-1 (5 men and 2 women, age range 32–48 yr), and 6 healthy donors (10 men and 2 women, age range 28–52 yr) who were negative for tuberculin purified protein derivative (PPD) and HIV-1.

Full details regarding patient selection are provided in Table E1 in the data supplement, and the experimental setup is described in the data supplement.

CD8+ T-Cell Proliferation Induced by Mtb Peptide
Peripheral blood mononuclear cells (PBMCs) were labeled with CFSE (5 mM; Molecular Probes) and 1–2 × 105 cells were stimulated with peptide 53–61 of Mtb Rv1484 protein at a concentration of 10 µg/ml in RPMI 1640 medium supplemented with 10% heat-inactivated pooled human AB+ serum, 2 mM L-glutamine, 20 mM HEPES, 100 U/ml penicillin, 100 µg/ml streptomycin, 5 × 10−5 M 2-mercaptoethanol, and 5 ng/ml IL-7 (Peprotech) as described previously (23). Positive (PHA, 1 µg/ml; Life Technologies) and negative (medium only) controls were included in each assay. On Day 7 of culture, cells were harvested. Replicates (n = 6) were pooled and stained using CD3-PerCP, CD8-APC, and CD56-PE (BD Biosciences) before acquisition on a FACSCanto and analyzed using FlowJo software (BD Biosciences). The percentage of proliferation was calculated as described previously (23).

In some experiments, a proliferation assay was performed in the presence of the anti–PD-1 (programmed cell death protein 1) monoclonal antibody (mAb) nivolumab at a final concentration of 20 µg/ml (24) or an isotype-matched mAb of irrelevant specificity at the same final concentration. Apoptosis was evaluated with the use of an Annexin-V-FLUOS staining kit (Roche Diagnostics).

Generation of CD8+ T-Cell Lines and Functional Assays
PBMCs from three patients with active TB were cultured with 10 µg/ml of Mtb-derived peptide as described previously (25). After 15 days, the cultures were restimulated weekly with an equal number of peptide-pulsed, irradiated (120 Gy from a cesium source) allogeneic feeder cells in the presence of 40 U/ml of IL-2, and 10 ng/ml each of IL-7 and IL-15. After four to five cycles of restimulation, the enriched population contained >80% CD8+ T cells.

Peptide-specific CD8+ T-cell lines were cocultured with uninfected, or Mtb- or HIV-1–infected or coinfected THP-1 cell line and monocyte-derived macrophages (MDMs) at an E:T ratio of 10:1. After 6 hours of coculture, the cytotoxicity of target cells was assessed by flow cytometry as previously described (25), after incubation with the Annexin-V-FLUOS staining kit. Mixtures of target and effector cells were lysed with 0.1% saponin and sonicated for 20 seconds. The number of colony-forming units was counted as previously described (25). HIV-1 p24 Gag levels in supernatants were determined by ELISA (26).

The HLA-A*0201–restricted CD8+ T-cell clone NFA2-16 and the HLA-E–restricted T-cell clone MV-14E were generated as previously described (27, 28) and used as positive controls.

Statistics
The nonparametric Mann-Whitney U test was used to determine statistical differences in the distribution of the results. P values of <0.05 were considered significant. Data were analyzed using statistical software (SYSTAT 11; Systat Software).
Results

Differential Downregulation of HLA-A2 and HLA-E Cell-Surface Molecules by Mtb/HIV-1 Coinfection

It is known that HIV-1 promotes downregulation of HLA class Ia molecules (particularly the HLA-A molecule) but does not affect HLA-E molecule expression (18, 19, 22). We initially investigated the expression of HLA-A2 and HLA-E molecules on the surface of MDMs obtained from PBMCs from HDs and the THP-1 monocyctic cell line upon in vitro infection with HIV-1 and Mtb, either alone or in combination. HIV-1 replication and Mtb growth in macrophages were assessed in parallel. As shown in Figures 1A and E1A, Mtb grew efficiently both in THP-1 cells and in MDMs obtained from three different HDs typed as HLA-A*0201, and HIV-1 similarly replicated in both target cells. Coinfection enhanced HIV-1 replication and Mtb growth. The synergistic effect of the two pathogens was more evident in MDMs than in THP-1 cells (Figures 1A and E1A) and attained statistical significance as compared with infection by each pathogen alone.

Virtually no change in HLA-A2 and HLA-E molecule expression was observed when either MDMs or THP-1 cells were infected with Mtb alone (Figures 1B and E1B). In contrast, coinfection by Mtb and HIV-1 provoked marked downregulation of the HLA-A2 molecule on the cell surface that was evident 1 day after infection and peaked at Day 3 (Figures 1C and E1C). Of note, HIV-1 caused early and almost complete downregulation of the HLA-A2 molecule in MDMs from all three tested HLA-A*0201 HDs (Figures 1B and 1C). As expected, neither HIV-1 nor Mtb, either singly or together, caused downregulation of the HLA-E molecule, which remained stably expressed on the surface of MDMs and THP-1 cells (Figures 1B and E1B).

Effect of Mtb/HIV-1 Coinfection on Recognition by HLA-A2– and HLA-E-restricted CD8+ T Cells

The differential HLA-A2 versus HLA-E downregulation capability of Mtb/HIV-1 coinfection might have consequences for CD8+ T-cell recognition of Mtb antigens presented on the surface of macrophages coinfected with Mtb and HIV-1. Therefore, we initially used the HLA-A*0201–restricted CD8+ T-cell clone NFA2-16, which recognizes epitope 120-128 of the Mtb Acr antigen (22, 25, 29) and the HLA-E–restricted T-cell clone MV-14E, which recognizes epitope 53-61 of Mtb Rv1484 (23). The latter epitope was shown to have the highest affinity for the HLA-E molecule in a stabilization assay with TAP-deficient RMA-S cells transfected with HLA-E (23, 29). Moreover, the crystal structure of HLA-E with bound peptide 53-61 of Mtb Rv1484 was recently reported (30). Both clones recognize and kill human macrophages infected with the pathogenic Mtb strain H37Rv in an antigen-specific and genetically restricted manner (29) (our unpublished results). When tested for their cytotoxic and microbicidal responses, both the NFA2-16 and MV-14E clones were able to kill both THP-1 and MDMs from HLA-A*0201–typed individuals infected with the pathogenic Mtb strain H37Rv (Figures 2A and 2B, E2A, and E2B), and consistently reduced the viability of intracellular Mtb in infected cells (Figure 2C, left panel). However, when targets were coinfected with Mtb and HIV-1, clone MV-14E retained its cytotoxic and microbicidal potential, but NFA2-16 failed to kill THP-1 and MDMs coinfected with Mtb and HIV-1 (Figures 2A and 2B, E2A, and E2B) or to reduce the growth of intracellular Mtb (Figure 2C, right panel). Altogether, these results demonstrate that coinfection with Mtb and HIV-1 results in downregulation of the HLA-A2 molecule, resistance to lysis by HLA-A2–restricted CD8+ T cells, and failure to restrict the growth of intracellular pathogenic Mtb. Conversely, HLA-E surface expression and HLA-E–restricted cytolytic and microbicidal responses are not affected by HIV-1/Mtb coinfection.

Even though the NFA2-16 and MV-14E CD8+ T-cell clones were generated under neutral culture conditions (i.e., in the presence of IL-2, IL-7, and IL-15, and in the absence of polarizing cytokines), we cannot exclude the possibility that their different cytotoxic and microbicidal potential toward Mtb/HIV-1–coinfected target macrophages was biased by the prolonged in vitro stimulation. Therefore, we decided to redefine our analysis using short-term polyclonal CD8+ T-cell lines recognizing peptide 120-128 of the Mtb Acr antigen in association with HLA-A*0201, or peptide 53-61 of Mtb Rv1484 in association with HLA-E.

CD8+ T-cell lines were obtained upon ex vivo stimulation with Mtb peptides from PBMCs from three patients who had active TB disease and were typed as HLA-A*0201. CD8+ T-cell lines specific for peptide 120-128 of the Mtb Acr antigen were able to kill Mtb-infected MDMs and THP-1 cells but failed to kill both targets coinfected with Mtb and HIV-1 (Figures 3A and E2C). Moreover, they reduced the growth of Mtb only when macrophages were infected by Mtb alone, and not when they were coinfected by HIV-1 and Mtb. Conversely, the three cytotoxic CD8+ T-cell lines obtained by stimulation with the HLA-E–restricted peptide 53-61 of the Mtb Rv1484 antigen efficiently killed MDMs and THP-1 target cells either infected with virulent Mtb alone or coinfected with Mtb and HIV-1 (Figures 3B and E2D), and reduced the viability of intracellular Mtb in both conditions.

These results indicate that Mtb/HIV-1 coinfection has a profound impact on the recognition of infected targets by HLA-A2–restricted CD8+ T cells, but does not impair cytolytic and microbicidal activities by HLA-E–restricted CD8+ T cells.

HLA-E-restricted CD8+ T Cells Are Expanded in the Circulation of Mtb/HIV-1–coinfected Patients

We next evaluated the size of HLA-A2– and HLA-E–restricted and Mtb peptide–specific CD8+ T cells in PBMCs from 6 PPD- and HIV-1–negative HDs, 10 patients with active TB, and 7 TB/HIV–1–coinfected patients, all of whom were typed as HLA-A*0201, by direct ex vivo binding of HLA-A2 and HLA-E tetramers (TM) loaded with peptide 120-128 of Mtb Acr or peptide 53-61 of the Mtb Rv1484 antigen, respectively, to CD8+ T cells. Figure 4A shows the gating strategy used to identify TM+ CD8+ T cells. The ex vivo frequency of HLA-A2–/Mtb-peptide TM+ CD8+ T cells was higher in patients with active TB disease than in HDs (Figure 4B), but TB/HIV–1–coinfected patients had a lower frequency of HLA-A2–/Mtb-peptide TM+ CD8+ T cells than patients with active TB, although the difference did not attain statistical significance.

Confirming our own previous work with other Mtb epitopes (25), the ex vivo frequency of HLA-E–/Mtb-peptide TM+ CD8+ T cells was higher in patients with active TB (Figure 4B) and, as expected, the highest frequency of HLA-E–/Mtb-peptide...
TM+ CD8+ T cells was found in TB/HIV-1-coinfected patients. We conducted an additional comparison of the relative intraindividual frequencies of HLA-A2 and HLA-E-restricted CD8+ T cells in patients with active TB disease and coinfection by HIV-1 (see Figure 4B). HLA-E-restricted CD8+ T cells recognizing peptide 53-61 of Mtb Rv1484 antigen were on average 50-fold more abundant than HLA-A2-restricted CD8+ T cells that recognized peptide 120-128 of Mtb Acr antigen (Figure 4B).

We also analyzed the memory phenotype of HLA-A2-/Mtb-peptide and HLA-E-/Mtb-peptide TM+ CD8+ T cells in the circulation of patients with active TB and HIV-1 coinfection. The majority of circulating HLA-A2-/Mtb-peptide TM+ CD8+ T cells had an effector-memory profile, consisting of 40% effector memory T cell and 30% terminally differentiated effector memory T cell (TEMRA) phenotypes, both in patients with active TB disease and in patients with TB/HIV-1 coinfection (Figure 4C).

In contrast, whereas a mean 45% of HLA-E-/Mtb-peptide TM+ CD8+ T cells were composed of TEMRA cells in patients with active TB disease, 70% of HLA-E-/Mtb-peptide TM+ CD8+ T cells in TB/HIV-1-coinfected patients were composed of TEMRA cells (Figure 4C). Thus, the HLA-E-restricted and Mtb-specific CD8+ T-cell response in Mtb/HIV-1-coinfected patients appears to be largely dominated by a TEMRA phenotype.

HLA-E-restricted CD8+ T Cells Express an Exhausted Phenotype in HIV-1/Mtb-coinfected Patients and Are Partially Restored by PD-1/PD-L1 Blockade

We previously reported that the frequency of Mtb-specific and HLA-E-restricted CD8+ T cells from patients with active TB greatly expanded in culture after stimulation with Mtb peptides (25). Here, we confirm that finding and show (Figure 5A) that HLA-E-restricted CD8+ T cells expanded in vitro upon specific peptide stimulation and had an average
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1 and tested for their ability to kill MDMs infected with Mtb alone or coinfe cted with Mtb.

T Cells in TB

B

CD8

A1

Aet al.

T Cells display potent cytotoxic and antimicrobial activities in vitro/Mtb-peptide TM

CD8

T cells because previous restricted ex vivo CD8 T cells from HIV-1 T cells from patients with TB or T cells (mean 78%) CD8 restricted T cells from T cells. For T cells from HIV-1/TB coinfecte d patients did not apoptosis of total and virus-coinfe cted CD8 T cells upon polyclonal stimulation.

Coinfected patients showed a restricted antiviral behavior similar to that of HLA-E

Tc e l l s

Figure 2. HLA-E-restricted CD8* T-cell clones display potent cytotoxic and antimicrobial activities toward Mtb/HIV-1-coinfe cted target cells. (A and B) The HLA-A*0201–restricted CD8* T-cell clone NFA2-16 (A), which recognizes epitope 120–128 of Mtb Acr antigen, and the HLA-E–restricted T-cell clone MV-14E (B), which recognizes epitope 53–61 of Mtb Rv1484, were obtained as described in Methods and tested for their ability to kill MDMs infected with Mtb alone or coinfe cted with Mtb and HIV-1 for 5 days. Pooled data from five independent experiments are shown. Bars represent mean ± SD. (C) The ability of NFA2-16 and MV-14E T-cell clones to inhibit the growth of intracellular Mtb in MDMs, either infected with Mtb alone or coinfe cted with Mtb and HIV-1, is shown (data from one experiment, representative of five independent experiments). Control group refers to Mtb-infected or Mtb/HIV-1–coinfe cted MDMs cultured in medium. *P < 0.05 compared with the control group.

17% apoptosis rate. As shown in Figure 5A, upon in vitro HLA-E peptide–specific stimulation, HLA-E TM CD8+ T cells from TB/HIV-1–coinfe cted patients did not expand in terms of absolute numbers (left panel) and showed an extensive apoptosis/mortality rate (right panel). Total CD8+ T cells from patients with TB or TB/HIV-1–coinfe cted patients showed a behavior similar to that of HLA-E–restricted CD8+ T cells upon polyclonal stimulation. Given that HLA-E–restricted CD8+ T cells are capable of controlling Mtb multiplication in the presence of HIV-1/Mtb coinfection, we believed it was important to investigate why Mtb-specific and HLA-E–restricted T cells from coinfe cted patients could not be specifically expanded, and how to sustain this CD8+ T-cell subset. We investigated PD-1 expression on Mtb-specific and HLA-E–restricted CD8+ T cells because previous studies have shown that PD-1 expression is associated with increased susceptibility to ex vivo apoptosis of total and virus-specific CD8+ T cells from HIV-1–infected donors, irrespective of antigen specificity (24). As shown in Figure 5B, PD-1 expression was low in total CD8+ T cells from patients with TB, but it was more frequent in total CD8+ T cells from TB/HIV-1–coinfe cted patients. We next assessed PD-1 expression on HLA-E–restricted and Mtb-specific CD8+ T cells. As shown in Figure 5B, a mean 18% of HLA-E–/Mtb-peptide TM+ CD8+ T cells from patients with TB expressed PD-1, whereas a mean 60% of HLA-E–/Mtb-peptide TM+ CD8+ T cells stained as PD-1+ in TB/HIV-1–coinfe cted patients. Similar patterns of PD-1 expression with respect to Mtb-peptide specificity were obtained when PD-1 expression was analyzed by the mean fluorescence intensity (data not shown).

We next investigated whether blocking the PD-1/PD-L1 pathway could restore ex vivo expansion of HLA-E–restricted and Mtb-specific CD8+ T cells. For this purpose, PBMCs from TB/HIV-1–coinfe cted patients were stimulated with peptide 53–61 of Mtb Rv1484 in the presence or absence of mAb against PD-1. After 7 days, the proliferation and apoptosis of HLA-E–/Mtb-peptide TM+ CD8+ T cells were measured and compared with values obtained at the beginning of culture. Peptide stimulation of CD8+ T cells from HIV-1/TB–coinfe cted patients with Mtb peptide resulted in a limited proliferation (mean 33%) of HLA-E–/Mtb-peptide TM+ CD8+ T cells (Figure 5C) and a mean 50% apoptosis (Figure 5D). Addition to cultures of the anti–PD-1 mAb nivolumab consistently improved proliferation of HLA-E–/Mtb-peptide TM+ CD8+ T cells (mean 78%) and decreased the apoptosis rate (mean 21%) (Figures 5C and 5D show representative results from a TB/HIV-1–coinfe cted patient, and Figure 5E shows cumulative data from three different experiments).

Taken together, these results demonstrate that HLA-E–restricted and Mtb-specific CD8+ T cells in the circulation of TB/HIV-1–coinfe cted patients are exhausted, and that blocking the PD-1/PD-L1 pathway can partially restore their functionality and survival.

Discussion

HIV-1 infection is the major risk factor that predisposes for Mtb progression from latent TB infection to active TB disease, and ~1.3 million individuals worldwide are coinfe cted by these two pathogens (31, 32). HIV-1 has evolved various mechanisms to evade HLA class I–restricted antiviral immunity, including downregulation of
HL class I molecules from the infected cell surface (18). In particular, the HIV-1 protein Nef downregulates HLA-A and HLA-B molecules (33–35) by binding of their cytoplasmic domains in conjunction with the μ1 subunit of host API (adaptor protein 1) (36), whereas the HIV-1 protein Vpu downregulates HLA-C (37). Moreover, HIV-1 Nef downregulates HLA-A more efficiently than HLA-B in vitro (22, 38, 39), which provides a mechanistic explanation for the dominant influence of HLA-B on the antiviral cytotoxic CD8⁺ T-cell response (19, 38). In contrast, HLA-E expression is not downregulated by HIV-1 Nef protein (20). Therefore, we hypothesized that Mtb/HIV-1 coinfection could affect the expression of HLA-A and HLA-E molecules differently on the cell surface, and this in turn might affect the recognition of infected targets by HLA-A2–restricted CD8⁺ T cells, but not by HLA-E–restricted CD8⁺ T cells.

The results reported here show that HIV-1 and Mtb coinfection enhanced replication of both pathogens, particularly in MDMs, and caused a significant downregulation of the HLA-A2 molecule as early as 1 day after infection. Conversely, expression of the HLA-E molecule was not affected by HIV-1 or Mtb alone, or by Mtb/HIV-1 coinfection, and in some instances it was even upregulated on the surface of coinfected MDMs. Because infection by Mtb alone did not influence HLA-A2 expression, it is likely that the downregulation of the HLA-A2 molecule observed during coinfection is largely due to HIV-1, as has been reported in other studies.

By using a reporter cell assay it was observed that HIV-1–mediated downregulation of the HLA-A2 molecule correlated with reduced antigen recognition by CD8⁺ T cells and resistance of infected cells to killing by cytotoxic CD8⁺ T cells (19). Accordingly, we show here that downmodulation of the HLA-A2 molecule by Mtb and HIV-1 coinfection correlates with resistance of coinfected targets to lysis by HLA-A2–restricted CD8⁺ T cells and failure to restrict the growth of intracellular pathogenic Mtb. This was convincingly demonstrated in two different in vitro models of coinfection (primary MDMs established from uninfected, healthy individuals and the THP-1 cell line) and with peptide-specific CD8⁺ T-cell clones or short-term polyclonal CD8⁺ T-cell lines generated under neutral, nonpolarizing conditions.

Conversely, coinfection of MDMs by HIV-1 and Mtb did not modulate HLA-E surface expression and hence did not affect cytolytic and microbicidal responses by HLA-E–restricted CD8⁺ T-cell clones and short-term polyclonal CD8⁺ T-cell lines generated under neutral, nonpolarizing conditions.

Given that HIV-1/Mtb coinfection escapes recognition and killing by HLA-A–restricted CD8⁺ T cells, but is efficiently recognized and killed by HLA-E–restricted CD8⁺ T cells, we became interested in deeply analyzing HLA-E–restricted antimycobacterial CD8⁺ T-cell responses in patients coinfected with HIV-1 and Mtb.

The ex vivo frequency of HLA-E–/Mtb-peptide TM⁺ CD8⁺ T cells was higher in patients with active TB and significantly higher in TB/HIV-1–coinfected patients. The ex vivo frequency of HLA-A2–/Mtb-peptide TM⁺ CD8⁺ T cells showed the opposite pattern, with the highest values detected in patients with active TB and lower values found in TB/HIV-1–coinfected patients.

These quantitative differences were associated with qualitative differences in the memory subset composition of CD8⁺ T cells. In fact, the vast majority (70%) of HLA-E–/Mtb-peptide TM⁺ CD8⁺ T cells consisted of TEMRA cells in TB/HIV-1–coinfected patients, but TEMRA cells only accounted for 40–45% of HLA-E–/Mtb-peptide TM⁺ CD8⁺ T cells in the circulation of patients with TB, and of HLA-A2–/Mtb-peptide TM⁺ CD8⁺ T cells both in patients with TB and in TB/HIV-1–coinfected patients. Thus, the memory subset repertoire of HLA-E–/Mtb-peptide TM⁺ CD8⁺ T cells is largely dominated by a TEMRA phenotype.

CD8⁺ TEMRA cells are a major player in the host protective immune response against Mtb in humans (40, 41), and depletion of CD8⁺ TEMRA cells during therapy with the anti–TNF-α biologic infliximab is associated with reactivation of latent TB infection (41). A role for...
CD8⁺ TTEMRA cells in TB was initially suggested by our own study in which we measured Mtb-specific responses by pentamer staining. In healthy, latently infected children, the majority of antigen-specific CD8⁺ T cells were TEMRA cells (42), whereas in patients with active TB who failed to control tubercle bacilli, antigen-specific CD8⁺ T cells were predominantly central memory T cells (42). Moreover, the frequency of CD8⁺ TEMRA cells is positively correlated with efficient control of HIV-1 infection (43, 44). A recent study in human leprosy identified a subset of CD8⁺ TEMRA cells, defined by the coexpression of three cytotoxic granule proteins, as well as by enrichment of the activating receptor NKG2C (45). This CD8⁺ T-cell subset was functionally capable of T-cell receptor–dependent and NKG2C-dependent release of cytotoxic granule proteins that mediate potent killing of intracellular bacteria (45). NKG2C, when complexed with CD94, binds to human HLA-E loaded with nonamer peptides derived from the signal sequence of other HLA class I molecules (9). Therefore, triggering of NKG2C by HLA-E–Mtb-peptide complexes might similarly activate HLA-E–restricted CD8⁺ T cells that recognize Mtb antigens in a T-cell receptor–independent manner. However, previous results from our laboratories tend to exclude this possibility because

**Figure 4.** Ex vivo analysis of the frequency and memory phenotype of HLA-E–Mtb-peptide tetramer (TM⁺) CD8⁺ T cells. Peripheral blood mononuclear cells (PBMCs) from tuberculin purified protein derivative (PPD)-and HIV-1–negative healthy donors (HDs, n = 6), patients with active TB (TB, n = 10), and patients with TB coinfected with HIV-1 (TB/HIV-1, n = 7), all of whom were typed as HLA-A*0201, were stained using HLA-A2 and HLA-E–Mtb-peptide TMs, followed by viability staining and cell-surface marker staining. Each experiment used samples from different clinical groups and a total of 12 experiments were performed to analyze all samples. (A) Gating strategy. The initial gate was on lymphocytes on the basis of forward scatter (FSC) and side scatter (SSC) followed by selection of live cells using Zombie NIR cell viability dye and gating on single cells. CD3⁺ cells were gated as T cells and further selected for CD8 expression and then for TM frequency. (B) Frequency and (C) memory profile analysis of ex vivo HLA-A2– and HLA-E–restricted CD8⁺ T cells in patients with TB or TB/HIV-1 coinfection. Memory populations were defined based on the expression of CCR7 and CD45RA. (D) Contour plot analysis of the distribution of the memory phenotype of HLA-A2 and HLA-E TM⁺ CD8⁺ T cells; representative of one patient with TB (upper panels) and one patient with TB/HIV-1 coinfection (lower panels). *P < 0.05, **P < 0.01, and ***P < 0.001. T_CM = central memory T cells; T_EM = effector memory T cells; TEMRA = terminally differentiated effector memory T cells; T_N = naive T cells.
1) HLA-E–restricted and Mtb-specific T-cell lines (25) and clones (46) only minimally express the NKG2C receptor or do not express it at all, as evaluated by flow cytometry (25, 46) and RNA expression analysis (46); 2) mAbs to NKG2C fail to inhibit activation of HLA-E–restricted CD8+ T cells by Mtb peptides (25); and 3) NK-cell clones that selectively express NKG2C fail to recognize HLA-E–/Mtb-peptide complexes (25). This indicates that HLA-E–/Mtb-peptide complexes may have very low or no affinity for the CD94/NKG2C receptor.

Although Mtb-specific and HLA-E–restricted CD8+ T cells were found in very high levels in the circulation of TB/HIV-1–coinfected patients, they showed a severely suppressed capacity to expand in vitro after stimulation with Mtb peptide. This was accompanied by extensive apoptosis and high expression of PD-1, which was rarely expressed in total CD8+ T cells or HLA-E–/Mtb-peptide TM+ CD8+ T cells from patients with TB or HIV-1. Manipulation of the PD-1/PD-L1 pathway in vitro with the anti–PD-1 mAb nivolumab partially restored expansion of Mtb-specific and HLA-E–restricted CD8+ T cells from TB/HIV-1–coinfected patients. Taken together, our data demonstrate that Mtb-specific...
and HLA-E–restricted CD8+ T cells are abundant but exhausted in the circulation of TB/HIV–1–coinfected patients, and this correlates with high levels of PD-1 expression. Hence, manipulation of this axis may lead to at least partial restoration of Mb-specific and HLA-E–restricted CD8+ T-cell numbers and function in patients with TB/HIV-1 co-infection. However, this approach should be viewed with caution, as recent reports have indicated that anti-PD-1 treatment in patients with cancer can lead to reactivation of latent TB infection (47, 48). Therefore, additional clinical studies in patients with different types of Mtb infection/disease, with or without HIV coinfection, are needed to establish the potential of immune checkpoint blockade.

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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