Tuberculosis-Associated Immune Restoration Syndrome in HIV-1-Infected Patients Involves Tuberculin-Specific CD4 Th1 Cells and KIR-Negative γδ T Cells

Anne Bourgarit, Guislaine Carcelain, Assia Samri, Christophe Parizot, Matthieu Lafaurie, Sophie Abgrall, Veronique Delcey, Eric Vicaut, Daniel Sereni and Brigitte Autran

*J Immunol* 2009; 183:3915-3923; doi: 10.4049/jimmunol.0804020

http://www.jimmunol.org/content/183/6/3915

---

Why *The JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

References This article **cites 42 articles**, 15 of which you can access for free at:

http://www.jimmunol.org/content/183/6/3915.full#ref-list-1

Subscription Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

Permissions Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Tuberculosis-Associated Immune Restoration Syndrome in HIV-1-Infected Patients Involves Tuberculin-Specific CD4 Th1 Cells and KIR-Negative γδ T Cells

Anne Bourgarit,*† Guislaine Carcelain,* Assia Samri,* Christophe Parizot,* Matthieu Lafaurie,‡ Sophie Abgrall,§ Veronique Delcey,¶ Eric Vicaut,‖ Daniel Sereni,† Brigitte Autran,*‡ and the PARADOX Study Group

Tuberculosis (TB)-associated immune restoration syndrome (IRS) is a frequent event (10 to 30%) in HIV-1-infected patients receiving antiretroviral treatment and is associated with an increased number of IFN-γ-producing tuberculin-specific cells. To further understand the immune mechanisms of TB-IRS and to identify predictive factors, we prospectively analyzed the T1 and TCRγδ T cells known to be involved in mycobacterial defenses and dendritic cells at baseline and after antiretroviral and TB treatment in 24 HIV-1+ patients, 11 with and 13 without IRS. At baseline, these two groups differed by significantly lower proportions of TCRγδ and Vδ2+ T cells displaying the inhibitory receptors CD94/NKG2 and CD158ah,b in IRS patients. The two groups did not differ in the baseline characteristics of CD8 or CD4 T cells or TLR-2 expression on monocytes or myeloid/plasmacytoid dendritic cells. During IRS, the increase in tuberculin-specific IFN-γ-producing cells involved only highly activated effector memory multifunctional (IFN-γ+TNF-α+IL-2+) CD4 T cells, whereas activated HLA-DR+CD4+ T cells also increased during IRS. In contrast, dendritic cells decreased significantly during IRS and there were no changes in TLR-2 expression. Finally, the Vδ2+ T cells, mostly killer Ig-related receptor (KIR) (CD94/NKG2− and CD158−), significantly peaked during IRS but not in non-IRS patients. In conclusion, IRS is associated with an increase in the number of activated tuberculin-specific effector memory CD4 T cells and of KIR− Vδ2+ TCRγδ+ T cells. Higher proportions of Vδ2+ TCRγδ+ T cells lacking KIR expression are present as baseline and distinguish patients who will develop IRS from those who will not. The Journal of Immunology, 2009, 183: 3915–3923.

Immune restoration syndrome (IRS)1 is observed in 10 to 30% of patients coinfected with HIV-1 and Mycobacterium tuberculosis (Mtb) after they begin highly active antiretroviral therapy (HAART) (1). It is characterized by clinical symptoms revealing exacerbation of granulomatous lesions (2). The pathophysiology of IRS is not well understood but is thought to reflect the sudden restoration of immune competence against a coexisting pathogen, such as Mtb, in HIV-1-infected patients with profound immune deficiency. During IRS, recovery of a strong tuberculin skin test reaction in previously anergic patients suggests the restoration of effective antimycobacterial immunity (3). We previously reported a strong increase in the number of tuberculin-specific T cells in patients developing IRS, compared with patients without this syndrome (4). This phenomenon was associated with a massive inflammatory and Th1 cytokine storm. The nature of these IFN-γ-producing cells is thus far unknown, and the involvement of other cells in this IFN-γ- and Th1-mediated granuloma formation remains hypothetical. Nonetheless, anti-tuberculosis (TB) immune reaction and granuloma formation classically involve macrophages, dendritic cells (DC), NK cells, TCRγδ, and CD4+ and CD8+ αβ T cells (5). Both HIV-1 and TB infections impair this cellular immunity, which recovers after the pathogens are controlled by both antiviral and antimycobacterial therapies.

Tuberculin-specific T cells are known to recover rapidly, both qualitatively and quantitatively, within 3 to 6 mo of HAART initiation and successful HIV-1 control (6, 7). In TB-coinfected patients, these cells have been shown to reach proportions up to 33% of the peripheral CD4+ T cells after 9 mo of HAART and to have an effector memory CD45RA−CD62L− phenotype (8). Moreover, T cell recovery is associated with recovery of delayed-type hypersensitivity to tuberculin (9). Taken together, these findings suggest that acute restoration of functional CD4+ T cells specific for Mtb may be massively involved in the pathophysiology of IRS.

The stark increase in tuberculin-specific T cells may also reveal the existence of a restoration process for APCs. CD11c+CD123+ myeloid DC (mDC) are involved in early activation of specific anti-TB Th1 immune response (10). The mycobacterial TLR-2 receptor involvement is responsible for DC maturation (CD83) and activation (CD86) and IL-12 production. Interaction of Mtb with

*Laboratory of Cellular Immunology, INSERM, Pitie-Salpetriere Hospital, Assistance Publique des Hopitaux de Paris (APHP), Universite Pierre et Marie Curie, Paris, France; †Department of Internal Medicine, and ‡Department of Infectious Diseases, Saint-Louis Hospital, APHP, Universite Denis-Diderot Paris, France; §Department of Infectious Diseases, Avicenne Hospital, APHP, Bobigny, France; and ¶Department of Internal Medicine, Lariboisiere Hospital, Clinical Research Unit, Fernand Widal Hospital, APHP, Paris, France

Received for publication December 2, 2008. Accepted for publication June 14, 2009.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Agence Nationale pour la Recherche sur le Sida et les hepatites virales. A.B. was a SIDACTION fellow.

2 Address correspondence and reprint requests to Dr. Brigitte Autran, Cellular and Tissular Immunology Laboratory, Hôpital Pitie-Salpetrière, Batiment CERRV, 47-83, Boulevard de l’Hôpital, 75013 Paris, France. E-mail address: brigitte.autran@psl.aphp.fr

3 Abbreviations used in this paper: IRS, immune restoration syndrome; DC, dendritic cell; HAART, highly active antiretroviral therapy; IQR, interquartile range; KIR, killer Ig-related receptor; mDC, myeloid DC; MFI, mean fluorescence intensity; Mtb, Mycobacterium tuberculosis; pDC, plasmacytoid DC; PPD, purified protein derivative; SFC, spot-forming cell; TB, tuberculosis.

Copyright © 2009 by The American Association of Immunologists, Inc. 0022-1767/09/852.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0804020
TLR-2 on monocytes can both provoke and inhibit proinflammatory macrophage activation (11–13). Both HIV-1 and Mtb can regulate immune response orientation by DC; their interaction affects the balance of the TLR-2/DC-SIGN pathway (14). HIV-1 infection has been associated with defects in circulating DC, both myeloid and plasmacytoid (15), defects that are partially restored by anti-retroviral and anti-tuberculosis therapy (16–18). TLR-2 membrane expression seems to be modulated by proinflammatory cytokine expression and viral antigens (19). This raises the question, not yet studied, of the involvement of these cells and their receptors in the IRS phenomenon.

Finally, TCRγδ T cells, another T cell subset, appear to play a predominant role against Mtb infection. These T cells produce IFN-γ strongly and early, in response to mycobacterial phosphoantigens (20–23). They are quantitatively and functionally impaired by both HIV-1 (24) and Mtb infection (25), which create an imbalance between the Vδ1 and the normally predominant Vδ2+ subsets (26) and Vδ2+ energy (27, 28). After HAART and anti-TB treatment, these defects, already associated with inflammatory syndromes in SIV-infected macaques (29), recover, except for the amplification of the Vδ1+ population (30). Moreover, the TCRγδ T cell effector function is known to be modulated by inhibitory and activating MHC class-I ligand receptors that belong to the NK receptor family. These modulations permit pathogens to escape the immune reaction. TB increases NKG2A inhibitor expression on Vδ2+ cells (31), whereas HIV-1 increases NKG2C activation on Vδ1+ T cells (32). These characteristics raised the question of the killer Ig-related receptor (KIR) involvement in the pathophysiology of IRS.

To improve our understanding of the pathophysiology of TB-associated IRS and identify predictive factors for it, we prospectively analyzed the characteristics of the major actors of Mtb-related granulomatous responses in patients with and without IRS, both before and after initiation of HAART.

Materials and Methods

Patients

Thirty-five consecutive untreated patients coinfected with TB and HIV-1 were prospectively included when they began anti-TB treatment. Inclusion criteria were: HIV-1 infection, no previous HAART, CD4 count below 200 cells/mm³, anti-TB therapy initiated, and indications for HAART. Inclusion was confirmed when the Mtb infection was proven (positive culture or histological findings). Patients were evaluated when they began antmycobacterial therapy (T10) and HAART (T0) and at 1, 3, 6, and 12 mo after beginning HAART (M1,3,6,12). In addition, patients with IRS were evaluated when it was first diagnosed (T1IRS) and 20 days later. IRS was defined (33) as recurrence of inflammatory reaction (fever with elevated C-reactive protein), enlargement of pre-existing lesions, or development of new lesions (lymph nodes or pleuritis) with no mycobacterium resistance and no other apparent cause, in a patient responding to HAART (HIV-1-RNA decrease >1 log copies/ml). Patients who did not experience IRS within 3 months of HAART initiation were considered not to have it. Eleven patients initially included were excluded: TB infection was not confirmed for four, four changed medical center, two were lost to follow-up within three months of starting HAART, and one was transferred early to ICU for seizures. The immunological investigations were therefore performed on the 24 patients who could be analyzed.

The study was approved by the institutional review board at Saint Louis Hospital, and all patients provided written informed consent. Preliminary data of some of these patients have already been published (4).

Methods

ELISPOT assay for quantification of mycobacterial-specific Th1 cells

Ag-specific Th1 cells producing IFN-γ were prospectively quantified on fresh PBMCs by ELISPOT as described (4), after a 40-h stimulation with mycobacterial extracts (tuberculin, 1 µg/ml; Statens Serum Institute). Controls were PHA (Murex) and medium alone. Spots were counted with an ELISPOT reader (Zeiss), and data were expressed as spot-forming cells (SFC)/10⁶ PBMCs. Results were considered positive when they exceeded 50 SFC/10⁶ PBMCs after subtraction of the mean background obtained with cells alone.

Membrane phenotyping

PBMCs were phenotyped on fresh whole blood by four-color flow cytometry with standard methods (5). The different circulating populations were defined and characterized as follows:

αβ T cells were stained with the following Ab combinations: anti-HLA-DR-FTTC (Beckman Coulter), anti-CD25-PE (BD Biosciences), anti-CD4-PerCPCy5 (BD Biosciences); or anti-CD8-PerCP (BD Bioscience) and anti-CD3-allophycocyanin (BD Bioscience), anti-45RA-FTTC (Beckman Coulter), anti-62L-PE (Pharmingen), and anti-CD7-PE (Beckmann Coulter).

γδ T lymphocytes were defined as CD3α-CD3γδ lymphocytes with pan-γδ TCR expression or PE-CYS (Beckman Coulter). The subpopulations were characterized by anti-TCRγδ-FITC (Beckman Coulter) and their function determined by HLA-DR expression (allophycocyanin and PeCys; BD Biosciences) and by membrane expression of NKR CD94/NKG2-P (HP-3B1; Beckman Coulter), C158a,h-PE (A09778, anti-KIR 2DL1, 2DS1), and C158b-PE (H1846, anti-KIR 2DL2, 2DL3, 2DS2) (Beckman Coulter). For one patient, we were able to have the kinetics of the functional subpopulations characterized in fresh blood by CD45RA-FC5 (Beckmann Coulter) and CD27-PE (BD Biosciences).

DC were defined as HLA-DR+ Lin1(CD3-14-16-19-20-CD56)+ PBMC (anti-HLA-DR-PerCys; BD Biosciences; anti-CD14-FTTC, BD Biosciences). Subpopulations were characterized as CD11c+CD123- for mDC and CD11c+CD123+ for plasmacytoid DC (PD). After washings, cells were incubated for 15 min with the following fluorescent-labeled conjugated mAbs: anti-CD27-FTTC (BD Biosciences), anti-CD8-FTTC, anti-CD45RA-PE (Beckman Coulter), anti-HLA-DR-FTTC (BD Biosciences), anti-CD11c-PE, and anti-CD1c-PE (BD Biosciences). TLR-2 membrane expression was measured as mean fluorescence intensity (MFI) (anti-TLR-2 PE, Bioscience).

Monococyte-macrophages were characterized as CD14+ CD3– PBMCs (anti-CD14 FITC; Beckman Coulter; anti-CD3 allophycocyanin; BD Biosciences). The TLR-2 membrane expression was measured as MFI (anti-TLR2 PE, Bioscience).

Flow cytometry analyses were performed on a FACSCalibur with CellQuest Pro software (BD Biosciences). A mean of 10,000 events was acquired. When populations of interest were identified, subsequent analysis of differentiation or activation status of the rarest populations was considered only if at least 100 positive cells were detectable in the appropriate gate.

Intracytoplasmatic cytokine staining

αβ T cells. Frozen PBMCs with viability above 80% after thawing were cultured for 4 h in RPMI 1640 medium (5% FCS). Cells were then washed and 1 x 10⁶ cells were stimulated for 2 h with tuberculin (10 µg/ml) in a 24-well flat-bottom plate. Cells stimulated with medium alone and PHA (1 µg/ml) were used as negative and positive controls, respectively. Brefeldin A (20 µg/ml) was added after 2 h and the incubation continued for another 18 h. After washings, cells were incubated for 15 min with the following fluorescent-labeled conjugated mAbs: anti-CD27-FTTC (BD Biosciences), anti-CD8-FTTC, anti-CD45RA-PE (Beckman Coulter), anti-HLA-DR-FTTC (BD Biosciences), anti-TNF-α-PE, anti-CD4-PECy5 (BD Biosciences), and anti-CD8-PercP (BD Bioscience). After washings, cells were fixed with PFA 4%, washed in PBS-0.5% BSA-0.1% saponin buffer and then stained for 15 min at room temperature with allophycocyanin-conjugated IFN-γ Ab (BD Biosciences). Flow cytometric analyses were performed on FACSCalibur with CellQuest Pro software (BD Biosciences) on ~500,000 events.

TCRγδ T cells. Frozen PBMCs with a cell viability above 80% after thawing were cultured overnight in RPMI 1640 medium (5% FCS). Cells were then washed and 1 x 10⁶ cells were stimulated for 4 h with coated anti-CD3 (5 µg/ml) in a 24-well flat-bottom plate. Cells stimulated with medium alone were used as negative controls. Brefeldin A (20 µl/ml) was immediately added. Staining was performed as above with a FACSCalibur with CellQuest Pro software (BD Biosciences) on ~500,000 events.

Statistical analysis

Groups were compared with nonparametric tests (Fisher’s exact test and Mann-Whitney as appropriate) at baseline, M1/T1IRS, and at the data peak or nadir after treatment began. Generalized linear models were used to compare the changes of different parameters with time. Two-sided significant
Table I. Clinical characteristics of patients with and without IRS

<table>
<thead>
<tr>
<th></th>
<th>IRS (n = 11)</th>
<th>Non-IRS (n = 13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>6/5</td>
<td>9/4</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>41 (30–56)</td>
<td>37 (26–63)</td>
<td>NS</td>
</tr>
<tr>
<td>TB infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary TB</td>
<td>2</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Disseminated TB</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Smeary positive</td>
<td>5</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>HIV infection (M0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 (*/mm³)</td>
<td>37 (3–123)</td>
<td>56 (13–330)</td>
<td>NS</td>
</tr>
<tr>
<td>VL (log)</td>
<td>5.7 (4.6–6.5)</td>
<td>5.2 (4.3–5.9)</td>
<td>NS</td>
</tr>
<tr>
<td>M1HAART Days from TBK</td>
<td>36 (7–77)</td>
<td>50 (14–111)</td>
<td>NS</td>
</tr>
<tr>
<td>TIRS/M1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days from M1HAART</td>
<td>26 (7–85)</td>
<td>91 (25–305)</td>
<td>NS</td>
</tr>
<tr>
<td>CD4 (*/mm³)</td>
<td>108 (59–430)</td>
<td>163 (9–580)</td>
<td>NS</td>
</tr>
<tr>
<td>ΔCD4 from M1HAART</td>
<td>54 (–1; 393)</td>
<td>77 (50; 250)</td>
<td>NS</td>
</tr>
<tr>
<td>M3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 (*/mm³)</td>
<td>118 (58–399)</td>
<td>132 (49–410)</td>
<td>NS</td>
</tr>
<tr>
<td>ΔCD4 from M1HAART</td>
<td>86 (–74; 367)</td>
<td>73 (88; 354)</td>
<td>NS</td>
</tr>
<tr>
<td>VL &lt; 200 cp/ml (n)</td>
<td>7/10</td>
<td>8/11</td>
<td></td>
</tr>
</tbody>
</table>

Mann-Whitney nonparametric tests comparing patients with and without IRS. VL, Viral load.

Results

Patients

Our prospectively analysis showed that 11 (46%) of the 24 patients developed IRS symptoms in a median of 26 days (range, 7–85) after HAART began. As shown in Table I, patients with and without IRS did not differ for age, sex ratio, country of birth, HIV-1 infection status, baseline (TBK and M0) CD4 count and HIV-1 viral load, TB clinical presentation, percentage of smear-positive patients, or resistance to anti-TB treatment. Patients with and without IRS did not differ for quantitative CD4 T cell restoration or viral control at IRS onset or M1, respectively.

Baseline characteristics of immune cells involved in Th1 pathways

All immunologic characteristics were studied at initiation of HAART in a median of 40 (range, 7–111) days after initiation of TB treatment. In conventional T cells, we detected similar levels of tuberculin-specific IFN-γ producing T cells in both groups, with an overall median of 52 (interquartile range (IQR), 19–182) SFC/10⁶ PBMCs (Fig. 1A). Intracellular staining showed those cells were CD4⁺ (Fig. 1B). In addition, we observed no difference in activation (HLA-DR) or differentiation markers (CD45RA and CD62L) on either CD4⁺ or CD8⁺ T cells (Table II).

When analyzing innate immune cells such as APCs, circulating DCs accounted for a median of 0.46% PBMCs overall with a CD11c⁺CD123⁺mDC/CD11c⁺CD123⁻pDC subpopulation ratio of 4:1. Neither parameter differed between the two groups (Table II). Similarly, the maturation/activation status of DC, as measured by the level of membrane CD83/CD86 expression, did not differ (Table II), nor did the number of peripheral CD14⁺ monocytes. Membrane expression of TLR-2, as shown by the MFI, also did not differ between IRS and non-IRS patients on both monocytes (MFI, 50 (IQR, 17–47) vs 31 (23–43)) and mDC (MFI, 11 (IQR, 5–20) vs 9 (6–18); Table II).

Finally, TCRγδ⁺ peripheral T cells accounted for a median of 5.6% (IQR, 1.5–14.4) of the CD3⁺ cells at baseline in the entire study group. This proportion did not differ between patients who did and did not develop IRS, nor did expression of the HLA-DR activation marker. As expected, the balance between the Vβ2⁺ and Vβ2⁻ TCRγδ⁺ T cells was inverted, with more Vβ2⁻ T cells than Vβ2⁺ in both groups. However, the proportions of Vβ2⁺ cells observed in patients who later developed IRS were significantly higher than in the patients who did not (median, 23% (IQR, 11–42) of TCRγδ⁺ T cells vs 12% (IQR, 9–24), respectively (p = 0.02); Table II, Fig. 3E). Moreover, surface expression of the lectin-like heterodimer CD94/NKG2 and the KIRs CD158a,h and b was measured on all subsets of CD3⁺/γδ⁺ T cells and Vβ2⁺ T cells (Fig. 2). CD158a,h and b expression did not differ on Vβ2⁺ γδ⁺ CD3⁺, γδ⁺ CD3⁻, or CD3⁻ T cells between the two groups. In contrast, patients who later developed IRS had significantly lower frequencies of Vβ2⁺ γδ⁺ T cells positive for CD158a,h and b and the number of peripheral CD158a,h and b. The stronger IFN-γ production induced by CD3 triggering in CD94/NKG2² or CD158⁻ Vβ1⁺ and Vβ2⁺ TCRγδ⁺ T cells compared with CD94/NKG2² or CD158⁺ cells demonstrates the negative CD94/NKG2 and CD158 signaling (Fig. 2D).

In conclusion, at baseline the only immunological difference between patients who will and will not develop IRS was that the former had a lower proportion of TCRγδ⁺ T cells displaying the inhibitory receptors CD158 and CD94/NKG2, particularly among Vβ2⁺ TCRγδ⁺ T cells involved against Mtb. These groups did not, however, differ for the conventional CD4⁺ Th1 cells specific for tuberculosis.

Characterization of IFN-γ-producing tuberculin-specific T cells during IRS

We first confirmed in a larger group of patients (n = 24) that TB-associated IRS is associated with a peak in tuberculin-specific cells producing IFN-γ; these cells, measured by ELISPOT, reached a median value of 3462 (IQR, 1422–3562) vs 453 (120–1676) SFC/10⁶ PBMC in patients without IRS (p < 0.005, Fig. 1A). The peak values occurred within a median of 197 days (range 23–390) after HAART initiation in IRS vs 119 (range, 28–377) in non-IRS compared with a median of 26 days for clinical symptoms. This acute restoration of purified protein derivative (PPD)-specific T cells was significantly more rapid in IRS patients as assessed by a significantly higher increase of PPD-specific T cells at time of IRS (in IRS patients) compared with M1 (in control non-IRS patients) with a median increase between baseline and T-IRS or M1 of 593 SFC/10⁶ PBMC (range, –42–+3286) in IRS vs 58 SFC/10⁶ PBMC in non-IRS patients (range, –102–+2136; p = 0.034; Fig. 1A). We then used intracellular staining to investigate the nature of these cells that produce IFN-γ in response to tuberculin. At the time of clinical IRS, tuberculin-specific IFN-γ⁺ T cells were mostly CD4⁺ T cells representing a median of 73% (59, 70, 76, 89, 97) of all IFN-γ⁺ CD3 T cells. They represented from 0.8 to 22% (median, 3.2% (0.8, 2, 3, 2, 13.2, 21.8); Fig. 1C) of all CD4⁺ T cells. In addition, all these cells coproduced TNF-α (Fig. 1D) but not IL-2. These tuberculin-specific CD4⁺ T cells had an extremely homogeneous differentiation profile of activated (median, 99% (38, 97, 99, 99, 99) HLA-DR⁺) effector memory (84% (79, 84, 89) CD45RA⁻CD27⁻) IFN-γ⁺TNF-α⁺IL-2⁺ T cells as observed in three to five IRS patients.

This involvement of activated tuberculin-specific IFN-γ-producing CD4 T cells was associated with changes in the proportions of total CD4⁺ and CD8⁺ HLA-DR⁺-activated T cells (Fig. 3A). The
proportions of activated CD4+ T cells increased both in IRS and non-IRS patients about a month after HAART began, as described during immune restoration (7), while activated CD8+ T cells increased only in IRS patients. The magnitude of these increases, however, differed significantly at peak for patients with and without IRS, with medians of 34% (IQR, 24–42) vs 18% (IQR, 7–25) (p = 0.02) and 10% (IQR, 2–20) vs 0 (IQR, 0–7) (p = 0.01) of CD4+ and CD8+ T cells, respectively. The CD4+HLA-DR− T cells remained significantly higher in IRS patients throughout follow-up (p = 0.02; Fig. 3A). In contrast, CD25 expression on CD4 T cells remained lower in IRS patients (p = 0.023; Table II) although there was no difference in the number of CD4+CD25+HLA-DR−-activated cells or CD25high T cells neither at time of IRS nor afterward (data not shown).

At IRS, the proportion of total DC decreased from a median of 0.64% to 0.2% of PBMCs (p = 0.02; Fig. 3B). This decrease involved both mDC and pDC. However, only the proportions of pDC differed between IRS and non-IRS patients (p = 0.04; Fig. 3B). Peripheral proportions of CD14+ monocytes did not differ over time or at IRS, and there was no significant modulation of TLR-2 expression on CD14+ monocytes (Fig. 3C) or on mDC (data not shown).

Finally, after HAART initiation, circulating Vβ2+TCRγδ+ T cells’ proportions remained higher (p = 0.02; Fig. 3D) and peaked
with a higher amplitude in IRS compared with non-IRS patients (13 vs 3.5% of TCR\(^{y8}\) T cells, respectively; \(p = 0.02; \) Fig. 3E). At that peak, 6% (IQR, 4–10) V\(^{2}\) T cells displayed the CD158 receptors and 31% (IQR, 26–43) TCR\(^{y8}\) T cells were CD94/NKG2\(^{+}\) in the IRS patients. The CD94/NKG2 expression on TCR\(^{y8}\) T cells remained significantly lower during follow-up in IRS than in non-IRS patients \((p < 0.0001; \) Fig. 2C). We did not detect any change in cell activation status, as measured by HLA-DR.

In conclusion, IRS is associated with expansion of KIR\(^{-}\) V\(^{2}\) TCR\(^{y8}\) T cells in addition to the expansion of activated effector memory CD4 T cells specific for mycobacteria.

### Discussion

Our study shows two major characteristics of IFN-\(\gamma\)-producing T cells associated with TB-IRS: tuberculin-specific T cells are multifunctional CD4\(^{+}\) effector memory T cells and the involvement of V\(^{2}\) TCR\(^{y8}\) T cells that do not express KIR. This latter characteristic can distinguish at baseline patients who will develop IRS from those who will not.

The IRS frequency in this study appears to be higher than that reported in the literature (1, 34), although we used the same criteria. However, a similar high frequency is currently being observed in an ongoing clinical trial evaluating a new antiretroviral strategy in HIV-infected patients with TB France (BKVIR trial) (O. Lorholary, personal communication) and might reflect a better knowledge of this now well recognized complication, leading to a more frequent clinical diagnosis.

Our results provide a clear definition of the T cells involved in the tuberculin-specific IFN-\(\gamma\) production previously associated with IRS. We confirm and extend prior findings by our group (4) and others (35) that IFN-\(\gamma\)-producing tuberculin-specific T cells are highly amplified in patients with IRS compared with those without it. These findings differ, however, from those of groups (36) that did not detect this increase in specific T cells during IRS.

It is important to note that in our study the significant differences in the peak number of IFN-\(\gamma\)-producing cells at restoration did not always coincide exactly with the IRS clinical symptoms. In all patients tested, both at baseline and during IRS, tuberculin-specific T cells were, either mainly or only, CD4 T cells. They had all the characteristics, both CD45RA CD27 and multifunctional (IFN-\(\gamma\), TNF-\(\alpha\), IL-2), of activated HLA-DR\(^{+}\) effector memory T cells. The mechanism of this expansion may be redistribution or proliferation, as already shown in immune restoration after HAART (6). In accordance with the fact that tuberculin is a combination of hundreds of Ags, we could show in one patient that this expansion was not clonal (data not shown). During IRS, after treatment of these disseminated TB infections in HIV-1 infected patients, we did not find the functional IFN-\(\gamma\)-IL-2-TCM conversion of tuberculin-specific cells that Millington et al. found in TB independently of HIV-1 and IRS, that might represent the persistent Ag spreading (37). These differences might reflect the profound immune deficiency status of our patients. In addition to this tuberculin-specific phenomenon, the peak of total activated CD4 and CD8 PBMCs was suggestive of additional mechanisms involving non-Ag-specific T cell activation, as observed in granuloma lesion (38).

Another major hallmark of these TB-associated IRS in this exploratory study of HIV-1 infected patients is characterized by the involvement of the V\(^{2}\) TCR\(^{y8}\) T cells that do not express KIR. As established in HIV-1 infection (24) and in HIV-1-TB coinfection (25), the TCR\(^{y8}\) T cells V\(^{2}\) subpopulation was markedly reduced in PBMCs of all patients, whereas the usual tissular V\(^{2}\) subpopulation of y8 T cells expanded. In this paper, we show that at baseline and throughout the follow-up, patients with IRS had more circulating V\(^{2}\) TCR\(^{y8}\) T cells than patients without it, a highly interesting finding because these cells have been described as specific for Mtb (20, 21, 23). We thus hypothesize that they may be involved in IRS pathogenesis, together with conventional CD4 Th1 cells. Gioia et al. (28) showed the quantitative and qualitative restoration of V\(^{2}\) effector memory T cells after TB control. Shen et al. (29) have already shown a recovery after HAART of immune responses directed against mycobacterial phosphohantigens, at the same time that the number of tuberculin-specific IFN-\(\gamma\)-producing CD4 T cells increases in bacillus Calmette-Guérin-SIV coinfected monkeys receiving antiretrovirals. In this paper, we add to this body of knowledge the recovery of effector V\(^{2}\) TCR\(^{y8}\) T cells during IRS, a finding that supports the involvement of these cells in the inflammatory syndrome.

Even more importantly, the significantly lower levels of KIR\(^{-}\) and NKR lectin-like CD94/NKG2\(^{+}\) TCR\(^{y8}\) T cells in IRS patients compared with those without IRS also points to differences in the modulation of signaling pathways in these patients. Because the Abs we used characterize NK cell receptors belonging to the family of KIR or lectin-like molecules but cannot distinguish between the inhibitory or activating properties of these receptors, we conducted a functional analysis of IFN-\(\gamma\) production after CD3 stimulation in those cells. Results clearly show these receptors were mediating negative signals on all y8 CD3\(^{+}\) T cells tested that were CD158\(^{+}\) or CD94\(^{+}\) but not on the CD158\(^{-}\) or CD94\(^{-}\) counterparts and are therefore in accordance with the reported inhibitory function of CD158 or NKG2A in V\(^{2}\) y8 T cells during TB (31). As almost 90% of these cells are known to be specific for the mycobacterial Ag isopentenyl pyrophosphate, our results provide a known mechanism for the amplification of the dysregulated immune response to TB that would cause the inflammatory syndrome. This lower expression appeared to be specific to the V\(^{2}\) subset of TCR\(^{y8}\) T cells and was not observed on conventional
CD3⁺ T cells nor on CD3⁻ lymphocytes, suggesting this phenomenon is not genetically determined. However, we cannot exclude a trapping of the KIR⁺ TCRγδ T cells in the granuloma because our observations were made in the peripheral blood cells. It is therefore difficult to assign this phenomenon a specific pathogenic role. Our findings may complement those of Price et al. (39), who found an increased frequency of activating NKR haplotypes but no difference in inhibitory haplotypes in patients experiencing virus-associated IRS.

Although the value of peripheral blood analyses of DC and monocytes is limited for these tissue cells, we observed a decrease rather than the expected quantitative restoration of mDC in patients with, but not those without, IRS. We suggest that this phenomenon may reflect their recruitment at inflammatory sites. We saw no other differences in DC, monocyte activation, or pattern recognition receptors at baseline or during IRS that could explain the uncontrolled Th1 reaction storm seen in IRS.

**FIGURE 2.** Phenotypic and functional characterization of NKR on CD3⁺ and CD3⁻ lymphocytes and γδ T cells at baseline. Data were established in 11 patients who will (black) and 13 patients who will not (white) experience IRS. A, Baseline (M₀) frequencies of CD158a,h⁺ (left) and b⁻ (right) cells. B, Baseline (M₀) frequencies of CD94/NKG2 heterodimer expression measured as CD94 positive cells. C, Kinetic of CD94/NKG2⁺ TCRγδ⁺ T cells in patients with (black) and without (white) IRS (median). D, Anti-CD3 stimulation of TCRγδ⁺ T cells in one IRS patient at baseline. p, Mann-Whitney nonparametric test; §, general linear model.
During IRS, even in the context of the proinflammatory cytokine storm already described (4, 19). Of note, we did not measure DC-SIGN expression, which is known to be up-regulated in TB. The balance between TLR-2-activation and DC-SIGN-inhibition during IRS may require further exploration (40, 41). We therefore cannot definitively identify or rule out roles for these innate immune-competent cells in triggering this exacerbated inflammatory syndrome.

Finally, the only immune characteristic that appears to predict IRS at initiation of HAART is the lower proportion of KIR$^+$

**FIGURE 3.** Kinetics of changes in circulating immune competent cells after HAART initiation. Results are expressed as median values in 11 patients with (black) and 13 patients without (white) IRS. A, Median HLA-DR$^+$ CD4$^+$ and CD8$^+$ T cells after HAART initiation. Arrow, Peak of HLA-DR$^+$ CD4$^+$ and CD8$^+$ T cells; *, $p = 0.02$ (CD4$^+$) and $p = 0.01$ (CD8$^+$); §, general linear model of CD4$^+$/HLA-DR$^+$ between IRS and non-IRS patients, $p = 0.02$. B, DC and pDC subpopulations. Arrow, Significantly different nadir in IRS vs non-IRS at time of IRS, $p = 0.02$. C, CD14$^+$ monocyte frequency and TLR2 membrane expression. D, Proportion of V$\beta$2$^+$ and V$\beta$2$^+$ TCR$\gamma\delta$ T cells in IRS and non-IRS patients (median). E, Proportion of V$\beta$2$^+$ TCR$\gamma\delta$ T cells at baseline, at peak of increase after HAART initiation. The changes from baseline are represented as $\Delta$. $p$, Mann-Whitney nonparametric test comparing patients with and without IRS; §, general linear model.
TCRγδ+ T cells, whereas, unlike Boulware et al. (42) and Tan et al. (35), we found no difference in T cell activation at baseline. Overall, the number of parameters explored in this relatively small study does not allow us to draw definitive conclusions on the respective role of these parameters. However, after adjusting our statistical analysis according to the Hochberg and Benjamini method (43), the magnitude of tuberculin-specific T cells during IRS and the low proportion of CD94/NKG2α TCR γδ+ T cells at baseline still remain significant.

In conclusion, we provide two clear and independent mechanisms for the storm of IFN-γ-producing cells that is associated with IRS in HIV-TB coinfected patients with an acute amplification of conventional tuberculin-specific multifunctional CD4 effector-memory T cells and an amplification of KIR Vα2 TCRγδ+ T cells that is already present before HAART initiation and appears to distinguish patients who will develop IRS from those who will not.

Acknowledgements


