

# Explosion of Tuberculin-specific CD4 Th1 Responses Induces Immune Restoration Syndrome in Tuberculosis and HIV Co-infected Patients

Anne Bourgarit<sup>a,b,\*</sup>, Guislaine Carcelain<sup>a</sup>, Valerie Martinez<sup>a</sup>, Caroline Lascoux<sup>b</sup>, Matthieu Lafaurie<sup>c</sup>, Brigitte Gicquel<sup>e</sup>, Eric Vicaut<sup>f</sup>, Philippe H. Lagrange<sup>d</sup>, Daniel Sereni<sup>b</sup>, Brigitte Autran<sup>a</sup> and the PARADOX-TB Study group.

<sup>a</sup> Cell Immunology Inserm U543 Pitié-Salpêtrière Hospital, Paris, France  
<sup>b</sup> Department of Internal Medicine, Saint-Louis Hospital, Paris, France  
<sup>c</sup> Department of Infectious Diseases, Saint-Louis Hospital, Paris, France  
<sup>d</sup> Department of Microbiology, Saint-Louis Hospital, Paris, France  
<sup>e</sup> Mycobacterial genetic unit, Institut Pasteur, Paris, France  
<sup>f</sup> Department of Clinical Research Unit, Fernand Widal Hospital, Paris, France

Corresponding Author:  
Pr Brigitte Autran  
Cell Immunology INSERM U543  
85013 PARIS, France  
brigitte.autran@piti-sap.fr

## ABSTRACT

**Background:** Simultaneous antiretroviral and anti-mycobacterial treatments in patients co-infected with HIV and tuberculosis (TB) frequently cause Immune Reconstitution Syndrome (IRS). To test the hypothesis that an acute exacerbation of mycobacteria-specific Th1 response after HIV-infection control by Highly Active Antiretroviral Therapy (HAART) causes IRS, we prospectively analyzed the kinetics of TB-specific Th1 immune response in TB-HIV co-infected patients receiving anti-TB then anti-HIV therapy.

**Methods:** Prospective, multicenter study of 22 consecutive untreated HIV-TB coinfected patients included when initiating antimycobacterial therapy and sequentially evaluated during HAART and at time of IRS. IRS was defined according to classical clinical diagnostic criteria. Patients were declared IRS- if no IRS occurred within 3 months after HAART initiation. Mycobacteria-specific (tuberculin/PPD, ESAT-6, 85B) Th1 IFN- $\gamma$  producing cells were quantified by ELISpot, intracellular cytokine analysis (ICS) and in-vitro production of 25 cytokines/chemokines in antigen-stimulated PBMC-superantigens quantified by chemiluminescence. Comparisons between groups were made using non-parametric Fisher exact and Mann-Whitney tests.

**Results:** Nine patients (41%) experienced IRS (IRS+) within a median of 23 days after HAART onset (M0). M0 median CD4 counts were 37/mm<sup>3</sup> for IRS+ vs. 56/mm<sup>3</sup> for IRS- (p=0.09) and rose at M3 by 97/mm<sup>3</sup> vs. 62/mm<sup>3</sup> in IRS+/IRS- patients (p=0.1). PPD-specific Th1 IFN- $\gamma$ -producing CD4 cells increased sharply during IRS from a baseline median of 56 up to a maxima of 3462 SFC/10<sup>6</sup>PBMC, but not CMV-specific responses tested as control. Those PPD-specific cells represented up to 35% of CD4 cells by ICS and all expressed activation marker (HLA-DR). Only 3 IRS+ patients had ESAT-6, but no 85B-specific responses at time of IRS. IRS- patients did not develop acute PPD-specific responses except in one case. In addition, at time of IRS a peak of PPD-specific Th1 cytokines/chemokines (IL-2, IL-12, IFN- $\gamma$ , IP10 and MIG) without Th2 cytokines (IL-4, IL-5, IL-13, IL-15), and a peak of non-specific inflammatory cytokines/chemokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IL-10, RANTES and MCP-1) occurred.

**Conclusion:** Immune restoration concomitant to CD4 T-cell exposure to mycobacterial antigens contained in tuberculin but not in living TB pathogens appears to cause IRS in patients co-infected with HIV and TB. This key event provides new evidence valuable for the diagnosis and treatment of IRS.

## BACKGROUND AND OBJECTIVE

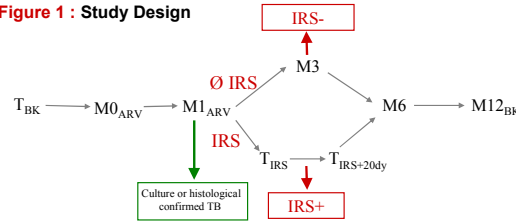
The aim of the study was to determine whether IRS in HIV-TB co-infected patients is due to an acute restoration of a Th1 TB-specific response, which causes its characteristic major inflammatory syndrome. We conducted a prospective multicenter study of patients co-infected with HIV and TB initiating both anti-infectious therapies. We describe our preliminary results.

## SUBJECTS and METHODS

**Patients:** Untreated HIV-TB co-infected patients were prospectively included when initiating anti-mycobacterial therapy and sequentially evaluated during HAART. Inclusion was secondly confirmed when *M. tuberculosis* infection was proved. Nine (41%) patients experienced IRS defined by recurrence of inflammatory reaction, enlargement of pre-existing lesions or development of new lesions with no mycobacterium resistance, no positive culture specimen, no other diagnosis and with response to HAART (HIV-RNA decrease of >1 log copies/ml). The 13 other patients, who did not experience IRS within three months after HAART initiation were defined as IRS-.

**Methods:** Fresh peripheral blood-mononuclear-cells (PBMC) were analyzed for Antigen-specific Th1 cells producing IFN- $\gamma$  quantification by: ELISpot after 40-hour stimulation with either mycobacterial extracts (PPD, 1 $\mu$ g/ml, Staten Institute), ESAT-6 (1 $\mu$ g/ml), 85B, Erp or controls: CMV extracts (Behring), HIVp24, PHA and medium alone. (positive threshold>50 SFC/10<sup>6</sup> PBMC above background); **IntraCellular Staining (ICS)** of IFN- $\gamma$ -producing-cell was measured after 16-hour stimulation with or without PPD (10 $\mu$ g/ml), secretion blocked by brefeldine A, analyzed on a FACSCalibur. **Cytokine/chemokine** production by antigen-stimulated fresh PBMC was measured in supernatant of four patients (1 IRS- and 3 IRS+) using the multiplexed sandwich immunoassay (Human-25-plex $\Phi$ , Biosource) of Luminex  $\Phi$ .

**Figure 1 : Study Design**



**Study design:** 22 untreated TB-HIV patients fulfilled inclusion criteria and began anti-mycobacterial then ARV therapy.

**IRS definition:** recurrence of inflammatory reaction, enlargement of pre-existing lesions or development of new lesions with no mycobacterium resistance, no positive culture specimen, no other diagnosis and with response to HAART (HIV-RNA decrease of >1 log cp/mL).

9 (41%) experienced IRS within 3 months after HAART initiation  
13 (59%) did not

Patient	Age	sex	origin	TB localisation	CD4	VL	Time to HAART (dy)	HAART	ACD4	IRS	Clinical manifestations
1	30	F	Cameroon	pleuritis	26	4.7	44	3TC TTV EFV	86	None	Peritonitis, tubal granuloma
2	41	M	Cameroon	liver, lymph nodes	9	5.7	-1	AZI 3TC ZNO	125	None	Fever, hepatitis
3	43	M	Congo	lung	5.6	5.04	14	AZI 3TC EFV	15	None	Pericarditis
4	32	F	Indonesia	lung, bone marrow, lymph nodes, liver	15	6.5	63	3TC AZI NVP	107	None	Fever, abdominal lymph node swelling
5	35	M	Congo	lung, lymph nodes	115	5.8	40	AZI 3TC EFV	94	None	Fever, peritonitis, abdominal lymph node swelling
6	32	F	Guinea	lung	24	5.7	27	FICZ FV EFV	367	None	Fever, alveolar pneumonitis, lymph node swelling
7	49	M	Congo	myliary, perihaptic lymph nodes	6	5.6	10	TFV FICZ LPV/ rV	14*	None	Fever, abdominal pain in
8	35	F	Congo	Cameroon, abdominal lymph nodes	90	5.1	77	AZI 3TC LPV/rv	-74	None	Fever, abdominal lymph node swelling
9	45	F	Cameroon	Medial stinal lymph nodes	77	5.7	71	FICZ FTV LPV/rv	274	None	Fever, pericarditis
A	36	M	Congo	lymph nodes	15	4.96	39	AZI 3TC ABC	71	None	None
B	38	M	Cote d'Ivoire	lung, lymph nodes	8	5.6	20	AZI 3TC NVP	53	None	None
C	38	M	Cape Verde	lymph nodes	22	5.7	27	AZI 3TC EFV	20	None	None
D	37	M	Mali	lung	32	5.4	14	AZI 3TC EFV	49	None	None
E	30	F	Zambia	lung, liver, spleen	43	4.8	61	AZI 3TC ABC	-7	None	None
F	26	F	Cameroon	abdominal lymph nodes, sinitis	47	5.0	76	AZI 3TC LPV/rv	47	None	None
G	34	M	Senegal	abdominal lymph nodes	131	5.1	50	TFV 3TC AZI/rv	80	None	None
H	31	M	Guinea	lung	198	5.9	24	AZI 3TC EFV	-88	None	None
I	61	M	Cameroon	lung, lymph node	166	5.5	111	3TC DDJ LPV	18	None	None
J	52	M	Mali	lung	219	4.7	53	3TC ABC TTV	80	None	None
K	35	M	Congo	lung	267	5.2	23	AZI 3TC ABC	74	None	None
L	63	M	Algeria	lymph nodes, lung, gastrointestary	60	5.7	89	AZI 3TC EFV	354	None	None
M	56	M	Algeria	lung	54	8	50	TFV FICZ EFV	198	None	None

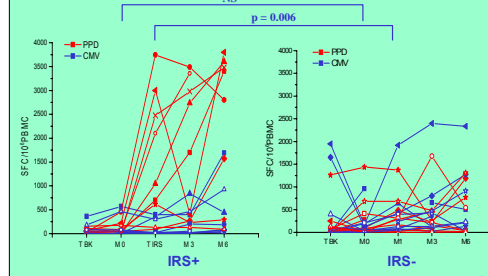
**Table 1 : Patients Characteristics**

	IRS+	IRS-	p
N	9	13	
Gender (M/F)	5/4	10/3	NS
Age (yr)	35 (30-49)	37 (26-63)	NS
Pulmonary disease	6/9	10/13	NS
Disseminated tuberculosis	7/9	9/13	NS
T <sub>HB</sub> CD4 (mm <sup>3</sup> )	26 (6-116)	47 (15-267)	NS
T <sub>HB</sub> VL (cp/ml)	500 000	250 000	NS
Time to HAART (dy)	36 (7-77)	50 (14-111)	NS
Time to IRS/HAART (dy)	23 (12-85)		
CD4 M1	108 (59-193)	147 (9-580)	NS
ACD4 M1-M0 (mm <sup>3</sup> )	53 (-1;-161)	71 (-50;-90)	NS
CD4 M3	120 (58-399)	119 (49-410)	NS
ACD4 M3-M0 (mm <sup>3</sup> )	97 (-74;-367)	62 (-88;-120)	NS
M3 VL-200 cp/ml (N)	4/7	8/11	NS

**Table 2 : Groups Characteristics**

**Results:** Patients with IRS (IRS+) and without (IRS-) did not differ for ethnicity, age, sex, dissemination of tuberculosis, baseline median CD4 count and VL, nor time between TBK and M<sub>0</sub>ARV. After HAART, quantitative immuno-virological outcome was not different between the two groups of patients.

**Figure 1: Kinetics of frequencies of tuberculin (PPD) and CMV specific T-cells expressed as SFC per million of PBMC (IFN- $\gamma$ ELISpot assay) in the IRS+ and IRS- patients.**



**Table 3: Median frequencies of specific T cells (IFN- $\gamma$ ELISpot assay) expressed as SFC per million of PBMC (italic) and number of responders (bold) in the IRS+ and IRS- patients.**

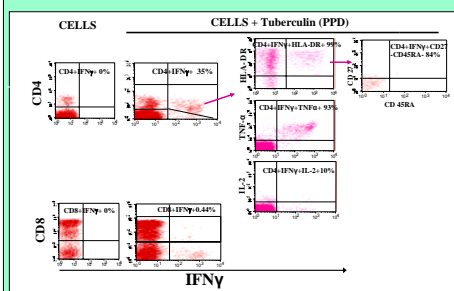
	M0	M1/TIRS	M3	M6	Max-M0					
	IRS+	IRS-	IRS+	IRS-	IRS+	IRS-				
<b>PPD</b>	<b>5/9</b>	<b>6/13</b>	<b>9/9</b>	<b>8/10</b>	<b>6/6</b>	<b>7/10</b>	<b>8/8</b>	<b>5/10</b>		
<i>SFC/10<sup>6</sup></i>	<i>56</i>	<i>36</i>	<i>1044</i>	<i>298</i>	<i>1501</i>	<i>218</i>	<i>3132</i>	<i>658</i>	<i>3462</i>	<i>453</i>
<b>ESAT-6</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>1</b>	<b>3</b>	<b>2</b>	<b>1</b>		
<i>SFC/10<sup>6</sup></i>	<i>4</i>	<i>7</i>	<i>4</i>	<i>20</i>	<i>18</i>	<i>12</i>	<i>13</i>	<i>6</i>	<i>16</i>	<i>13</i>
<b>85 B</b>	<b>0/5</b>	<b>0/10</b>	<b>0/4</b>	<b>0/5</b>	<b>0/2</b>	<b>0/6</b>	<b>0/2</b>	<b>0/5</b>		
<i>SFC/10<sup>6</sup></i>	<i>0/6</i>	<i>0/9</i>	<i>0/4</i>	<i>0/9</i>	<i>0/2</i>	<i>0/6</i>	<i>0/2</i>	<i>0/6</i>		
<b>CMV</b>	<b>4</b>	<b>8</b>	<b>3*</b>	<b>9</b>	<b>4</b>	<b>8</b>	<b>6</b>	<b>8</b>		
<i>SFC/10<sup>6</sup></i>	<i>40</i>	<i>60</i>	<i>27</i>	<i>184</i>	<i>126</i>	<i>124</i>	<i>134</i>	<i>220</i>	<i>200</i>	<i>353</i>
<b>P24</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>0</b>	<b>0</b>	<b>0</b>		
<i>SFC/10<sup>6</sup></i>	<i>2</i>	<i>7</i>	<i>8</i>	<i>24</i>	<i>31</i>	<i>9</i>	<i>19</i>	<i>14</i>	<i>29</i>	<i>47</i>

p comparison of IRS+/IRS-; \*p<0.05; \*\*<0.006, <=0.05

**Results:** The number of PPD-specific cells producing IFN- $\gamma$  did not differ at baseline (M0) between both groups but their proportion increased sharply during IRS with a median 35-fold amplification and reached a maximum of 3462 SFC/10<sup>6</sup>PBMC (median range 129-3744) in the 9 IRS+ patients. This explosive reactivity did not affect the CMV-specific responses from IRS- patients. The IRS- patients did not display a similar acute increase in PPD-specific responses (maximum 453 SFC/10<sup>6</sup>PBMC (median), p=0.0056) even in the sub-group (n=7) with a CD4 gain above 50 cells/mm<sup>3</sup> at M3.

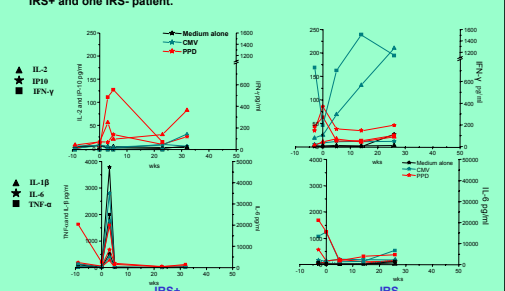
Only 3 IRS+ patients had ESAT-6-specific positive responses during IRS, none had 85B Antigen, neither Erp responses.

**Figure 2: Characterization of PPD specific IFN- $\gamma$ -producing-T-cells by ICS at T<sub>IRS</sub> in one patient.**



**Results:** At time of IRS 14% of the PPD-specific cells are CD8+, 80% are CD4+, which represents up to 35% of the CD4. These CD4 are activated (100% HLA-DR+), co-produce TNF- $\alpha$  not IL-2 and are effector memory cells (84% CD45RA-CD27-).

**Figure 3: Kinetic of proteomic analysis of in-vitro production of 25 Th1/Th2 cytokines/chemokines in PPD- or CMV- or non-stimulated PBMC supernatant of one IRS+ and one IRS- patient.**



**Results:** Two distinct patterns were observed during IRS:  
 \* peak of PPD-specific IFN- $\gamma$  and other Th1 cytokines-chemokines (IL-2, IL-12, IP-10, MIG) without production of Th2 cytokines/chemokines (IL-4, IL-5, IL-13, IL-15, Eotaxin).  
 \* peak of non-specific inflammatory cytokine/chemokines (TNF- $\alpha$ , IL-6, IL-1 $\beta$ , IL-10, RANTES, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ ) by both un-stimulated and antigen-stimulated PBMC.

## CONCLUSIONS

Immune reconstitution syndrome (IRS) in HIV and TB co-infected patients appears to be the consequence of an acute exacerbation of Th1 responses against some mycobacterial antigens associated with a large peak of non-specific inflammatory cytokines/chemokines. This response is mostly mediated by effector memory CD4 cells. We observed poor response to the immunodominant secreted mycobacterial antigens ESAT-6 and 85B compared to the large response to PPD.

These results provide new evidences valuable for the diagnosis and the treatment of IRS.

**The PARADOX STUDY Group:**  
 Abgrall S, Baoukili A, Béglé A-M, Bergmann JF, Bese F, Bolens D, Bouchaud O, Bursacchi P, Cadranet J, Camuset J, Chakvetadze C, Delecq V, Delgado J, Diemer M, Dupont B, Elmarsafy S, Faix O, Fonguerue L, Furco A, Girard P-M, Grillot-Courvalin C, Grivols JF, Guignat A, Guilleminot MC, Herrmann J-L, Jentilis V, Joly V, Jouan M, Joins V, Katlama C, Klutse P, Lacombe K, Lafaurie M, Labourot R, Lavoie A, Lefebvre B, Lefort A, Letellier E, Lortholary O, Metro A, Trucman M, Meynard J-L, Meyhas M-C, Molina J-M, Obenga G, Parnellino M, Pelet O, Pialoux G, Pintado C, Ponscarre D, Rami A, Rozenbaum W, Sahli H, Samri A, Sellier P, Slama L, Courial S, Tubiana R, Strimcman J, Tassi S, Taulera O, Toutou H, Vacher I, Vincent F, Yeni P.