EDITORIAL

SIDE EFFECTS OF THE IMMUNE SYSTEM: LESSONS FROM TUBERCULOSIS-RELATED IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

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Immune reconstitution inflammatory syndrome (IRIS) is a recently described syndrome among human immunodeficiency virus (HIV)-infected patients attributable to the recovery of the immune system during antiretroviral therapy. A growing number of researches on this syndrome have been conducted in recent years, but IRIS in children has not been widely studied. We report the case of a 4.5 month-old, tuberculosis (TB)-HIV co-infected girl who developed IRIS two months after beginning antiretroviral and anti-TB medications. We moreover review the immunopathogenesis of TB-HIV coinfection and IRIS, with particular regard to TB-related IRIS.

The resurgence of TB has been widely influenced by human immunodeficiency virus (HIV) infection. Cellular immunity is the link between these diseases: CD4 T cells are a key component in preventing progression of TB from latent infection to active disease, but they are impaired by HIV.

Highly active antiretroviral therapy (HAART) has been shown to reduce the incidence of TB by more than 80% in HIV-infected adults. This effect is presumably due to the restoration of *Mycobacterium tuberculosis* (Mtb)-specific immunity. However, the impact of HAART on the immune system of HIV-infected patients is not yet completely understood: some patients present a worsening of a previously controlled TB infection or even a new diagnosis of TB shortly after starting the antiretroviral therapy (ART). It has been argued that in some patients ART probably induces a strong and disregulated T cell-mediated immune response in patients with latent, subclinical or treated TB infection which could promote the development of active TB or a relapse of previously controlled TB. This scenario is known as Immune Reconstitution Inflammatory Syndrome (IRIS).

We report the case of a 4.5 month-old, TB-HIV co-infected girl who developed IRIS two months after beginning antiretroviral and anti-TB medications. We moreover review the immunopathogenesis of TB-HIV coinfection and IRIS, with particular regard to TB-related IRIS.

Case description

A 4.5-month-old girl (2.5 months of post-conceptional age) was admitted to the pediatric intensive care unit (PICU) of an academic hospital because of the development of acute respiratory

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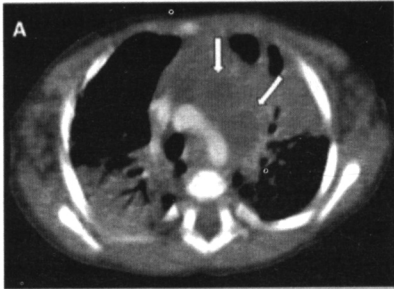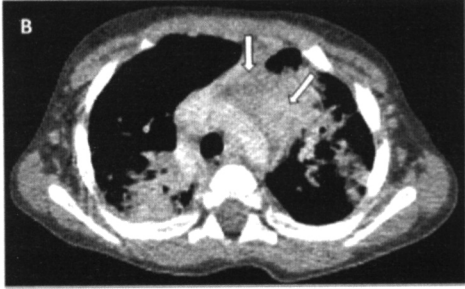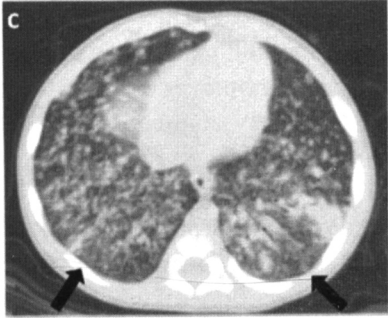Tel: +39 3319819715
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Table I. *The patient's signs and symptoms, laboratory data, images and medications.*

Clinical, laboratory and radiologic findings	4.5 month-old: Diagnosis of TB-HIV coinfecion	7 month-old: Clinical deterioration
Signs and symptoms	Fever, acute respiratory failure	Fever, failure to thrive, loss of appetite, tachipnea and tachycardia, night sweats, frequent crying
CD4+ T cell count	173 per cubic milliliter	1684 per cubic milliliter
HIV viral load	> 500000 copies per milliliter	< 50 copies per milliliter
Medications	Anti-TB medications ART Prednison	Anti-TB medications ART
CT scans: (lymph node findings) Panel A shows necrotic lymph nodes in the anterior mediastinum (white arrows). Lymph nodes are decresed in size and not necrotic (white arrows) in Panel B		
CT scans (lung findings) Panel C is compared with Panel D, which shows a worsening of lung consolidations in posterior segments of lower lobes (black arrows).		

failure. A diagnosis of TB (QuantiFERON TB Gold In-Tube test, bacterioscopic analysis, cultures and polymerase chain reaction (PCR) assay for Mtb on the bronchoalveolar lavage (BAL), urine and gastric washings gave positive results) and HIV-1 (HIV viral load (VL) >500.000 copies/ml, CD4 T-cell count 173/μl) co-infection was performed. A pulmonary computed tomography (CT) scan showed nodular areas of consolidation throughout lungs, large opacities in the dorsal apical

segment of the right lower lobe and in the lingula; mediastinal lymphadenopathy; marked hypodensity in mediastinal lymph nodes, consistent with a necrotic process (Table I, panels A and C). Liver ultrasonography showed two round hypoechoic lesions in the IV and V segments. On the same occasion, a diagnosis of TB-HIV co-infection was made also of the mother.

Anti-TB drugs (isoniazid, pyrazinamide, ethambutol and rifampicin), ART (zidovudine, lamivudine, lopinavir-ritonavir), corticosteroids and trimetropim-sulfametoxazole were administered, with a prompt stabilization of her clinical conditions.

The patient was transferred to the pediatric infectious diseases unit of our hospital when she was 4.5 months of PCA for a clinical and laboratory monitoring of treatment. Weight was 5050 g (3rd percentile). CD4 T-cell count was 1118/µl, HIV VL was 52 copies/ml.

One month later the child presented a deterioration of her clinical conditions. On examination, the infant appeared irritable, with frequent crying, loss of appetite and low interaction. Temperature was 39°C. Laboratory analysis revealed a white-cell count of 20050/µl (79.6% neutrophils). Levels of serum electrolytes, glucose, tests of hepatic and renal function and urinalysis were normal. C-reactive protein (CRP) was 65.7mg/l (normal range, <3mg/l), D-dimer level was 356 ng/ml. CD4 T-cell count was 1684/µl, HIV VL was <50 copies/ml.

Viral, bacterial and fungal infections were ruled out, as well as autoimmune disorders. Staining of gastric washings, blood, BAL and CSF for acid-fast bacilli and PCR assays for Mtb were negative. The abdominal ultrasonography, cerebral Magnetic Resonance Imaging and bone marrow aspirate showed no anomalies. A chest CT scan demonstrated areas of consolidation in the posterior basal segments of the lower lobes, micronodules throughout lungs, mediastinal lymphadenopathy (Table I, panel B and D). Compared with the first CT scan performed two months before, the latter showed a strong decrease in lymph node size and variation in their density, suggesting necrosis resolution. Nevertheless, the two areas of consolidation in the posterior basal segments of lower lobes were not shown in the first CT scan.

Because of the association of worsening radiological findings, clinical deterioration that could not be explained by a previous or newly-acquired infectious or non-infectious condition or by adverse effects of therapy, the sensitivity of Mtb and HIV to administered drugs and the evidence of immune recovery, a diagnosis of paradoxical TB-associated IRIS was made. Prednisone (2 mg/kg for 2 weeks, tapered during 6 weeks) was begun. A few days after the end of the steroid therapy, she presented the same signs and symptoms of the first IRIS episode. A diagnosis of relapsed IRIS was made after the exclusion of opportunistic infections. The same steroid treatment was initiated, with prompt stabilization of her clinical conditions. At the end of corticosteroid therapy, she had no relapse. Chest X-ray showed an improvement of previous findings.

At one-year follow-up (18 months of PCA), she was in good clinical conditions. Her weight was 9400g (between the 15th and the 50th percentile), her length 77 cm (3rd percentile).

DISCUSSION

The presented case of Tb-related IRIS highlights the difficulties in the management of TB-HIV co-infection. In these cases, a physician not only needs to deal with a difficult-to-manage therapy (due to drug interactions between rifampin-based anti-TB regimens and non-nucleoside reverse transcriptase inhibitors and protease inhibitors), but also needs to deal with the extremely complex immune responses implicated in TB-HIV co-infection. On one hand HIV favor TB progression, on the other hand a reconstituting immune system can initiate an inflammatory process that may compromise the patient's clinical condition; a kind of "side effect of the immune system".

In both TB and HIV infections, and particularly TB-HIV co-infection, the immune system plays a primary role, both in disease containment and disease progression. Taking the cue from the reported case, we review basic concepts of the pathogenesis of TB and HIV infections, TB-HIV coinfection and IRIS.

TB immunology: basic concepts

Mtb infects via the respiratory route, encountering alveolar macrophages in the airways and transiting to the lung parenchyma, where innate and adaptive immune responses shape the evolution toward either

disease development or infection control.

After infection, Mtb resides in macrophages and replicates in the endosomal compartment (1). Detection of Mtb by myeloid cells via pattern recognition receptors (PRRs) and processing mycobacterial antigens enable antigen-presenting cells (APCs) to activate T lymphocytes, critical mediators of acquired immune control of TB infection. Cytokines produced during the immune response polarize naïve T-helper (Th) cells into Th1, Th17, or T-regulatory (Treg) cell phenotypes. Human dendritic cells favor the secretion of interleukin (IL)-23/IL-12p70 versus IL-10 (2).

Th1 cytokines [via interferon (IFN)-γ and tumor necrosis factor (TNF)-α activation and characterized by IL-12 production] activate macrophages (imprinting an effector phenotype), a critical step for disease containment. Activated macrophages are characterized by nitric oxide (NO) radical synthesis (upregulated by IFN-γ signaling), efficient in inhibiting Mtb growth restriction. This cluster of infected myeloid cells - once activated by T cells - cooperates to generate the granuloma, an accumulation of macrophages, both infected and uninfected, in various stages of activation directed by T cells surrounded by fibrous tissue (3).

It is believed that active caseation is a necessary sacrifice of tissue to wall off Mtb bacilli. Events after development of caseum determine whether the host develops active TB or remains latently infected. Caseous granulomas that have successfully contained bacterial growth become encapsulated by a fibrotic layer and show macrophages of epithelioid, foamy, or multinucleated appearance (4).

In non-human models of infection a hypoxic environment in the granuloma has been demonstrated. Within granulomas, Mtb is therefore subjected to both nutrient deprivation and hypoxia but, as a highly evolved pathogen, responds by altering patterns of gene transcription to adopt a dormant lifestyle at the lowest metabolic activity (5). This bacterial lifestyle continues until breakdown of host immunity, with subsequent disruption of the structure or function of granuloma, reactivation of dormant bacilli, their dissemination and development of active disease (6). HIV is the leading cause of the disregulation of anti-TB immunity and the best example of how the impairment of the immune system can inhibit bacilli

growth and the development of active disease.

TB-HIV co-infection immunology: how HIV inhibit TB progression

A number of hypothesis have been proposed to explain how HIV could impair the ability to control Mtb infection (7).

HIV causes a strong reduction in peripheral, mucosal, and gut CD4 T cells shortly after infection by preferentially infecting activated CD4 T cells and resting memory CD4 T cells (7). However, immunologic changes during HIV/TB coinfection seem to be more complex. It has been proposed that the worsening of pathology associated with HIV-Mtb co-infection is caused by a functional disruption of the immune response within granulomas (8), with a decrease in the ability to contain Mtb, leading to increased bacterial growth, thus mycobacterial dissemination and severe pathology.

Studies have suggested that HIV infects activated (HLA DR+) alveolar macrophages (CD14+ CD36+), as well as lymphocytes (CD26+), in the pleural fluid or airways of co-infected individuals (9). Since CD4 T cells and macrophages are major components of the granuloma and most of these T cells are likely to be activated, granulomas seem to be an ideal site for HIV replication. Also proinflammatory cytokines induced by TB-host contact seem to favor HIV replication within granulomas (10).

It has also been demonstrated that peripheral blood mononuclear cells of TB-HIV co-infected individuals stimulated with heat-killed Mtb proliferated significantly less and released less IFN-γ than those of TB-only patients (11).

IRIS: Definition, epidemiology, risk factors and diagnostic criteria

Antiretroviral treatment in TB-HIV co-infected individuals leads to an increase in the percentage of naive (CD27$^+$ CD45RA$^+$) CD4 T cells at 36 weeks after antiretroviral therapy and a sustained increase in central memory (CD27$^+$ CD45RA$^-$) CD4 T cells by 12-week post-treatment (12). This increase in Mtb-specific T cell responses is significantly weaker than that of individuals with TB alone and, moreover, it may not always ameliorate TB pathology and may, sometimes, exacerbate a latent, subclinical or even treated TB infection. This scenario is known as IRIS.

Table II. *Studies on pediatric IRIS.*

Author	Type of pubblication	Country	Number of IRIS events	Number of TB-IRIS events	Mean age	Time on HAART	Baseline CD4	IRIS scenario	Deaths from IRIS
Orikiiriza et al (17)	Original article	Uganda	62	25	6 yr	1 to 6 months	<15%	11 – P 14 – UNM	0
Innes et al (18)	Case report	South Africa	1	1	7 m	3 months	908/µl	?	0
Wamalwa et al (19)	Original article	Kenia	ND	ND	4.9 yr	ND	286	ND	1
Smith et al (20)	Original article	South Africa	34	12	7 yr	24 weeks	<15%	ND	1
Walters et al (21)	Retrospective study	South Africa	10	10	11.5 m	2 to 4 months	19%	ND	4
Puthanakit et al 2006 (16)	Original article	Thailand	32 (29 children)	14	8.2 yr	4 wk	3.1 % (29/µl)	3 – P 11 – UNM	2
Zampoli et al (22)	Case series	South Africa	11	11	7.6 yr	ND	354/µl	4 – P 7 - UNM	1
Puthanakit et al 2007 (23)	Original article	Thailand	25	6	7.6 yr (0,4-14,8)	<24 wk	5.2%	ND	4 (0 TB-IRIS)
Bakeera-kitaka et al (24)	Retrospective study	Uganda	106	106	ND	< 6m	<200/ml	104 – UNM 2 – P	ND
Wang et al (25)	Original article	Perù	18	4	5.6 yr	6.6 wk	288/µl	1 – P 4 – UNM	0
Rabie et al (26)	Case report	South Africa	1	1	ND	ND	ND	P	0
Shah I (27)	Original Article	India	7	2	5.8 m	2m	ND	P	0
Present paper	Case report	Italy	2 (1 child)	2 (1 child)	6.5m	2m	173	P	0

P: paradoxic TB-IRIS; UNM: unmasking TB-IRIS; ND: not-defined; TB-IRIS: tuberculosis related IRIS.

IRIS has been defined as "a paradoxical deterioration in clinical status attributable to the recovery of the immune system during HAART (13)." Patients with this syndrome 1) have been diagnosed as having AIDS, 2) have laboratory evidence of immune recovery, 3) exhibit symptoms of an infectious or inflammatory condition while receiving ART, 4) have symptoms that cannot be explained by a newly-acquired infectious or not infectious condition, by the expected clinical course of a previously recognized infectious agent, or by adverse effects of therapy (13).

Two clinical scenarios have been described: "unmasking" IRIS, in which there is an underlying subclinical infection, occult or latent, when starting HIV therapy, with an accelerated presentation after the beginning of HAART, and "paradoxical" IRIS, in which there is a symptomatic relapse of a previously known, treated infection (14).

The incidence of IRIS is 10–15% among adults with AIDS in North America and Europe, 20–25% in resource-limited regions (15). Unlike adults, there are few data on IRIS in the pediatric population. The majority of published pediatric IRIS works originate from Thailand, with an incidence rate of 19% in 153 symptomatic HIV-infected children starting HAART (16), and from Uganda, with a reported prevalence of 38% among 162 children with a median age of 6 years (17).

Table II shows characteristics of studies on IRIS in children starting ART for HIV infection.

A number of risk factors associated with TB-IRIS have been identified: 1) a previous diagnosis of extrapulmonary or disseminated TB; 2) time interval between initiation of TB therapy and starting HAART < 2 months; 3) a baseline low CD4 T cell count and high VL, associated with a good viro-immunological response to HAART (28).

Different IRIS working groups have published consensus criteria for the diagnosis of TB-IRIS (29-32), and they have been already validated (33). Table III summarizes the most used definitions for IRIS.

IRIS: pathogenesis

IRIS is secondary to immunological change due to ART. These changes may provide the opportunity for pathogen-specific cells to gain access to sites of previous infection and engage in the host an inflammatory response to foreign antigens, since the presence of viable organisms is no longer necessary (35).

Coinfected individuals on HAART may have excessive inflammation during immune reconstitution. This is believed to be the result of increased inflammation in tissues, which can enhance symptoms of TB or possibly even trigger its reactivation. The excessive inflammation may be caused by: an increase in the antigenic burden, perhaps by reconstituting CD4 T-cell effector function in the granuloma, which can kill bacilli and release antigens; dysregulation of cytokine responses (36); an increase in T cell migration and activation at the site of infection (37).

Haddow et al. recently evaluated inflammatory biomarker profiles during paradoxical and unmasking TB-IRIS (38). Firstly, they found that paradoxical TB-IRIS typically had a biomarker profile of lower IL-10 and monocyte chemotactic protein (MCP)-1 concentrations, higher CRP: IL-10 ratio and undetectable IL-12p70 and GM-CSF during clinical events when compared to TB-infected individuals experiencing non-IRIS inflammatory events after the same duration of ART. Increased production of MCP-1 could probably contribute to monocyte and T-cell responses during TB infection, while IL-10 seems to downregulate many immune functions, including production of Th1 cytokines and has a suppressant effect on immune responses to TB. Suggested hypotheses to explain this biomarker profile (lower MCP-1 and IL-10 concentrations) are: reduced IL-10 concentrations may indicate slower reconstitution of Mtb-specific Treg function relative to effector function leading to loss of immunoregulatory control of the response against TB; reduced MCP-1 levels may reflect focal rather than disseminated disease; and reductions in both markers may result from reduced monocyte activity (38). Secondly, they found that unmasking TB-IRIS was associated with higher pre-ART CRP and IFN-γ levels when compared with non-TB-infected individuals who did not develop IRIS. These differences might be due to the presence of occult, subclinical or undiagnosed TB infection (38). Thirdly, during both paradoxical and unmasking TB-IRIS, circulating CRP levels and CRP:IL-10 ratios were higher in patients with IRIS than in patients who did not develop IRIS, which is

Table III. *Diagnostic criteria for IRIS, adapted by Muller et al. (34), Lancet Infect Dis 2010; 10(4):251-61.*

Author	Number of IRIS cases evaluated	Clinical criteria
Wendel et al., 2001 (29)	28 TB-IRIS	• Documented worsening of signs or symptoms of tuberculosis or exacerbation of disease at other extrapulmonary sites during appropriate treatment • Worsening of pulmonary infiltrates on chest X-ray or CT without other aetiology
Shelburne et al., 2002 (30)	9 IRIS cases related to various infections	• HIV-infected patient • Receipt of effective ART as shown by a decrease in HIV RNA VL or an increase in CD4 T-cell count from baseline • Symptoms consistent with inflammatory process • Clinical course not consistent with expected course of previously or newly diagnosed opportunistic infection, or with toxic effects of medications
French et al, 2004 (31) • *Both major criteria or* *1 major + 2 minor criteria*	IRIS cases related to various infections	*Major criteria* • Atypical presentation of opportunistic infections or tumours in patients responding to ART: exaggerated and atypical inflammatory reaction; progressive organ dysfunction or enlargement of pre-existing lesions after definite clinical improvement with pathogen-specific therapy before starting of ART; or exclusion of alternative causes (toxic effects of drug treatment, newly acquired infection or tumour, or treatment failure) • Decrease in plasma HIV RNA VL by >1 log *Minor criteria* • Increase in blood CD4 cell count after ART • Increase in an immune response specific to the relevant pathogen (e.g., delayed type hypersensitivity response to mycobacterial antigens) • Spontaneous resolution of disease without specific antimicrobial therapy or tumour chemotherapy with continuation of ART
Meintjes et al., 2008 (32)	27 TB-IRIS cases in resource-poor settings	*Antecedents* • Tuberculosis diagnosis according to WHO guidelines before starting of ART • Tuberculosis should have stabilised or improved before starting of ART *Clinical criteria* • New enlarging lymph nodes, cold abscesses, or other focal tissue involvement • New or worsening radiological features of tuberculosis • New or worsening CNS tuberculosis • New or worsening serositis *Exclusion of alternative causes* • Failure of TB treatment (non-compliance or resistance) • Opportunistic infections or neoplasms • Toxic effects of drug treatment

consistent with an acute phase response.

Bougarit et al. showed that IRIS is associated with an increase in the number of activated tuberculin-specific effector memory CD4 T cells and KIR-negative Vγ2+TCRγδ+ T cells. Higher proportions of Vγ2+TCRγδ+ T cells lacking KIR expression are present at baseline and could therefore distinguish patients who will develop IRIS from those who will not (39).

IRIS: treatment

Because of this immunological background, it has been suggested that IRIS treatment should be based primarily on anti-inflammatory measures. For

mild cases, observation and clinical and laboratory monitoring may suffice, because IRIS can be self-limited. For moderate cases, non-steroidal anti-inflammatory drugs have been successfully used. Life-threatening forms can be managed with corticosteroids or temporary discontinuation of ART. The method of delivery, dosing, or duration of therapy are not well established. WHO has recommended prednisone (1–2 mg/kg for 1–2 weeks, then gradually decreasing doses during the following 6 weeks) for TB-IRIS when severe paradoxical reactions occur (14).

Meintjes et al. conducted a double-blind placebo-controlled randomized clinical trial (RCT) in South Africa to determine whether a short course of oral prednisone (1.5 mg/kg/day for 2 weeks, followed by 0.75 mg/kg/day for 2 weeks) might be of value in patients with TB-IRIS (median age, 32). Compared with the placebo group, the prednisone group had significantly fewer days of hospitalization or outpatient therapeutic procedures, more rapid improvement in symptoms, chest X-ray findings, quality of life, and CRP levels. Eight patients in the prednisone arm and three in the placebo arm had adverse events that could be attributed to prednisone therapy. There were 27 infections in the prednisone group *versus* 17 in the placebo group, but there was no difference in severe infections (2 *vs* 4, respectively; $P = 0.40$) (40).

However, a number of concerns remain: 1) the differentiation between mild, moderate and severe disease is undefined, above all in children; 2) the impact of long-term inflammatory conditions in children below 1 year of age can be devastating, because this period of life is marked by physical growth, maturation, acquisition of competences, and psychological reorganization, affecting a child's behavior and social relationships. For these reasons, there are many concerns regarding who needs to be treated or not, and about potential conditions of use, since IRIS could be both a self-limiting phenomenon or a life-threatening condition; 3) long-term steroid-based treatment is a double-edge sword, because steroids can also modify growth and development of body systems, with short- and long-term consequences. Moreover, all the above-mentioned researches have been conducted only on the adult population.

CONCLUSIONS

The incidence of IRIS related to various infections (above all, those TB-related) is high among HIV-infected patients in TB endemic settings, also among the pediatric population. The geographic expansion of the HIV epidemic and the re-emergence of TB around the world have created a favorable situation for TB-HIV coinfection also in developed countries. For these reasons, in the future there could be a higher rate of these infections and their complications (such as IRIS) in TB-HIV non-endemic countries.

This unfortunate side effect of HAART demonstrates that preventing TB is not as straightforward as simply replacing CD4 T cells. Instead, a balance of pro- and anti-inflammatory responses is necessary for optimal control of Mtb. The restoration of the immune response following HAART is likely deficient in reconstituting that balance in some individuals.

There are still several unanswered questions regarding TB in general and IRIS in particular: comprehension of immunity and inflammation in TB; understanding IRIS pathogenesis in order to develop new biomarkers to identify those patients at the greatest risk of developing IRIS; comprehension of the immunopathology of unmasking TB-IRIS, which is particularly poorly understood; how to decrease IRIS rates in high-risk patients; how to develop IRIS-animal models; developing prospective cohort studies and RCTs in order to validate prevention and treatment strategies aimed at returning the immune system to homeostasis following initiation of ART. All these aspects need to be evaluated specifically also in pediatric populations.

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