Pathogenesis of the immune reconstitution inflammatory syndrome affecting the central nervous system in patients infected with HIV

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Anti-retroviral therapy partially restores the immune function of patients infected with human immunodeficiency virus, thereby drastically reducing morbidity and mortality. However, the clinical condition of a subset of patients on anti-retroviral therapy secondarily deteriorates due to an inflammatory process termed immune reconstitution inflammatory syndrome. This condition results from the restoration of the immune system that upon activation can be detrimental to the host. Among the various clinical manifestations, central nervous system involvement is associated with greater morbidity and mortality. This review covers the pathogenesis of this novel neuroinflammatory disease, including the nature of the provoking pathogens and the composition and specificity of the evoked immune responses. Our current perception of this neuroinflammatory disease supports therapeutic strategies aimed at modulating immune aggression without dampening the life-saving restoration of the immune response.

Introduction

Patients infected with HIV are often affected by a spectrum of neurological disorders. This can be a direct consequence of HIV infection of the CNS, where HIV can reside in microglia and perivascular macrophages, or of the immune response to the virus (Gonzalez-Scarano and Martin-Garcia, 2005). Furthermore, the HIV-mediated immunodeficiency predisposes infection of the CNS by many opportunistic pathogens. These opportunistic infections cause further injury to the CNS, independent of inflammation. After the initiation of anti-retroviral therapy, the control of HIV replication permits partial recovery of the immune system. This restores the host defence against opportunistic pathogens (Autran et al., 1997) and has led to a drastic reduction in the...
incidence of acquired AIDS-associated morbidity and mortality, in particular of AIDS-associated CNS diseases (Mocroft et al., 2003; d'Arminio Monforte et al., 2004).

However, anti-retroviral therapy has inadvertently created a novel clinical complication in which the restored immune system causes severe tissue damage to HIV-infected patients. This clinical complication is not unique to AIDS, as the withdrawal of immunosuppressive drugs in other diseases can cause a similar secondary inflammatory syndrome, even in the absence of infection (Coles et al., 2008; Sun and Singh, 2009; Clifford et al., 2010). Anti-retroviral therapy-induced deterioration was first reported in 1992 (French et al., 1992). Since then, immune reconstitution inflammatory syndrome (IRIS) has become a distinct clinical condition. IRIS is now an emerging health concern that affects a growing number of patients due to the increasing use of anti-retroviral therapy worldwide (UNAIDS/WHO statistics, 2008 update, http://www.who.int/hiv/pub/epidemiology/pubfacts/en/). The frequency of IRIS among HIV-infected patients starting anti-retroviral therapy is estimated at 16% (11.1–22.9) (Muller et al., 2010). This is fatal for 4.5% (2.1–8.6) of cases (Muller et al., 2010). Neuroinflammatory diseases driven by IRIS (neuro-IRIS) are estimated to occur in 0.9–1.5% of patients initiating anti-retroviral therapy (McCombe et al., 2009). Neuro-IRIS is of specific interest due to its clinical severity and unfavourable prognosis, frequently resulting in severe neurologic disability or death (French, 2009).

Here, we will examine the current literature pertaining to neuro-IRIS. We have structured our review to focus on (i) the clinical expression; (ii) the pathogenesis; and (iii) the implications for diagnosis and therapeutic intervention of neuro-IRIS.

Clinical expression of neuro-immune reconstitution inflammatory syndrome

Neuro-IRIS is a polymorphic condition with heterogeneous clinical manifestations. This disease is associated with a variety of different pathogens (Table 1). As there are no specific biomarkers, neuro-IRIS is presently diagnosed on the basis of a multiparametric assessment (Johnson and Nath, 2010). First, HIV-infected patients must respond positively to anti-retroviral therapy, with evidence of controlled HIV replication. Second, there must be neurological manifestations with (i) a close temporal relationship between anti-retroviral therapy initiation and disease onset; (ii) evidence of an inflammatory reaction demonstrated by MRI and/or CNS histopathology; and (iii) exclusion of a differential diagnosis.

Clinical scenarios of neuro-immune reconstitution inflammatory syndrome

IRIS can develop through two distinct scenarios that differ in their clinical expression and disease management, which are referred to as ‘unmasking’ or ‘paradoxical’ IRIS (French, 2009). During unmasking IRIS, anti-retroviral therapy reveals a subclinical and previously undiagnosed opportunistic infection (French, 2009). Consequently, the immune restoration leads to an immune response against a living pathogen, which can generally be isolated by microbiological analyses. Tissue damage is due to both the replicating pathogen and the ensuing immune response, as is common for pathogenic microbial infections (Casadevall and Pirofski, 2003). Despite these two pathogenic arms, current management of unmasking IRIS focuses mainly on the elimination of the opportunistic pathogen using approved antimicrobial treatments.

Paradoxical IRIS describes a clinical condition where a patient that was recently successfully treated for an opportunistic infection unexpectedly deteriorates after anti-retroviral therapy initiation (French, 2009). The diagnosis of paradoxical IRIS relies partly on negative microbiologic analyses, testifying that the previous opportunistic infection has been controlled by the antimicrobial therapy and that there is no newly acquired infection. In paradoxical IRIS, the recovering immune response is thought to target persisting pathogen-derived antigens or, potentially, self-antigens, causing tissue damage. Management of paradoxical IRIS therefore requires therapeutic strategies aimed at controlling immune aggression. However, there are presently no approved methods for treating the dysregulated immune response driving paradoxical IRIS.

Neuro-immune reconstitution inflammatory syndrome associated with opportunistic infections

Viral pathogens associated with neuro-immune reconstitution inflammatory syndrome

The JC polyomavirus establishes persistent asymptomatic infection in up to 68% of people by the age of 59 years (Egli et al., 2009). Consecutive to immunodeficiency, viral reactivation allows the lytic infection of oligodendrocytes, leading to demyelination and progressive multifocal leukoencephalopathy (PML) (Cinque et al., 2009). PML is the second most prevalent opportunistic infection of the CNS in industrialized countries (Antinori et al., 2001) and the second cause of AIDS-related death (Lewden et al., 2008). Since the introduction of anti-retroviral therapy, the median survival time of HIV-infected patients with PML has improved from 0.4 to 1.8 years (Engsig et al., 2009) and anti-retroviral therapy remains to date the only available treatment for AIDS-associated PML (Cinque et al., 2009). Its clinical benefit is probably immune-mediated, as the detection of circulating JC virus-specific CD8 T cells correlates with improved survival (Marzocchetti et al., 2009).

Nevertheless, immune restoration induced by anti-retroviral therapy is not always beneficial and 16.7% (2.3–50.7) of patients with PML worsen due to severe neuro-inflammation (PML-IRIS) (Muller et al., 2010). The contribution of IRIS to the clinical worsening of PML under anti-retroviral therapy is difficult to distinguish from an unfavourable natural history of classical AIDS-associated PML. Moreover, the precise contribution of IRIS to PML prognosis remains a controversial issue (Cinque et al., 2003; Du Pasquier and Koralnik, 2003; Falco et al., 2008; Tan et al., 2009). However, the distinction between these two JC
### Table 1 IRIS manifestations associated with infection by neurotropic pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incidence and mortality of neuro-IRIS</th>
<th>Neuro-IRIS manifestations</th>
<th>Non-neurological IRIS manifestations</th>
<th>Risk factor</th>
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<tr>
<td><strong>Viral infections</strong></td>
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<tr>
<td>JC virus</td>
<td>16.7% (2.3–50.7) (Muller et al., 2010)</td>
<td>Inflammatory form of PML (Tan et al., 2009)</td>
<td>None</td>
<td>Unknown</td>
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<tr>
<td>HIV</td>
<td>Rarely reported (Miller et al., 2004; Gray et al., 2005; Venkataramana et al., 2006; Holmes et al., 2010)</td>
<td>HIV-associated neurocognitive disorder (Miller et al., 2004; Gray et al., 2005; Venkataramana et al., 2006; Holmes et al., 2010)</td>
<td>Myocarditis (Rogers et al., 2008)</td>
<td>Unknown</td>
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<tr>
<td><strong>Herpes viruses</strong></td>
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<td>Herpes simplex virus</td>
<td>Anecdotic cases of neuro-IRIS (French et al., 2000; Clark et al., 2004; Janowicz et al., 2009; Newsome and Nath, 2009; Anderson et al., 2010)</td>
<td>Encephalitis, myelitis (French et al., 2000)</td>
<td>Genital ulceration (Couppie et al., 2006)</td>
<td>Unknown</td>
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<tr>
<td>Varicella-Zoster virus</td>
<td></td>
<td>Myelitis, brain vasculitis (Clark et al., 2004; Newsome and Nath, 2009)</td>
<td>Dermatomal zoster (Domingo et al., 2001)</td>
<td>Unknown</td>
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<tr>
<td>Cytomegalovirus</td>
<td></td>
<td>Brain vasculitis (Anderson et al., 2010), ventriculitis (Janowicz et al., 2005)</td>
<td>Vitritis, uveitis (Karavellas et al., 2010), colitis (von Both et al., 2008)</td>
<td>Unknown</td>
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<td>Parvovirus B19</td>
<td></td>
<td>Encephalitis (Nolan et al., 2003)</td>
<td>Pure red cell anemia (Watanabe et al., 2010)</td>
<td>Unknown</td>
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<td><strong>Mycobacterial infections</strong></td>
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<td>Mycobacterium tuberculosis</td>
<td>15.7% (9.7–24.5) (Muller et al., 2010), among which 19.5% develop neuro-IRIS (Pepper et al., 2009)</td>
<td>Meningitis, tuberculoma, radiculo-myelitis (Pepper et al., 2009)</td>
<td>Lymphadenitis, pulmonary infiltrates, pleural effusion, cutaneous abscess (Lawn et al., 2005a)</td>
<td>Early ART initiation after treatment, Low CD4 T cell count, Disseminated tuberculosis (Lawn et al., 2005a)</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>Anecdotic</td>
<td>Tuberculoma (Kishida and Ajsawa, 2008)</td>
<td>Lymphadenitis, pulmonary infiltrates, pleural effusion, cutaneous abscess (Phillips et al., 2005)</td>
<td>Unknown</td>
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<td><strong>Fungal infections</strong></td>
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<td>Cryptococcus neoformans</td>
<td>19.5% (6.7–44.8) (Muller et al., 2010)</td>
<td>Meningitis (or aseptic recurrence of meningitis for paradoxical IRIS), cryptococcoma (Lortholary et al., 2005)</td>
<td>Lymphadenitis, cavitating pneumonia, skin lesions (Lortholary et al., 2005)</td>
<td>Early ART initiation after treatment, Low CD4 T cell count, Disseminated cryptococcosis (Lortholary et al., 2005)</td>
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<td>Candida spp</td>
<td>Anecdotic</td>
<td>Meningitis with vasculitis (Berkeley et al., 2008)</td>
<td>None</td>
<td>Unknown</td>
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<td><strong>Parasitic infections</strong></td>
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<td>Toxoplasma gondii</td>
<td>Rarely reported (Berkeley et al., 2008)</td>
<td>Toxoplasmic encephalitis during immune restoration (Martin-Blondel et al., 2010)</td>
<td>Retinitis (Sendi et al., 2006)</td>
<td>Unknown</td>
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ART = anti-retroviral therapy.
polyomavirus-associated CNS diseases is necessary, as treatment strategies are likely to differ. PML-IRIS requires the therapeutic control of the antiviral inflammatory response, whereas the treatment of PML aims to restore the immune system to afford viral clearance. In patients with PML-IRIS the extent of CNS inflammation is usually detected by gadolinium enhancement and/or a mass effect by MRI (Fig. 1). Unfortunately, this approach is of limited use, since gadolinium enhancement is frequently (43%) lacking at the onset of PML-IRIS (Tan et al., 2009). An accurate diagnosis currently requires a brain biopsy to detect the pronounced perivascular and sometimes parenchymal infiltration of predominantly CD8 T cells and macrophages that is characteristic of PML-IRIS (Gray et al., 2005) (Fig. 2). Potential complications of this invasive strategy stress the need for alternative diagnostic markers. Furthermore, the therapy for PML-IRIS is currently limited to corticosteroids, with conflicting results on their efficacy (Vendrely et al., 2005; Martinez et al., 2006; Tan et al., 2009).

Figure 1  Brain MRI of PML-IRIS. A 40-year-old anti-retroviral therapy-naïve female infected with HIV was admitted for mild cognitive impairment and progressive left brachio-facial hemiparesis. Her CD4 T cell count was 43/mm³ (8%) and the HIV viral load was $6 \log_{10}$ copies/ml. Axial fluid-attenuated inversion-recovery imaging showed multiple patchy areas of subcortical hyper-intensities, without a mass effect (A). Axial T₁ with gadolinium showed marked right frontal subcortical hypo-intensity, without enhancement (B). The cerebrospinal fluid was positive for JC virus and negative for other pathogens. PML was diagnosed and anti-retroviral therapy, based on non-nucleoside reverse transcriptase inhibitors, was started. Eighteen days after starting anti-retroviral therapy, her condition worsened sharply with left hemiplegia and dysarthria. Her CD4 T cell count was 68/mm³ (19%) and the HIV viral load was $3.4 \log_{10}$ copies/ml. Axial fluid-attenuated inversion-recovery showed extension of hyper-intensities and right frontal sulcal effacement, suggesting a mass effect (C). Axial T₁ with gadolinium revealed patchy areas of bilateral contrast enhancement (D). Extended microbiological analysis of her cerebrospinal fluid showed only JC virus. No brain biopsy was taken. PML-IRIS was diagnosed and methylprednisolone was started (1 g/day for 3 days) and then gradually tapered. Anti-retroviral therapy was continued. Her clinical condition gradually improved, with a mild residual cognitive impairment after 13 months of follow-up.
Other viruses are less frequently associated with neuro-IRIS, including HIV itself. A small number of reports have described the occurrence or worsening of HIV-associated neurocognitive disorder after anti-retroviral therapy initiation (Miller et al., 2004; Gray et al., 2005; Venkataramana et al., 2006; Holmes et al., 2010). HIV was the only pathogen detected in the CSF and/or brain and therefore the inflammatory response was thought to target HIV itself. The inflammatory lesions demonstrated activated microglia and a diffuse infiltration of macrophages and cytotoxic CD8 T cells in the white and grey matter. Herpesviridae are only anecdotally associated with neuro-IRIS (Nolan et al., 2003; Janowicz et al., 2005; Anderson et al., 2010), but are commonly associated with non-neurologic IRIS such as uveitis for cytomegalovirus (Karavellas et al., 2001), viral-related skin manifestations for Herpes-simplex virus and Varicella-Zoster virus (Ratnam et al., 2006) and Kaposi sarcoma for Human Herpes virus-8 (Bower et al., 2005).

**Figure 2** Histological aspects of PML-IRIS. A 45-year-old anti-retroviral therapy-naïve male infected with HIV was admitted for a progressive cerebellar syndrome. His CD4 T cell count was 115/mm³ (8%) and the HIV viral load was 5.3 log₁₀ copies/ml. A brain MRI showed non-enhancing T₁ hypo-intense and T₂ hyper-intense lesions of the posterior fossa without a mass effect or gadolinium enhancement. Analysis of the cerebrospinal fluid showed no evidence of JC virus. A probable PML was diagnosed and anti-retroviral therapy based on protease inhibitors was started. He was re-admitted 22 days after starting anti-retroviral therapy for rapid worsening of the cerebellar syndrome. His CD4 T cell count was 224/mm³ (13%) and the HIV viral load was 2.8 log₁₀ copies/ml. A brain MRI showed that the lesions had increased in size with a cerebellar mass effect, without gadolinium enhancement. A cerebellar stereotactic biopsy was taken. Positive simian virus 40 immunohistochemistry confirmed the PML diagnosis. Haematoxylin and eosin staining showed lymphocyte infiltration of brain tissue, particularly in the perivascular area (original magnification ×20 (A) and ×40 (B)). CD68 and CD3 immunohistochemistry demonstrated parenchymal macrophagic infiltration (C) and marked perivascular T cell infiltration (D) (brown nuclei, original magnification ×40). PML-IRIS was diagnosed. Anti-retroviral therapy was adjusted and his clinical condition gradually improved without the need for corticosteroids.

**Mycobacteria-associated neuro-immune reconstitution inflammatory syndrome**

CNS tuberculosis is a major cause of morbidity and mortality among patients living with HIV worldwide, mostly in highly endemic areas such as sub-Saharan Africa (Lawn et al., 2007). Current concepts of the pathogenesis of CNS tuberculosis indicate that *Mycobacterium tuberculosis* productively infect microglia, a population of CNS cells closely related to macrophages (Curto et al., 2004; Rock et al., 2004).

After anti-retroviral therapy initiation, tuberculosis-IRIS affects 15.7% (9.7–24.5) of patients co-infected with tuberculosis and HIV (Muller et al., 2010). Manifestations are variable, with mostly pulmonary involvement and lymphadenitis (Lawn et al., 2007). Neurological involvement can be observed in 19% of patients with tuberculosis-IRIS (Pepper et al., 2009). Manifestations include meningitis, tuberculoma and radiculomyelopathy, sometimes associated with non-neurological manifestations.
The mortality rate at 6 months has been estimated at 13% and the rate of permanent neurological disability at 37.5% (Pepper et al., 2009). Although no firm conclusions can currently be drawn about the impact of corticosteroids on mortality and disability, 95% of patients that showed an initial improvement had been given corticosteroids (Pepper et al., 2009).

Finally, non-tuberculous mycobacteria such as *Mycobacterium avium* complex have also been associated with IRIS (Phillips et al., 2005), but very rarely with neuro-IRIS (Kishida and Ajisawa, 2008).

**Fungus-associated neuro-immune reconstitution inflammatory syndrome**

*Cryptococcus neoformans* is the major causative agent of fungal meningoencephalitis in HIV-infected patients (Helbok et al., 2006; Dromer et al., 2007). Dissemination of yeasts through the bloodstream leads to a systemic infection, with a predilection to infect the CNS where it causes cryptococcal meningoencephalitis (Lin, 2009). Infected monocytes are involved in blood-brain barrier crossing of *C. neoformans*, leading to brain invasion (Charlier et al., 2009). Brain lesions consist of accumulations of yeasts in the leptomeninges and Virchow-Robin spaces, either free or internalized in mononuclear cells (Chretien et al., 2002).

Paradoxical cryptococcal-IRIS affects 19.5% (6.7–44.8) of HIV-infected patients that initiated anti-retroviral therapy after treatment for neuro-meningeal cryptococcosis (Muller et al., 2010). The main neurological manifestation is the aseptic recurrence of meningitis. Features suggestive of IRIS include higher CSF opening pressures and white blood cell counts compared with the AIDS-related cryptococcal disease (Shelburne et al., 2005a). MRI can also reveal meningeal or choroid plexus gadolinium enhancement. Meningoradiculitis, brain abscesses (Fig. 3) or obstructive hydrocephaly also occur occasionally (Lortholary et al., 2005). The incidence of unmasking cryptococcal-IRIS is unknown. It occurs earlier after starting anti-retroviral therapy than the paradoxical form and causes culture-positive neuro-meningeal cryptococcosis (Shelburne et al., 2005a). It is estimated that 20.8% (5.0–52.7) of patients with cryptococcal-IRIS will die (Muller et al., 2010), mostly in low-income countries (Lawn et al., 2005b). Corticosteroids have been reported to relieve the symptoms (Lortholary et al., 2005), but their impact on the functional or vital prognosis is unknown.

One case of neuro-IRIS has been linked to basilar *Candida* meningitis, associated with vasculitis involving mainly CD8 T cell infiltration (Berkeley et al., 2008).

**Parasite-associated neuro-immune reconstitution inflammatory syndrome**

Despite the great prevalence of parasitic infections in developing countries, only few cases of neuro-IRIS have been described, all of them involving toxoplasmic encephalitis (Lawn and Wilkinson, 2006). *Toxoplasma gondii* is an obligate intracellular protozoan parasite. After a mainly asymptomatic acute infection, persisting

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**Figure 3** Brain MRI of paradoxical cryptococcal IRIS. A 38-year-old anti-retroviral therapy-naïve male infected with HIV was treated for disseminated cryptococcosis with meningitis, nodular pneumonitis and skin lesions. His CD4 T cell count was 14/mm³ (2%) and the HIV viral load was 5 log₁₀ copies/ml. His brain computed tomography was normal at the time of cryptococcosis onset. Cerebrospinal fluid analysis revealed fluconazole-sensitive *C. neoformans*. Antifungal therapy (amphotericin B and flucytosine) was started. His clinical condition promptly improved and his cerebrospinal fluid was sterilized after 2 weeks. Subsequent consolidation and maintenance antifungal therapy consisted of prolonged administration of fluconazole (>200 mg daily). Protease inhibitor-based anti-retroviral therapy was started 90 days after antifungal therapy initiation. The patient was readmitted 160 days after starting anti-retroviral therapy for partial seizures. His CD4 T cell count was 52/mm³ (4%) and his HIV viral load was <1.2 log₁₀ copies/ml. Sagittal T₁ with gadolinium showed a cerebellar lesion with annular enhancement and leptomeningeal enhancement, mainly in the posterior fossa (A). Extended microbiological cultures of all the sites sampled were negative, including the cerebrospinal fluid. The cryptococcal antigen titres in the blood and cerebrospinal fluid demonstrated a >4-fold decline from baseline. No brain biopsy was performed. Paradoxical cryptococcal IRIS was diagnosed. Fluconazole maintenance therapy and anti-retroviral therapy were continued and prednisone was started (1 mg/kg/day for 4 weeks) and then gradually tapered off over 2 months. His clinical condition gradually improved and sagittal T₁ with gadolinium showed a partial regression of contrast enhancement after 2 months on corticosteroids (B).
intracellular tissue cysts are disseminated and found mainly in the muscles and brain (Lyons et al., 2002). Toxoplasmic encephalitis most often results from reactivation of a persisting T. gondii infection in immunocompromised patients, due to the severely depressed T cell-mediated immune response (Lang et al., 2007). Toxoplasmic encephalitis is the most prevalent opportunistic infection of the CNS of the anti-retroviral therapy era (Antinori et al., 2004; d’Arminio Monforte et al., 2004). However, only nine cases of unmasking toxoplasmic encephalitis-IRIS (Martin-Blondel et al., 2010) and one of paradoxical toxoplasmic encephalitis-IRIS have been reported (Cabral et al., 2010). In addition to clinical and neuro-imaging features of classical toxoplasmic encephalitis, the unmasking toxoplasmic encephalitis-IRIS patients exhibited unusually high blood CD4 T cell counts (median count of 222/μl [IQ25–75 160–280]). Five of these patients were on chemoprophylaxis and the brain biopsies of two patients revealed an intense angiocentric inflammatory infiltrate dominated by CD8 T cells. The clinical outcome was favourable in all these cases even without corticosteroid treatment.

**Neuro-immune reconstitution inflammatory syndrome associated with autoimmunity**

When no CNS opportunistic infections can be identified, it is clearly difficult to distinguish between HIV-specific and self-reactive immune responses in HIV-infected patients who develop new neurological manifestations after anti-retroviral therapy. Immune dysregulation, including the hyperactivation of B cells, hypergammaglobulinaemia and the presence of autoantibodies are classical manifestations of HIV infection (Lane et al., 1983). However, autoantibody titres frequently increase during infections in patients without autoimmune disease, indicating that serological auto-reactivity is not synonymous of autoimmunity (Berlin et al., 2007). Nevertheless, clinical autoimmune diseases have been associated with anti-retroviral therapy-induced immune reconstitution, indicating that autoantigens can be targets of the deleterious immune response. Indeed, several autoimmune diseases such as autoimmune thyroid disease, Guillain–Barré syndrome, systemic lupus erythematosus or rheumatoid arthritis have been reported as bona fide manifestations of IRIS (Diri et al., 2000; Calza et al., 2003; Piliero et al., 2003; Chen et al., 2005). As for neuro-IRIS, few cases of cerebral vasculitis or inflammatory demyelination are consistent with an autoimmune neuroinflammatory disease (Ringelstein et al., 2009). However, autoimmune T or B cell responses were not formally proven to be involved in these cases and therefore their autoimmune origin is still questionable.

**Pathogenesis of neuro-immune reconstitution inflammatory syndrome**

The inflammatory syndromes associated with anti-retroviral therapy-induced immune reconstitution are an emerging research area and only few immunological studies have been performed to date, providing largely anecdotal or descriptive information. Based on these studies and according to the damage response framework of microbial pathogenesis (Casadevall and Pirofski, 2003), a consensus has emerged that IRIS is a pathogen-driven disease whose clinical expression depends on the susceptibility of the host, the intensity and quality of the antimicrobial immune response and the nature and characteristics of the provoking pathogen (Fig. 4).

**Central nervous system environment in HIV-infected patients**

Any analysis of the pathogenesis of neuro-IRIS must consider the relatively privileged immune status of the CNS (Galea et al., 2007a). Several passive mechanisms reduce the visibility of this organ to immune surveillance. These include the absence of dedicated lymphoid drainage, the reduced accessibility of immune cells and mediators due to the blood–brain barrier and the absence of local dendritic cells at steady state. Active mechanisms, including the local production of anti-inflammatory molecules, the expression of pro-apoptotic signals and activation of regulatory T cells further limit CNS immune responses (Cassan and Liblau, 2007). However, the CNS is not immune-deprived and microbial infections can be cleared efficiently from the CNS.

The CNS environment of HIV-infected patients is substantially modified by the local HIV infection and the resulting infiltration by antiviral T cells, which can be further accentuated by CNS opportunistic infections (Gonzalez-Scarano and Martin-Garcia, 2005). Neuroinvasion by HIV is an early event that occurs during primary infection, when HIV-infected monocytes and CD4 T cells cross the blood–brain barrier (Haase, 1986; Davis et al., 1992). Secondarily, HIV infects CNS blood-derived perivascular macrophages and microglial cells (Koenig et al., 1986). Infected cells produce pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) (Tyror et al., 1992) and chemokines (CCL3, CCL4, CCL5, CCL7), leading to increased blood–brain barrier permeability (Brabers and Nottet, 2006) and sustained recruitment of mononuclear cells to the CNS (Chaudhuri et al., 2008; Peng et al., 2008). Thus, the immune privilege of the CNS is disrupted early after HIV infection, prior to anti-retroviral therapy treatment. This basal immune activity is likely to increase the sensitivity of the CNS to the excessive immune reaction that occurs in neuro-IRIS.

**Pathogen-related factors**

**Pathogen tropism and survival strategy**

The tropism and survival strategy of the opportunistic pathogen, together with its efficacy of dissemination, will determine the intensity and the location of the inflammatory lesions.

Not every pathogen infecting the CNS as a result of impaired cellular immunity causes neuro-IRIS. As described previously, JC virus, M. tuberculosis, C. neoformans and T. gondii all productively infect the CNS, even if they cause neuro-IRIS with distinct frequencies. By contrast, herpes simplex virus establishes
latency within sensory ganglia during which spontaneous reactivation only occurs in a few neurons (Margolis et al., 2007). Viral reactivation leads to neuronal anterograde transport of virions to innervated mucocutaneous regions where viral replication is reinitiated, causing overt viral infection (Knipe and Cliffe, 2008). This tropism of herpes simplex virus for the skin is consistent with the predisposition to dermatitis observed during IRIS (Ratnam et al., 2006) and to its lack of association with neuro-IRIS. Neurotropism of the opportunistic pathogen, therefore, predisposes to neuro-IRIS.

However, variable survival strategies and immune evasion mechanisms of neurotropic pathogens may further influence the predisposition to neuro-IRIS. In this context, T. gondii is of interest given that it represents a common opportunistic infection of the CNS in AIDS, which is only rarely associated with neuro-IRIS. T. gondii has developed mechanisms either to reduce its visibility to the immune system or favour immune regulation. T. gondii inhibits effector cytokines, induces immunoregulatory cytokines (IL-10 and TGF-β) and favours the expansion of regulatory T cells (Table 2). Furthermore, during the slow-replicating bradyzoite stage, T. gondii decreases the synthesis of immunogenic surface proteins and reduces its metabolic activity (Bohne et al., 1999; Lyons et al., 2002). It is plausible that, collectively, these mechanisms contribute to reduce the predisposition of T. gondii infected patients to neuro-IRIS. In contrast M. tuberculosis and C. neoformans infect immunocompetent cells and remain visible to pathogen-specific CD4 T cells to establish granuloma formation. For JC virus infection, immune evasion is likely to be of less importance, given that immunodeficiency triggers lytic infection of oligodendrocytes, warranting an antiviral immune response (Cinque et al., 2009). These observations therefore suggest that the risk of developing neuro-IRIS is influenced by the efficiency of microbial survival strategy of neurotropic pathogens.

Antigenic load
Visibility of antigens to the immune system is critical for immune responses. Several studies have suggested that a high antigenic load at the time of anti-retroviral therapy initiation may predispose to IRIS. A greater risk of developing paradoxical IRIS after tuberculosis and cryptococcosis has been associated with disseminated and/or extra-pulmonary tuberculosis, fungaemia and elevated blood cryptococcal antigen titres (Lortholary et al., 2005; Michailidis et al., 2005; Sungkanuparph et al., 2009). Moreover, initiation of anti-retroviral therapy early after treatment of tuberculosis and cryptococcosis, when the load of pathogen-derived
antigens is likely to be elevated, increases the risk of developing IRIS (Lortholary et al., 2005a; Shelburne et al., 2005a; Lawn et al., 2007). These studies indirectly correlate a high pathogen burden, due to incomplete pathogen clearance or a wider distribution, with the development of IRIS.

**Host genetic susceptibility**

The clinical variations between individuals in a population infected with the same pathogen suggest that the genetic composition of the host influences the pathogenesis of infectious diseases (Alcais et al., 2009). Mendelian or polygenic differences can modulate the predisposition to infection by pathogens (Dean et al., 1996; Filipe-Santos et al., 2006). Human genes also influence infectious disease-related immunological and clinical phenotypes (Zerva et al., 1996). Only few genetic association studies on IRIS have been performed to date, all lacking functional insight. For example, 33% of patients who suffered from cytomegalovirus-related IRIS expressed HLA-A2, -B44 and -DR4, as compared with 14% of controls (Price et al., 2001). Furthermore, polymorphisms in inflammatory cytokine genes, such as those encoding for IL-12, IL-6 and TNF-α, have been associated with herpes virus-related and mycobacterial-related IRIS (Price et al., 2002). Certain major histocompatibility complex genotypes, together with polymorphisms of inflammatory cytokine genes, could thus influence the magnitude and quality of the immune response to pathogens and hence contribute to the development of IRIS. In addition, polymorphisms within the IL-21 gene region have been associated with the development of secondary autoimmune tissue damage after immune reconstitution (King et al., 2004; Jones et al., 2009). Further genetic investigations, including genome-wide association studies, are needed.

**Immune parameters**

It is essential to understand the mechanisms whereby the immune system is restored under anti-retroviral therapy in order to accurately describe the pathogenesis of IRIS. HIV infection is

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**Table 2 Survival strategies of neurotropic pathogens that could be associated with neuro-IRIS**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Neurotropism</th>
<th>Immune evasion mechanisms</th>
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<tbody>
<tr>
<td>Rarely associated with neuro-IRIS (Martin-Blondel et al., 2010)</td>
<td>Disseminated bradyzoites in brain (Lyons et al., 2002)</td>
<td>Reduced visibility to the immune system: (i) Reduced expression of immunogenic surface proteins and decreased metabolic activity of the bradyzoites (Bohne et al., 1999; Lyons et al., 2002)</td>
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<td>Frequently association with neuro-IRIS (Muller et al., 2010)</td>
<td>Infection of microglia (Curto et al., 2004)</td>
<td>Persistence within macrophages: (i) Avoids digestion by inhibiting phagosome maturation, avoiding phagolysosome fusion, and blocking phagosome acidification (Sturgill-Koszycki et al., 1994)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>Accumulation within leptomeningeal spaces either free or in mononuclear cells (Chretien et al., 2002)</td>
<td>Reduced visibility to the immune system: (i) Reduction of antigen presentation by interfering with the synthesis of major histocompatibility complex class II molecules (Harding and Boom, 2010)</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>Infection of oligodendrocytes and astrocytes (Cinque et al., 2009)</td>
<td>Immune regulation: (i) Survival within phagosomes by accumulating capsular polysaccharides (Feldmesser et al., 2001)</td>
</tr>
<tr>
<td>JC virus</td>
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<td>Persistence within macrophages: (i) Capsular polysaccharides interferes with DC maturation and activation (Vecchiarelli et al., 2003)</td>
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<td>Immune regulation: (i) Capsular polysaccharides suppress T cell proliferation and induce IL-10 production by splenocytes (Chiapello et al., 2004)</td>
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<td>Immunocompetent hosts: (i) Functional quiescence during latency (Cinque et al., 2009)</td>
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<td>Immunocompromised hosts: (i) Lytic infection of oligodendrocytes (Cinque et al., 2009)</td>
</tr>
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</table>

Summary of the survival mechanisms developed by T. Gondii and the three pathogens that are most frequently associated with neuro-IRIS. Toxoplasma gondii is a neurotropic pathogen that represents a frequent opportunistic infection during AIDS, but does not predispose to neuro-IRIS.
characterized by progressive immunosuppression that is reversed by anti-retroviral therapy (McCune, 2001; Battegay et al., 2006). The increase in the number of circulating T cells after the initiation of anti-retroviral therapy is biphasic, with an initial rapid increase in the first 3–6 months, followed by a second slower wave, which can last for up to 2 years (Ledegerber et al., 2004). The initial increase reflects the redistribution of CD45RO+ memory CD4 and CD8 T cells from lymphoid tissues to the periphery (Bucy et al., 1999). The second wave results from the de novo output of CD45RA+ CD62L+ naive CD4 and CD8 T cells by the thymus (Pakker et al., 1998). This quantitative recovery is associated with a partial recovery of cell-mediated immune functions, as measured by delayed-type hypersensitivity and in vitro T cell proliferation (Valdez et al., 2000). Innate immune responses are also restored, as indicated by the recovery of natural killer cell, macrophage and dendritic cell functions (Chehimi et al., 2007).

Restored effector immune mechanisms
Several immune parameters may act in synergy during immune restoration so that an antimicrobial immune response overshoots its goal and becomes detrimental to the host. The underlying effector mechanisms are those physiologically employed to control pathogen infection and differ according to the microbe encountered. This highlights that IRIS is the consequence of an overwhelming pathogen-specific cell-mediated immune response (French, 2009).

Innate immunity
Little is known about innate immunity in the pathogenesis of IRIS. Macrophages and microglia are major players in the pathogenesis of HIV-associated neurological disorders (Gonzalez-Scarano and Martin-Garcia, 2005). They also play a pivotal role in modulating innate and adaptive immune responses (Stout and Sutcliffe, 2004). Consequently, the predominant macrophage infiltration seen in lung lesions during mycobacterial-related IRIS has been proposed to play a key role in mycobacterial-IRIS. The excessive macrophage activation in the presence of mycobacterial antigens is likely to contribute to the tissue damage characteristic of IRIS (Lawn et al., 2009). Macrophages develop distinct phenotypes related to specialized functions that depend on the local environment. They can adopt one of at least three distinct phenotypes in response to cytokines or innate stimuli: classically activated, wound-healing or regulatory (Mosser and Edwards, 2008). Knowledge of the composition of macrophages/microglia in IRIS and the ability to direct their plasticity might prove valuable for IRIS.

TCRγδ T cells are a non-conventional T cell subset sharing several features with innate cells (Bonneville et al., 2010). These cells are known to produce IFN-γ in response to mycobacterial phosphoantigens. The activation of TCRγδ T cells is regulated by inhibitory and activating killer Ig-related receptors. One study showed that paradoxical tuberculosis-IRIS was associated with an increase in the number of Vβ2+ TCRγδ T cells that had down-modulated inhibitory killer Ig-related receptors (Bourgarit et al., 2009). An imbalance between the activating and inhibitory receptors on TCRγδ T cells might favour the exaggerated inflammatory response observed during IRIS.

Memory T cells
Most patients (75%) develop IRIS during the first 3 months of anti-retroviral therapy (Shelburne et al., 2005b), indicating that the pathogenesis of IRIS coincides with the first wave of immune restoration (Bucy et al., 1999). It is therefore very likely that the pathogenic T cell response in IRIS is derived from the memory T cell pool, which is characterized by previous antigen encounter. In agreement with this hypothesis, tuberculosis-IRIS has been associated with an increase in the number of activated, tuberculin-specific, effector-memory CD4 T cells (Bourgarit et al., 2009). Thus, the overwhelming response observed during immune restoration could be linked to the expansion of persisting memory T cells.

CD8 T cells
Experimental evidence suggests that CD8 T cells may transmigrate into the CNS using an antigen-dependent route (Galea et al., 2007b) and that they can directly contribute to local demyelination and tissue damage (Na et al., 2008; Saxena et al., 2008). CD8 T cells are essential for the host defence against viral infections and also underlie IRIS triggered by JC virus and HIV (Miller et al., 2004; Gray et al., 2005; Vendrely et al., 2005; Venkataramana et al., 2006). Neuropathological examinations of PML-IRIS have highlighted the dominance of CD8 T cells in the diffuse and focal perivascular mononuclear infiltrates (Gray et al., 2005; Vendrely et al., 2005). This response is thought to be virus-specific, as JC-specific CD8 T cells are detected in the blood of patients with PML-IRIS, concomitant with contrast enhancement on brain MRI and/or brain inflammation (Du Pasquier and Koralnik, 2003; Marzocchetti et al., 2009). Similarly, CD8 T cells are the major effectors of the natural immune response to T. gondii (Lang et al., 2007). The same effector mechanism underlies toxoplasmic encephalitis-IRIS, where neuro-inflammation is marked by the perivascular infiltration of CD8 T cells in the CNS (Pfeffer et al., 2009; Cabral et al., 2010; Martin-Blondel et al., 2010).

CD4 T cells
Th1-type CD4 T cells are implicated in the host defence against mycobacterial and cryptococcal infection (Co et al., 2004). Biopsies of tissues affected by mycobacterial and cryptococcal-related IRIS have shown granulomatous inflammation, consistent with a Th1-type CD4 T cell response (Lawn et al., 2005a; Lortholary et al., 2005). This immune response is specific, as tuberculin skin tests change from negative to strongly positive during tuberculosis-IRIS (Narita et al., 1998). In addition, two studies showed that tuberculosis-related and cryptococcosis-related IRIS were associated with an exacerbated Th1-type inflammatory response compared with patients who do not develop IRIS. This was demonstrated by enumerating IFN-γ producing T cells upon ex vivo antigen-specific stimulation (Bourgarit et al., 2006; Tan et al., 2008). These observations indicate that the
opportunist infection determines the effector T cell response driving IRIS.

**Regulatory T cell defects**

IRIS results from a protective immune response that becomes excessive, possibly due to inadequate regulation (French, 2009). Regulatory T cells are major players of the immune system homeostasis, limiting the magnitude of effector responses and collateral tissue damage (Belkaid and Tarbell, 2009). A loss of functional regulatory T cells leads to excessive T cell activation, reminiscent of the disproportionate inflammatory response seen in IRIS (Gavin et al., 2006). Few studies, and none in neuro-IRIS, have assessed the quantitative and qualitative changes in regulatory T cells in anti-retroviral therapy-treated patients who develop IRIS. Unexpectedly, the proportion of regulatory T cells increase in patients suffering from mycobacterial- or cryptococcal-related IRIS (Meintjes et al., 2008; Tan et al., 2008; Seddiki et al., 2009). This is accompanied by a burst of T and B cell responses to the initiating antigen(s), suggesting that the frequency of circulating regulatory T cells is not a reliable indicator of the regulatory mechanisms operating in the IRIS-affected tissues and/or that immune control mechanisms are ineffective in IRIS. In that respect, in vitro suppression experiments have illustrated a reduced suppressive capacity of regulatory T cells from IRIS patients, as well as the refractoriness of effector CD4 T cells to regulatory T cell control (Seddiki et al., 2009). Further studies, including analysis of regulatory T cells in the target tissue, are certainly needed to further determine the contribution of regulatory T cells to the pathogenesis of neuro-IRIS.

**Lymphopenia-induced proliferation**

Lymphocyte numbers remain remarkably stable throughout life despite the gradual loss of thymic output (Takada and Jameson, 2009). This process is referred to as T cell homeostasis and is maintained by a self-limiting multifaceted process that permits a space-driven expansion of peripheral T cells in the absence of full activation (Jameson, 2002). This homeostatic proliferation is antigen-driven and influenced by the affinity of the T cell receptor, as T cells with a high affinity for their peptide:MHC ligands accumulate more readily (Ge et al., 2001; Kieper et al., 2004). This mechanism is further accentuated in pathological lymphopenia, leading to lymphopenia-induced proliferation (Datta and Sarvetnick, 2009). Severe lymphopenia is a hallmark of HIV infection and the prolonged duration and depth of lymphopenia prior to anti-retroviral therapy predisposes a patient to IRIS (Stoll and Schmidt, 2004). During the first wave of immune restoration in HIV-infected patients, which is driven by lymphoid-resident memory T cells, high affinity peptide:MHC ligands could asymmetrically expand these memory T cell specificities that are already limited in diversity due to previous encounter with antigens (Mackall et al., 1996; Marleau and Sarvetnick, 2005). This risk might be even more significant for memory T cells that reside within tissues and are essential for the control of local latent infections (Gebhardt et al., 2009). This narrowing of T cell diversity can be driven by self-antigens, but also by HIV or opportunistic infection-derived epitopes. Future studies should be dedicated to address: (i) the relative impact of self-antigens, HIV antigens or opportunistic infection-derived epitopes during immune restoration following anti-retroviral therapy introduction; (ii) if narrowing of the T cell repertoire predisposes to IRIS; and (iii) if the magnitude of this repertoire skewing is shared between the conventional T cell and regulatory T cell compartments.

Moreover, when naïve T cells undergo lymphopenia-induced proliferation, they convert into memory-like cells with a CD44hiCD62Llo phenotype, but which do not differentiate into effector cells (Goldrath et al., 2000). However, the conditions are less favourable during anti-retroviral therapy in HIV-infected patients. Indeed, the persistence of microbial antigens might promote full T cell activation. Lymphopenia-induced proliferation by high-affinity ligands promotes T cell expansion, but is also more permissive to effector commitment (Min et al., 2005). Hence, foreign antigens, including residual microbial antigens, might inadvertently activate high-affinity T cells. Pro-inflammatory cytokines that are detected during IRIS, notably IL-6, further stimulate effector T cell differentiation during lymphopenia-induced proliferation (Stone et al., 2001, 2002; Tajima et al., 2008). Furthermore, T cells undergoing lymphopenia-induced proliferation in anti-retroviral therapy-treated HIV-infected patients are enriched in memory T cells, whose activation threshold is lower than that of naïve T cells (Rogers et al., 2000). This carries a particular risk, as the concomitant lymphopenia-induced proliferation of both CD4 T cells and memory CD8 T cells with shared auto-antigen specificity permits the differentiation of memory CD8 T cells into cytotoxic effector cells, leading to pathogenicity in animal models (Le Saout et al., 2008). This is likely to extend to anti-infectious responses as lymphopenia-induced proliferation-induced memory-like CD8 T cells provide protection that is as robust as conventional memory cells against pathogens (Hamilton et al., 2006; Cheung et al., 2009). Thus, HIV-related lymphopenia and the subsequent lymphopenia-induced proliferation are important factors contributing to the exacerbated immune response that is thought to underlie neuro-IRIS.

**Contribution of autoimmunity to neuro-immune reconstitution inflammatory syndrome**

An excessive destructive immune response against a persisting pathogen is difficult to distinguish from a genuine autoimmune disease in which the pathogenic lymphocytes recognize self-antigens. Moreover, these two processes are not mutually exclusive. Lymphopenia and lymphopenia-induced proliferation are known to predispose to inflammatory disorders in the absence of infection. Early studies on irradiated and thymectomized rats revealed that immune restoration predispose to autoimmune thyroiditis and diabetes (Penhale et al., 1975; Stumbles and Penhale, 1993). Similar observations are made in humans, where immune restoration following hematopoietic stem cell transfer or lymphocyte depletion by alemtuzumab, a monoclonal antibody that targets CD52, is quite frequently associated with autoimmune diseases (Daikeler and Tyndall, 2007; Coles et al., 2008). These studies indicate that immune restoration carries an intrinsic risk of generating
pathogenic autoimmune responses. This propensity towards autoaggression involves the expansion of the autoreactive repertoire, together with additional factors that favour immune aggression (Krupica et al., 2006). Elegant studies by the laboratory of Don Mason identified that deficiencies in autoantigen-specific regulatory T cells favour the progression towards lymphopenia-induced autoimmunity (Fowell and Mason, 1993; Saoudi et al., 1996; Seddon and Mason, 1999). Furthermore, the cytokine IL-21 has been suggested to be of particular interest (Jones et al., 2009). Indeed, both in animal models and in humans, lymphopenia-induced autoimmunity is associated with the overproduction of IL-21, which drives increased cell cycling followed by reduced T cell survival (King et al., 2004; Jones et al., 2009). As persistent or strong T cell receptor signals can counter this mechanism, self-antigens are thought to rescue cycling T cells, favouring the enrichment and activation of autoreactive T cells (King et al., 2004). This mechanism also makes IL-21 an interesting candidate for immune dysregulation in paradoxical neuro-IRIS, where immune restoration is thought to generate pathogenic T cell responses against either residual antigens derived from previous opportunistic infections or potentially auto-antigens (French, 2009). The current literature pertaining to the specificity of the immune response correlates IRIS with increased antimicrobial T cell responses (Bourgarit et al., 2006; Tan et al., 2008; Elliott et al., 2009; Marzocchetti et al., 2009). However, a potential contribution of autoimmune responses should not be neglected as the microbial environment is known to influence autoimmunity (Chervonsky, 2010). Infections can both inhibit or promote autoimmune diseases. The former is illustrated by the correlation between the decline in infectious diseases and increase in autoimmune disorders in the western world over the past three decades (Bach, 2002). Tissue damage predisposes towards autoaggression by autoimmune mechanisms known as epitope spreading (Lehmann et al., 1992; Vanderlugt and Miller, 2002) or molecular mimicry (Fujinami and Oldstone, 1985). Conceptually, this situation is compatible with neuro-IRIS, where both HIV infection and local opportunistic infections will have caused significant local tissue damage to the CNS (Gonzalez-Scarano and Martin-Garcia, 2005). Persisting antigen-presenting cells loaded with self-antigens derived from debris and/or from the uptake of dying cells could allow the priming of T and B cell responses able to perpetuate autoimmune tissue damage (Munz et al., 2009). Addressing this mechanism in humans is challenging, as a secondary autoimmune response is generated and propagated autonomously within the CNS (McMahon et al., 2005). Consequently, analysis of the few lymphocytes within the CSF (Scotet et al., 1999) or revival of isolated CNS-infiltrating T cells from CNS biopsies (Seitz et al., 2006) would be required to identify the contribution of self-reactive T cells to neuro-IRIS.

**Perspectives**

Research should focus on improving our knowledge on the pathogenesis of neuro-IRIS, so as to improve diagnostic tests, prevention and treatment strategies.

**Diagnostic and predictive markers of neuro-immune reconstitution inflammatory syndrome**

Identifying reliable diagnostic markers is important, particularly for differentiating paradoxical neuro-IRIS from an alternative diagnostic. In addition, reliable predictive markers would allow taking specific prophylactic measures at the time of anti-retroviral therapy initiation for patients at risk of developing neuro-IRIS.

**Immunological and virological variables**

Immunological and virological variables have been suggested as predictors of IRIS. In particular, a ≥2 log_{10} copies/ml decrease in the HIV viral load in response to anti-retroviral therapy has been considered as a risk factor for IRIS (Shelburne et al., 2005b; Manabe et al., 2007). A low CD4 T cell count or frequency before initiating anti-retroviral therapy has been widely associated with the risk of IRIS (French et al., 2000; Manabe et al., 2007; Muller et al., 2010). However, the reports are conflicting regarding the predictive value of the slope and magnitude of change in the CD4 and CD8 T cell counts or frequency upon anti-retroviral therapy initiation (Shelburne et al., 2005b; Manabe et al., 2007). Therefore, monitoring changes in the numbers of circulating CD4 and CD8 T cells after initiating anti-retroviral therapy does not appear to be reliable for predicting IRIS. However, assessing their activation profile or the oligoclonal expansion of circulating CD8 T cells could represent a useful surrogate marker of the strong immune response underlying IRIS. Similarly, the circulating concentrations of cytokines and chemokines, particularly IL-6 (Stone et al., 2001, 2002) but possibly others (Bourgarit et al., 2006), may also be used to detect the exacerbation of the immune response. Changes in gene expression profiles in either unfractionated peripheral blood mononuclear cell or purified immune cell subsets may lead to the identification of an immune signature correlated with IRIS development. CD8 and CD4 T cells are obvious cellular candidates, as they are intimately involved in the pathogenesis of viral, mycobacterial and cryptococcal IRIS.

**Assessment of functional antimicrobial immune response**

During HIV infection, persistence of HIV antigens and defects in CD4 T cell help disrupt T cell memory, impacting negatively on repertoire diversity (El-Far et al., 2008). It is therefore possible that patients may be at greater risk of paradoxical IRIS if the T cell repertoire diversity is narrowed towards reactivity to the pathogen. Therefore, an improved prediction and diagnosis of neuro-IRIS could be afforded by the definition of blood or CSF markers that would allow quantifying and enumerating pathogen-specific immune responses at the time of starting anti-retroviral therapy and at the onset of clinical worsening. Indeed, positive tuberculin skin tests assessing the delayed-type hypersensitivity response or the enumeration of tuberculin-specific T cells could contribute elegantly to the diagnosis of paradoxical tuberculosis-IRIS.
(Bourgarit et al., 2006; Elliott et al., 2009). Likewise, a longitudinal retrospective study showed that patients with PML-IRIS had increased JC virus-specific CD8 T cells (odds ratio 7.8, 95% CI 1.16–52.35) (Marzocchetti et al., 2009).

Thus, assessing the reactivity of circulating T cells against the predominant IRIS-associated pathogens at the time of starting anti-retroviral therapy or after clinical worsening may represent an accessible method to improve the prediction and diagnosis of neuro-IRIS. This could be achieved by techniques such as enzyme-linked immunospot assays for IFN-γ producing cells, lymphocyte proliferation assays or major histocompatibility complex-peptide multimer stainings of T cells after anti-retroviral therapy-induced immune recovery (Lima et al., 2007; Furco et al., 2008; Tan et al., 2008).

Genetic polymorphisms

Although genetic polymorphisms may be risk factors for IRIS development, they may not be reliable predictive markers of IRIS. Indeed, the number of genes governing the intensity and quality of the immune response and the wide variety of targeted antigens make it highly unlikely that a combination of single-nucleotide polymorphisms can capture this heterogeneity.

Therapeutic strategies

Corticosteroids

Corticosteroids have been advocated for both preventive and curative treatment of neuro-IRIS. Indeed, corticosteroids may represent a preventive therapeutic option for patients with opportunistic infections associated with a high-risk of neuro-IRIS. The systematic use of pre-emptive corticosteroid therapy has been proposed for patients with multiple sclerosis developing PML during natalizumab therapy, to counter the evolution towards PML-IRIS after natalizumab withdrawal (Clifford et al., 2010). Although this immunosuppressive drug is promising in multiple sclerosis, its use in patients with HIV is likely to facilitate the occurrence of other opportunistic infections. Nevertheless, corticosteroids have been used to treat patients with neuro-IRIS, particularly those with severe manifestations (Lortholary et al., 2005; Pepper et al., 2009; Tan et al., 2009). However, studies that have investigated the efficacy of corticosteroids for treating IRIS suggest limited efficiency. A recent double-blind placebo-controlled randomized clinical trial of patients with paradoxical tuberculosis-IRIS found that prednisone (1.5 mg/kg/day for 2 weeks then 0.75 mg/kg/day for 2 weeks) provided clinical improvement without affecting mortality (Meintjes et al., 2010). However, patients with life-threatening tuberculosis-IRIS (neurological involvement, airway compression or respiratory failure) were excluded from this study, although they might have benefited from corticosteroid treatment. This limited therapeutic impact, together with the likely negative effect of steroids on immune restoration has inspired the development of novel approaches that would selectively alleviate inflammation without hampering immune restoration.

Blocking cell migration

Neuro-IRIS might be controlled by neutralizing the molecular cues that initiate inflammation or by blocking cell migration. Antagonists of the chemokine receptor CCR5, such as maraviroc, have recently entered the HIV armamentarium. This novel class of drugs inhibits HIV entry by blocking its hosts cellular receptors (Soriano et al., 2009). CCR5 is also implicated in the migration of lymphocytes to inflammatory sites, in particular to the CNS (Ubigou et al., 2006). Blocking the recruitment of CCR5+ cells to inflammatory lesions may therefore explain the immunomodulatory properties of maraviroc (Corbeau and Reyes, 2009). Thus, anti-retroviral therapy combined with maraviroc might improve the management of IRIS (Martin-Blondel et al., 2009).

Although it may seem provocative given its risk of initiating PML (Clifford et al., 2010), the transient use of low-doses of anti-α4 integrin monoclonal antibody (natalizumab) could blunt T cell and monocyte transmigration to the CNS in severe cases of neuro-IRIS. However, its protracted pharmacodynamics renders its use somewhat delicate (Miller et al., 2003). Another approach to prevent lymphocyte transmigration to the CNS is the oral use of FTY720. FTY720 is a functional antagonist of sphingosine1-phosphate receptors, which play an essential role for T cell egress from thymus and lymph nodes. FTY720 has been shown to inhibit experimental models of CNS inflammation (as well as other models of tissue inflammation) and has proved efficacious in multiple sclerosis (Cohen et al., 2010; Kappos et al., 2010). Although this molecule may help mitigate the deleterious inflammation in neuro-IRIS, FTY720 would oppose the second wave of T cell restoration following anti-retroviral therapy, by blocking thymic egress of newly generated T cells. Secondly, FTY720 differentially affects the recirculation of naïve, central memory (retained in lymph nodes) and effector memory T cells (less or not retained) (Mehling et al., 2008; Brinkmann, 2009). The potential efficiency of FTY720 in neuro-IRIS would, therefore, depend on the relative involvement of these T cell subsets in pathogenesis.

Neutralization of cytokines

Neutralization of cytokines is now widely used in the treatment of inflammatory diseases (Elliott et al., 1994; Cohen et al., 2002). Interestingly, TNF-α neutralization has successfully treated one case of neuro-IRIS cryptococcoma (Sitapati et al., 2010) and one case of a severe neurological paradoxical reaction after starting anti-tuberculosis therapy (Blackmore et al., 2008). Considering the importance of granulomatous lesions in mycobacterial and cryptococcal-related IRIS and the central role of TNF-α in granuloma formation (Bean et al., 1999), TNF-α antagonism could, therefore, be a relevant therapeutic strategy. The IL-1 receptor antagonist anakinra may be an alternative treatment, given its efficacy in treating autoinflammatory conditions (Cohen et al., 2002; Gabay et al., 2010). However, IL-6 might prove a more suitable target. This pleiotropic cytokine contributes to many aspects of inflammation, including the induction and regulation of acquired immune responses (Kishimoto, 2005). Consequently, blocking IL-6 signal has provided clinical benefit in a variety of inflammatory diseases (Nishimoto, 2010). Three observations suggest that neutralizing IL-6 signal would be appropriate in
Neuro-IRIS. First, IL-6 is one of the cytokines that favours the effector differentiation by T cells undergoing lymphopenia-induced proliferation (Stone et al., 2001, 2002; Tajima et al., 2008). Second, IL-6 has a negative impact on immune regulation by rendering effector T cells refractory to regulation by regulatory T cells (Pasare and Medzhitov, 2003). And third, in the absence of IL-6 the exposure to TGF-ß will differentiate naïve T cells into antigen-specific Foxp3 regulatory T cells, at the expense of effector or Th-17 cells (Bettelli et al., 2006). Therefore, neutralizing IL-6 might benefit neuro-IRIS by reducing systemic inflammation and supporting immune regulation. Further studies are needed to validate such therapeutic approaches.

Conclusions

Neuro-IRIS is an emerging CNS inflammatory disorder that arises during anti-retroviral therapy-induced immune restoration of HIV-infected patients. Its pathogenesis seems to be multifactorial and depends greatly on the pathogen involved. Opportunistic pathogens that are at high-risk of neuro-IRIS, such as M. tuberculosis, C. neoformans and JC virus, differ from low-risk pathogens, such as T. gondii by a lower ability to avoid immune recognition. Excessive tissue inflammation is favoured by defects in immune regulatory mechanisms. Lymphopenia and subsequent lymphopenia-induced proliferation, together with numerical or functional defects in regulatory T cells might compromise the mechanisms that normally serve to maintain host tolerance, thereby promoting the initiation and exacerbation of immune responses. Consequently, neuro-IRIS is coherent with a dysregulated host immune response provoked by either pathogen-derived antigens or auto-antigens that cause disproportionate tissue damage. This pathological scheme would propose that the quantification and enumeration of pathogen-specific immune cells prior and during anti-retroviral therapy would improve the diagnosis and prediction of neuro-IRIS. Furthermore, therapies should aim to interfere with inflammatory cytokines and/or lymphocyte transmigration, to selectively alleviate inflammation without blunting immune restoration. But, most of all, neuro-IRIS represents a supplementary argument to reinforce the initiation of anti-retroviral therapy prior to the establishment of immunosuppression. This strategy would reduce the risk of anti-retroviral therapy-induced immune dysregulation, thereby preventing neuro-IRIS.

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