The pathogenesis of Leishmania/HIV co-infection: cellular and immunological mechanisms

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The intracellular protozoan parasites of the genus Leishmania have been recognized as opportunistic pathogens in immunosuppressed individuals, including those infected with human immunodeficiency virus type-1 (HIV-1). Leishmaniasis and AIDS overlap in several sub-tropical and tropical regions around the world, including the Mediterranean area. In 1994, 3%–7% of HIV-1-infected individuals in southern Europe developed visceral leishmaniasis. In humans, interestingly, both HIV-1 and Leishmania interact with, invade, and multiply within cells of myeloid or lymphoid origin. The combined modulation of Leishmania- and HIV-1-related pathogenesis in the co-infected cases is therefore probably a realistic goal. In the light of the recent demonstration that L. donovani can up-regulate HIV-1 replication, both in monocytoid and lymphoid cells in vitro and in co-infected individuals, it is clear from the epidemiological data available that Leishmania can probably act as a powerful co-factor in the pathogenesis of HIV-1 infection. In those who are co-infected, complex mechanisms involving cytokine secretion and cellular-signalling events play pivotal roles in the Leishmania-mediated activation and pathogenesis of HIV-1. An overview of the recent findings concerning this Leishmania/HIV-1 interaction is presented here.

The aetiological role of the human immunodeficiency virus type-1 (HIV-1) in the pathogenesis of the acquired immunodeficiency syndrome (AIDS) is firmly established (Popovic et al., 1984; Levy, 1993). HIV-1 is known to propagate mainly in T-lymphocytes because these cells express the primary cellular receptor for viral entry into target cells, the surface molecule CD4 (Dalgleish et al., 1984; Klatzmann et al., 1984; McDougal, 1986). However, the macrophage has been recognized as the predominant cell line productively infected with HIV in the lymph nodes, lungs and central nervous system (Meltzer et al., 1990). Monocytes and macrophages represent an important reservoir for HIV and serve as vehicles that disseminate the virus throughout the host. In the infected individual, HIV-1 may replicate, undetected, at low levels for prolonged periods, with minimal clinical manifestation. An extended period of latency, the development of various and repetitive, opportunistic infections attributable to the state of immunodeficiency induced by the infection, and the period

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between time of infection and onset of full-blown AIDS (Melbye et al., 1986; Fauci, 1988a) are, indeed, the principal clinical characteristics of HIV-1 infection. Co-infection with certain opportunistic micro-organisms, including the protozoan parasites of the genus *Leishmania*, may lead not only to direct pathogenesis and morbidity but may also play an important and active role in the progression of HIV-1 infection toward AIDS. Such opportunistic infections induce inflammatory responses and cellular-signalling events in their host that could promote viral replication (Tremblay et al., 1996). Interestingly, it is known that certain stimuli can activate regulatory elements located within the long-terminal-repeat (LTR) sequences of HIV-1, and may induce HIV-1 gene expression from latent proviruses, thus accelerating viral replication and disease progression (Fauci, 1988a, b). It seems likely that cellular activation induced in the course of infection by opportunistic pathogens could lead to the activation of the regulatory elements of HIV-1.

Although the role of protozoan parasites as possible co-factors favouring the progression of HIV-1 infection towards AIDS still needs much study, Tremblay et al. (1996) discussed the putative participation of *Leishmania* in the pathogenesis of HIV-1 infection. Their ideas were based on the observation that the geographical distributions of HIV and *Leishmania* overlap in several countries. Many cases of *L. infantum*/HIV-1 co-infection have already been recorded around the Mediterranean basin, particularly in France, Spain, and Italy (Jeannel et al., 1989; Fillola et al., 1992). There are clinical and epidemiological reports implicating *Leishmania* as an opportunistic pathogen in immunosuppressed AIDS patients (Alvar et al., 1987). As leishmanial parasites constitute a major public-health risk throughout subtropical and tropical areas of the world (Jeronimo et al., 1994; Marty et al., 1994; Zijlstra et al., 1994), it should come as no surprise if *Leishmania*/HIV co-infection emerges in patients living in these regions.

Bernier et al. (1995) observed that *L. donovani*, its surface lipophosphoglycan (LPG) and diverse structural components of the parasite could each induce viral replication in several monocyteid cell lines chronically infected with HIV-1 [Fig. 1(a)]. The LPG molecule, a glycoconjugate, is one of the major constituents expressed on the surface of the *Leishmania* promastigote. When a promastigote is inoculated into a human host, by an infected sandfly, it is engulfed into the phagolysosome of a macrophage, where it rapidly differentiates into an amastigote. During its engulfment, the parasite loses most of its structural LPG at the surface of the phagocyte, and retains only the intramembrane component. This component, the phosphatidyl inositol core (core-PI), is solely encountered on the surface of the amastigote. The LPG surface molecule has been recognized as favouring the intracellular survival and establishment of the parasite (Turco, 1999). The alteration of signalling events, dependent on the second messenger protein kinase C (PKC), has been implicated in the LPG-mediated protection of the parasite within the macrophage phagolysosome and the LPG-induced inhibition of several important macrophage functions, such as the generation of oxygen radicals (Handman et al., 1986). Among other host-cell dysfunctions induced by *Leishmania* are inhibiting mechanisms (possibly LPG-independent) that can lead to the alteration of several signalling pathways in which protein tyrosine kinase (PTK), PKC and calcium ($Ca^{2+}$) participate (Olivier et al., 1992a, b, 1998).

In a recent Spanish study, which is discussed at length below, *L. infantum* LPG was found to be not only an excellent activator of HIV-1 replication within latently infected monocyteid cells but also a potent inducer of HIV-1 LTR transcription and viral replication in T-cells [Fig. 1(b); unpubl. obs.]. *In vitro*, rapid upregulation of HIV-1 LTR activity was seen following LPG addition, possibly indicating a direct effect on the LTR-dependent gene expression of HIV-1.
PATHOGENESIS OF Leishmania/HIV CO-INFECTION

FIG. 1. Induction of HIV-1 expression by lipophosphoglycan (LPG) in cells of the monocytoid and lymphoid lineages. (a) Induction of HIV-1 expression in monocytoid (U1) cells by Leishmania donovani (one parasite/U1 cell), 10 μM LPG — with or without antibodies to tumour necrosis factor-α (anti-TNF-α, at 2 μg/ml), or, as a positive control, 10 nM phorbol 12-myristate 13-acetate (PMA). (b) Activation of HIV-1 transcription in lymphoid T (1G5) cells by 10 μM LPG, 10 μM core-PI, or, as a positive control phytohaemagglutinin (PHA, at 3 μg/ml). (c) Signalling and activation of the HIV-1 long-terminal repeats in infected lymphoid T (1G5) cells subjected to signalling-inhibitor treatment 1 h prior to stimulation with 10 μM LPG; the inhibitors were herbimycin A (HerA, at 0.1 μM), H7 (5 μM), HA1004 (HA, 5 μM), MDL12330 (MDL, 125 μM), the membrane permeant, acetomethoxy-ester form of bis-(aminophenoxy)ethane-tetraacetic acid (BAPTA/AM, 5 μM) and W7 (5 μM). (d) Activation of the HIV-1 long-terminal repeats, in Jurkat cells that had been transfected with wild-type nuclear factor-κB (NF-κB; ■) or mutated constructs (□) of this factor, following stimulation for 24 h with PHA (at 3 μg/ml) or LPG (at 10 μM). For each experiment, the results shown are mean values, with s.e. shown as error bars. All the results for the test cultures differed significantly from those of the respective control cultures (P < 0.01). In each culture, viral load was measured as the level of reverse transcriptase activity (a) or, after attaching a luciferase gene to the long terminal repeat of the HIV, by measuring luciferase activity (b, c and d).

Various intracellular second messengers, whose involvement was crucial to the LPG-mediated activation of the HIV-1 LTR sequence, were also identified. Both PTK- and protein-kinase-A/cAMP-dependent signalling, for example, were key events in the LPG-induced activation of HIV-1 LTR transcription [Fig. 1(c)]. The divalent cation Ca²⁺ and the Ca²⁺-binding protein calmodulin were also important physiological effectors for the Leishmania-LPG-induced viral activation; Ca²⁺ is known to be a regulator of several second messengers in pivotal, transductional mechanisms (Lewis and Cahalan, 1995). Such observations were not surprising, as the Leishmania parasite and its surface LPG can rapidly modulate Ca²⁺ homeostasis and mobilization within
the phagocytic host cell (Olivier et al., 1992a; Olivier, 1996; Mansfield and Olivier, 2002).

Signalling events often result in increased gene expression, which must involve a transcriptional factor such as nuclear factor-κB (NF-κB). As expected, the LPG-mediated, HIV-1-LTR expression was found to involve the NF-κB binding region, the LTR activation induced by LPG being completely abolished in cells transiently transfected with a mutated NF-κB construct [Fig. 1(d); unpubl. obs.]. LPG is capable of inducing NF-κB translocation to the nucleus (Bernier et al., 1998). The fact that the regulation of NF-κB involves several protein kinases, such as protein kinases A and C, PTK and the Ca\(^{2+}\)-dependent phosphatase calcineurin (Frantz et al., 1994; Barbeau et al., 1997), helps to explain why so many second-messenger antagonists blocked LPG-induced HIV-1 LTR activation, through NF-κB-dependent events (Folks et al., 1987; Duh et al., 1989; Pomerantz et al., 1990). The *Leishmania* LPG molecule is not the only pathogenic component to activate NF-κB — bacterial lipopolysaccharide (LPS) and the lipoarabinomannan of *Mycobacterium tuberculosis* are also recognized as powerful inducers of NF-κB activation (Bernier et al., 1998; Zhang et al., 1995).

Like the LPG itself, the core-PI — the intramembrane structural component of LPG that is present on the amastigote surface — is also a powerful inducer of HIV-1 LTR activity. This is of prime importance considering that it is under the amastigote stage that leishmanial infection progresses within the human host. Repeated units of the *Leishmania* LPG molecule can be detected on the surface of *Leishmania*-infected, mononuclear, phagocytic cells (Turco, 1999). *Leishmania*-infected macrophages that are involved in cell–cell interactions with T-lymphocytes during the process of antigenic presentation (which may involve the presentation of different structural components of the LPG molecule) might therefore be sufficient to trigger signalling events in HIV-1-infected CD4\(^+\) T-lymphocytes. Although most of the relevant studies have been performed *in vitro*, it is realistic to believe that, during the course of *Leishmania* infection in an individual afflicted by HIV-1, free or phagocyte-presented parasite constituents could similarly exacerbate HIV-1 replication in cells of monocytoid or lymphoid origin.

Research by Easterbrook et al. (1995) demonstrated that interactions between LPG and T lymphoid cells lead to the inhibition of the HIV-1-induced formation of syncytia. As the formation of these giant multinucleated cells is an important event in the development of HIV-1 pathogenesis (Levy, 1993), some interest in the potential use of *Leishmania* LPG to inhibit the progression of HIV-1 infection has developed. However, any potential benefits from the use of LPG in the design of new therapeutic strategies against HIV-1 have to be carefully balanced against the potential disadvantages (Bernier et al., 1995, 1998). In addition to its capacity to activate HIV-1 replication, the LPG molecule can also stimulate the release of several inflammatory molecules, such as prostaglandin E\(_2\) (PGE\(_2\); Matte et al., 2001); PGE\(_2\) is reported to be secreted during the course of both *Leishmania* (Reiner and Malemud, 1984) and HIV-1 infection (Abel et al., 1992; Foley et al., 1992). It seems likely that PGE\(_2\) plays a role in the development of HIV-1-related pathogenesis, particularly in those co-infected with *Leishmania*, since this prostaglandin is a powerful inducer of HIV-1 replication (Dumais et al., 1998).

In summary, it has been established that *L. infantum* LPG and its core-PI moiety can, via complex biochemical pathways involving the participation of transcriptional factors such as NF-κB, activate HIV-1 LTR transcription in host cells of the monocytoid and lymphoid lineages. It is sufficient to expose HIV-1-infected T-cells or phagocytic cells to LPG or *Leishmania*-infected mononuclear phagocytes to trigger activation of latent provirus DNA (Fig. 2; unpubl. obs.). In those co-infected with *Leishmania* and HIV-1, the protozoan parasite seems to be an important co-factor that helps promote the switch from...
FIG. 2. Schematic representation of the signalling mechanisms involved in the Leishmania lipophosphoglycan-mediated HIV-1 expression by monocytes/macrophages and T lymphocytes. Ca^{2+}, calcium; EP4, prostaglandin-E2 receptor of type 4; IKB, nuclear-factor-xB-inhibitory molecule; LIPG, lipophosphoglycan; LTR, long terminal repeat; PGES2, prostaglandin E2-synthase 2; PKA, protein kinase A; PKC, protein kinase C; p65/p50, active elements of nuclear factor-xB; PTK, protein tyrosine kinase; R, receptor for LIPG and/or its phosphatidylinositol core; TNF-z, tumour necrosis factor-z; TNFR, TNF-z receptor.
a state of clinical latency (in terms of the HIV-1 infection) to virus-related disease. The elucidation of the cellular events that occur during *Leishmania* infection, HIV-1 infection and *Leishmania*/HIV-1 co-infection should speed the identification of specific cellular targets and the development of new therapeutic approaches that could control the associated diseases.

**TH1- AND TH2-TYPE RESPONSES**

Some of the interactions that occur between HIV infection and parasitic diseases can be anticipated from the immunology of parasitic infections in humans and the known effects of HIV on the immune system of its hosts. The viral infection may lead to the loss or non-development of protective immunity, permitting unrestricted proliferation of the parasites, whereas the stimulation by the parasites of host cells that are latently or chronically infected with the virus may lead to increased expression of HIV or its protein products (Morrow *et al.*, 1989). Leishmaniasis is one of a group of parasitic diseases in which the pattern of response by the T-helper (Th) cells of an immunocompetent host correlates well with the clinical picture and the severity of the disease (Reed and Scott, 1993). In immunocompetent humans and murine models, resistance to leishmaniasis and the self-limitation of the disease are associated with a Th1 cytokine profile (Barral-Neto *et al.*, 1995). Most patients who have localized and self-limited mucocutaneous leishmaniasis, for example, have good lymphoproliferative responses to leishmanial antigens and produce large amounts of interleukin-2 (IL-2) and interferon-γ (IFN-γ), even in the active phase of their disease. In contrast, susceptibility to the leishmanial infection and disease exacerbation are associated, in the immunocompetent, with cytokine responses of the Th2 type, in which IL-4, IL-5 and large amounts of IL-10 predominate (Pirmez *et al.*, 1993; Scott, 1993).

Although humans infected with HIV do not show evidence of a response that is predominantly of the Th1 or Th2 type (Graziosi *et al.*, 1994), some advances toward a better understanding of the immunopathogenesis of this life-threatening infection have been made since HIV-1 was discovered. A profound disruption in cell-mediated immunity is, of course, central to the immune defect in AIDS, but nearly every aspect of the host’s normal immune response is affected by HIV infection (Pantaleo *et al.*, 1993, 1994; Fauci, 1996).

HIV can interact with T-helper and T-suppressor lymphocytes, monocytes, macrophages, dendritic cells, B-cells, and microglia cells in the central nervous system (CNS). In addition, several chemokines and RANTES (‘regulated upon activation, normally T-cell expressed and secreted chemokine’) receptors are down-regulated during the primary infection (Fauci, 1996; Weissman and Fauci, 1997; Zaitseva *et al.*, 1998). The dynamics of HIV replication and its contribution to T-cell and macrophage destruction lead to the inevitable and irreversible incapacitation of the host’s immune system, leaving it unable to limit existing co-infections or to prevent opportunistic microbes from infecting the host and causing morbidity (Ho *et al.*, 1995; Mellors *et al.*, 1996; Perelson *et al.*, 1996). The level of destruction of CD4+ cells — as quantitatively expressed by counts of such cells in the peripheral blood — appears closely correlated with the risk that an opportunistic infection will have clinical manifestations (Mellors *et al.*, 1997). Most patients who have <100 CD4+ cells/μl in their peripheral blood have had at least one episode of illness as the result of an opportunistic infection (Frank *et al.*, 1998). The pattern and severity of several infectious diseases (leishmaniasis being one of the best examples) are markedly worsened by HIV co-infection although, curiously, the clinical manifestations of leishmanial infection in those with other immunosuppressive disease are generally similar to those seen in the immunocompetent (Badaró *et al.*, 1986a, b).
Almost all (90%) of the approximately 700 cases of Leishmania/HIV co-infection reviewed by the World Health Organization (1997) had fewer than 200 CD4+ cells/μl. In southern Europe, there are demographic, clinical and epidemiological differences between the immunocompetent who have visceral leishmaniasis (VL) and the HIV-infected cases of the disease (Desjeux and Alvar, 2003). Those co-infected with HIV may develop VL though infected with a Leishmania zymodeme that only causes cutaneous leishmaniasis in the immunocompetent, their leishmaniasis may be particularly severe and unresponsive to treatment, and they may have amastigotes in tissues, such as the intestine, that are never found infected in the immunocompetent (Badaró et al., 1986a; Badaró, 1997; Muñoz-Rodríguez et al., 1997; Sebastian et al., 1997; Moreno-Camacho et al., 1998; Vásquez-Pineiro et al., 1998; Wolday et al., 1998; Desjeux and Alvar, 2003). Those co-infected with HIV also have unusually high numbers of amastigotes in their reticulo-endothelial system (Martínez et al., 1993).

Exactly how HIV circumvents the host’s immune defences still baffles investigators. Both Leishmania and HIV share the same target cells. The results of in-vitro studies have demonstrated that L. infantum induces the expression of latent HIV-1 by up-regulating CCR5 and CXCR4 receptors and GP120 on the surface of CD4+ lymphocytes and macrophages (Bernier et al., 1998). Consequently, more virus infects the CD4+ cells, which are virtually destroyed by the L. infantum, whether the parasites are of a dermotropic or viscerotropic zymodeme. Thus, in those co-infected with HIV, severe forms of leishmaniasis can arise, irrespective of the L. infantum zymodeme involved (Jiménez et al., 1991, 1996; Gradoni and Gramiccia, 1994). Prolonged, Th2-type activation and increased viral replication have been documented in patients co-infected with Leishmania and HIV, despite treatment of their leishmaniasis (Cacopardo et al., 1996). The Th2-type cytokine profiles in Leishmania/HIV co-infection (with high levels of IL-4, IL-6 and IL-10) differ significantly from those seen in simple HIV infection (Preiser et al., 1996). When Nigro et al. (1999) used phytohaemagglutinin (PHA) to stimulate peripheral-blood mononuclear cells (PBMC) from HIV-positives, they found that the responses varied depending on whether the cells had come from a patient co-infected with Leishmania or not. Although there were marked increases in the production of both IFN-γ and IL-2R if the cells came from a patient without Leishmania infection, there was only a significant increase in the production of IL-2R if the cells came from a case of co-infection. Cultured PBMC from the cases of Leishmania/HIV co-infection also released greater quantities of IL-4 and IL-10 than the cells from patients who were not infected with Leishmania. These observations confirm that co-infection with HIV exacerbates the Th2-type responses of VL patients (Preiser et al., 1996). In addition, the observation that IL-4 is overproduced in the co-infected patients supports the hypothesis that HIV infection in association with other Th2-type inducing infections could represent an indirect feature of Th2 cell expansion among Leishmania/HIV co-infected subjects (Clerici et al., 1993; Klein et al., 1997; Nigro et al., 1999). If immunocompetent, acute cases of VL generally respond well to antileishmanial therapy, with resolution of the active infection and the complete regression of the signs and symptoms of the disease. VL patients with HIV co-infection, however, respond poorly to leishmaniasis treatment (Russo et al., 1996) and prophylactic therapy must be administered frequently to control the active disease. This bleak clinical picture supports the theory that irreversible defects in the T-cell-related immune response, triggered by Th2 cytokine over-expansion, are caused by the dual infection (Zumla and Croft, 1992; Nigro et al., 1999). Such defects favour the progression to AIDS in HIV-positive subjects.

Memory cells also appear to be affected in Leishmania/HIV co-infection. The delayed-type hypersensitivity reaction (DTH) is the
hallmark of hypersensitivity in tegumental leishmaniasis (Badaro, 1997) but several HIV-positive cases of mucocutaneous leishmaniasis have been found to give a negative result when tested with the leishmanin antigen (Badaro, 1997; De Souza et al., 1998). Hopefully, use of highly active antiretroviral therapy (HAART) will induce a reversal of cytokine profiles in HIV-positive patients, from type-2 to type-1 patterns, and enhance the efficacy of antileishmanial therapy in those co-infected with Leishmania (Nabors and Farrell, 1996; Kubar et al., 1998).

PRO-INFLAMMATORY CYTOKINES

As mentioned above, the life-cycle of HIV-1 infection is characterized by a prolonged and variable period of clinical latency, usually with a high viral turnover (Ho et al., 1995), progressive loss of T-helper-cell-mediated immunity, and depletion of CD4+ T-cells (Clerici et al., 1989). The sequential decline and ablation of cell-mediated immunity results in the development of opportunistic diseases. The opportunistic pathogens may further modify the immunological status of the host and influence the outcome of the HIV-related disease. A Th1 response, characterized by the production of IFN-γ and IL-2, is present during the early stages of HIV infection, the progression to AIDS being associated with a switch to a Th2 response, marked by the production of IL-4 and IL-10 (Clerici et al., 1993). Tumour necrosis factor-α (TNF-α) may also play a pivotal role in HIV-1 infection, since it can enhance the replication of the virus (Mellors et al., 1991), and high serum concentrations of TNF-α and TNF-β have been associated with a progression to AIDS (Aukrust et al., 1994; Medrano et al., 1998a).

Immunologically, VL is also accompanied by impaired (Leishmania-specific) cell-mediated immunity and by a decline in the number of CD4+ cells (Cenini et al., 1993). Susceptibility and resistance to Leishmania in experimental models have been associated with the Th2 and Th1 profiles of cytokine production, respectively (Reed and Scott, 1993). Intense production of TNF-α is also observed in patients during leishmanial infections (Cenini et al., 1993). Therefore, both VL and HIV-1 infection are associated with a depression in T-cell response and similar disturbances in cytokine patterns. To date, only one study has focused on the Th1/Th2 dynamic of the VL/HIV-1 co-infection (Cacopardo et al., 1996); increases in the serum concentrations of Th2 cytokines and HIV viraemia, together with a decrease in the serum concentrations of IL-2 and IL-12 (i.e. cytokines associated with the Th1 response), were observed in HIV-positive patients after they developed VL. Cacopardo et al. (1996) proposed that an irreversible switch from a type-1 to a type-2 response occurs during the co-infection. As the four patients included in this study had surprisingly high counts of CD4+ cells at baseline, however, it is difficult to extrapolate the results to the majority of HIV-infected patients who have lower counts. A more detailed account of the immunological status induced by the co-infection is still lacking.

The serum cytokine concentrations and peripheral T-cell sub-populations of eight HIV-1-infected patients have been studied before, during and after active VL and compared with those of appropriate controls (unpubl. obs.). In this investigation, the HIV-1-infected patients at VL diagnosis showed significantly higher serum concentrations of IFN-γ than the matched HIV-positive (but Leishmania-negative) controls, and lower serum concentrations of IL-10 than the immunocompetent VL controls (Fig. 3). These results show that the Th1/Th2 response induced by the co-infection differs from that induced by either single infection, and indicate an unexpected, non-synergistic effect of the two infections on Th1/Th2 polarity in vivo. In the co-infected cases, there were no relevant changes in the serum concentrations of IFN-γ or IL-10 during the 6 months of follow-up after the
FIG. 3. The serum concentrations of tumour necrosis factor-α (a), interferon-γ (b) and interleukin-10 (c) observed in cases of Leishmania infantum/HIV-1 co-infection (square; measured when visceral leishmaniasis was diagnosed) and, as controls, individuals who were Leishmania-negative/HIV-positive (open square), Leishmania-positive/HIV-negative (closed square) or Leishmania-negative and HIV-negative (open circle). For each experiment, the results shown are mean values, with s.e. shown as error bars.

The recent Spanish study had several other major findings (unpubl. obs.). Firstly, macrophages and lymphocytes (Aukrust et al., 1994). The release of pro-inflammatory cytokines is considered to be involved in the mechanism of defence against Leishmania and other intracellular pathogens (Barral-Netto et al., 1991; Cenini et al., 1993; Haug more importantly, that the serum levels of TNF-α in the co-infected cases remained high after recovery from VL (Fig. 5). Thirdly, that CD4+ counts in the co-infected cases fell as active VL developed and remained low thereafter. Fourthly, that there was progressive seroconversion, for the p24 HIV-1 antigen, throughout the follow-up period. An increase in serum HIV viraemia was also observed in the patients after the onset of acute VL (Fig. 5). As HIV-1 directly infects cells of the immune system and triggers a robust immune response, it is an important and persistent source of immune activation (Fauci, 1996) linked to TNF-α secretion by macrophages and lymphocytes (Aukrust et al., 1994). The release of pro-inflammatory cytokines is considered to be involved in the mechanism of defence against Leishmania and other intracellular pathogens (Barral-Netto et al., 1991; Cenini et al., 1993; Haug
TNF-\(\alpha\) release from monocytes latently infected with HIV-1 and promote viral replication. The results of the more recent study by Medrano et al. (1998b) indicate that this effect of leishmanial infection on TNF-\(\alpha\) production may also occur in vivo. These findings have important implications because strong activation of the TNF-\(\alpha\) system is considered to play an important role in the progression of HIV infection to AIDS. The deleterious immunological and virological effects of chronic activation of TNF-\(\alpha\) could explain the dynamics of the CD4+ cell decline, the increase in serum RNA viraemia and the p24 seroconversion observed, by Medrano et al. (1998b), in the co-infected patients during the course of their VL.

The data presented here demonstrate that an aberrant persistent activation of TNF-\(\alpha\) production, with possibly harmful immunological and virological consequences, occurs in patients with HIV-1 and Leishmania co-infection. Although it is not possible to discern clearly the exact cause–effect relationship between the parasite and the viral activation, the results of several studies (Bernier et al., 1995; Haug et al., 1996; Gazzinelli et al., 1995; Medrano et al., 1998b) support the hypothesis that various opportunistic infections may trigger the production of pro-inflammatory cytokines during immunodeficiency, and in this way accelerate the course of HIV-1 disease. Again, however, a more extensive knowledge of the immunological status induced by the co-infection will be necessary to determine the best procedures for treatment and control.

**FIG. 5.** Changes in the serum concentrations of tumour necrosis factor-\(\alpha\) (a), the numbers of CD4+ cells/\(\mu\)l blood (b) and the HIV-1 viraemia (c) in eight HIV-1-infected patients before and after they were found to have visceral leishmaniasis (VL). For each experiment, the results shown are mean values, with s.e. shown as error bars.

HIV-negative patients with VL have high serum concentrations of TNF-\(\alpha\), for example, that normalize after successful treatment (Barral-Netto et al., 1991; Cenini et al., 1993), and similar findings exist for other pathogens, such as mycobacteria (Haug et al., 1996). Consequently, an enhancement in TNF-\(\alpha\) production may be expected when the immune system of HIV-1-infected patients is activated by opportunistic pathogens.

In an in-vitro study, Bernier et al. (1995) demonstrated that Leishmania can trigger HUMORAL AND CELLULAR IMMUNE RESPONSES

In immunocompetent individuals, VL is associated with a clear humoral response, the result of polyclonal B-cell activation, and the suppression of cellular responses to the parasite — although these are restored after successful chemotherapy (Carvalho et al., 1981). The T-cell-mediated immune response
is, in fact, critical to the recovery process and to protection against other infections (Murray et al., 1989). HIV-infected patients with VL have lower titres of antileishmanial antibodies (Mary et al., 1992) and much higher frequencies of clinical relapse following antileishmanial treatment (Alvar, 1994) than the immunocompetent cases. Despite the numerous cases of co-infection that have now been reported, there appear to have been only two detailed investigations of the immune responses of such patients: one on two AIDS cases with American tegumentary leishmaniasis caused by *L. braziliensis* (Coutinho et al., 1996) and one on the changes in blood-cell subsets during *L. donovani/HIV* co-infection (Cenini et al., 1994).

Successful chemotherapy of VL in non-immunocompromised patients reduces the antigenic load through parasite destruction, resulting in decreases in the serum titres of specific and non-specific antibodies and the restoration of specific, T-cell-mediated immunity (Haldar et al., 1983). There seems to be no protective specific immune response in most of those with *L. infantum/HIV* co-infection, even following treatment with pentavalent antimonial compounds. This would explain why, in HIV-positives, the clinical and parasitological response to antileishmanial treatment is so poor (Medrano et al., 1992) and the frequency of post-treatment relapse is so high (Alvar, 1994).

Although the results of IFAT indicated that five out of the 14 cases of co-infection investigated recently had high titres of antileishmanial antibodies (unpubl. obs.; see Table), these antibodies were not able to control the spread of the parasite (Liew and O’Donnell, 1993). The other nine cases investigated had titres of specific antibodies that were low or undetectable in IFAT, indicating why IFAT are of such limited value in the diagnosis of leishmaniasis in HIV-positive patients (unpubl. obs.). More (11 of 13) of the cases appeared seropositive when they were checked by immunoblotting, which is a more sensitive technique than IFAT (Fig. 6) and clearly preferable for the early diagnosis, and therefore early treatment, of VL in HIV-positive subjects (Kubar et al., 1998). Immunoblotting also confirmed that the co-infected cases had a partial or weak humoral response in comparison to that

<p>| Table: Details of the 17 cases of Leishmania infantum/HIV co-infection investigated |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Risk group for HIV</th>
<th>Anti-Leishmania antibody titre</th>
<th>Immunoblot result</th>
<th>Stimulation index*</th>
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<td>IVDU</td>
<td>1/640</td>
<td>Polyclonal</td>
<td>0.92</td>
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<td>2</td>
<td>Homosexual</td>
<td>1/640</td>
<td>Polyclonal</td>
<td>2.03</td>
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<tr>
<td>3</td>
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*The -fold increase in lymphocyte proliferation resulting from mitogen induction, compared with that seen in unstimulated cells.

IVDU, Intravenous-drug user; ND, not determined.
however, because of the preferential death of memory cells upon activation in HIV-positive patients (Janossy et al., 1993). The observation that the four patients who appeared responsive to SLA in the recent Spanish study all relapsed (unpubl. obs.) supports the loss of specific, cell-mediated immunity.

Characterization of blast cells after stimulation showed that both CD4+ and CD8+ cells are able to respond to SLA (Fig. 7). CD4+ cells may have a protecting or exacerbating role in leishmaniasis (Liew and O’Donnell, 1993), and there is increasing evidence that CD8+ also have a specific role in the disease (Da-Cruz et al., 1994). In experimental VL, it has been shown that acquisition of resistance to infection with L. donovani requires both CD4+ and CD8+ cells, but CD8+ cells alone contribute to the host’s defences against other infections (Murray et al., 1992). Cure in patients with cutaneous leishmaniasis caused by

in immunocompetent cases of VL (Fig. 6), as previously reported by Mary et al. (1992). The low antibody titres generally observed are not the consequence of therapy, as they may have been in immunocompetent subjects, but the result of the impaired immunity induced by HIV infection. Oligoclonal B-cell responses may be consequent to the absence of T cells that are able to recognize specific Leishmania antigens or to stimulate B cells. This loss of specific antibodies has been described for other infections in HIV-infected patients (Biggar et al., 1987; Chamot et al., 1990).

Only one of the two AIDS cases with American tegumentary leishmaniasis described by Coutinho et al. (1996) showed a lymphoproliferative response after anti-leishmanial treatment. Clinical recovery of such patients and a positive response to soluble Leishmania antigen (SLA) in some of them would confirm that they are able to generate a specific T-cell response. This type of cellular response is probably lost,
*L. braziliensis* is associated with a high frequency of *L. braziliensis* reactivity among their CD8+ T cells, in antigen-stimulated cultures (Da-Cruz *et al.*, 1994) and in patients with the cutaneous leishmaniasis and AIDS (Coutinho *et al.*, 1996). For the four patients who were found to have lymphoproliferative responses to SLA in the recent study, the ability of their CD4+ or CD8+ cells to proliferate clearly did not induce acquired resistance, since all four suffered relapses (unpubl. obs.). Although more relevant data are needed, it is probable that the phenotype of the proliferating cells depends on the CD4+ or CD8+ phenotype of the recall cells that have not been lost (Janossy *et al.*, 1993).

HIV and *L. infantum* infections share common features in terms of the changes they induce in PBMC subpopulations, such as increasing the numbers of activated T- and B-cells while decreasing the numbers of CD4+ and natural-killer (NK) cells. An increase in the number of CD8+ cells only appears to occur with HIV infection, however, and increased expression of human leucocyte antigen-DR (HLA-DR) in lymphocytes only happens with VL (De Martini *et al.*, 1988; Cenini *et al.*, 1993). In those with both VL and HIV infection, the common effects often show synergy but the HIV-specific effects tend to predominate over the VL-specific (Cenini *et al.*, 1994). For the subjects of the recent study, the percentages of PBMC that were CD4+ were similar for those with *L. infantum*/HIV co-infection as for the HIV-positive patients without leishmanial infection (although the counts were made after antileishmanial treatment; Fig. 7; unpubl. obs.). That the percentage of PBMC that are CD8+ is relatively high in the cases of co-infection after antileishmanial treatment might indicate that these cells have a specific role in the post-treatment response to the parasite, or may simply reflect the replacement of the decreased circulating pool of CD4+ T-cells (Coutinho *et al.*, 1996).

The immunosuppression induced by HIV infection may allow latent *Leishmania* infections to re-activate and may facilitate the development of VL. Since both HIV and the *Leishmania* parasite can invade and replicate within macrophages, it is possible that the interactions between these pathogens could exacerbate both infections. Wolday *et al.* (1998) found, for example, that the addition of live or killed HIV-1 virions increased the multiplication of *L. donovani* in monocyte-derived cells. It has also been proposed that leishmanial infection can induce HIV replication in macrophages (Tremblay *et al.*, 1996). *In vitro*, the infection with *Leishmania* of macrophage lines already infected with HIV produces an increase in viral replication mediated by TNF-α (Bernier *et al.*, 1995). Although the HIV-positive patients investigated in the recent study were found to have similar serum concentrations of TNF-α, whether they had VL or not (Fig. 8; unpubl. obs.), this does not preclude a specific role for this cytokine *in vivo*, given the autocrine and paracrine effects of TNF-α on *Leishmania*-infected macrophages. In the study by Medrano *et al.* (1998b), serum concentrations were found to be higher in co-infected patients than in HIV-positive/*Leishmania*-negative patients or HIV-negative cases of VL, although the levels in the cases of co-infection declined after treatment, while HIV expression was significantly elevated. These results do not seem to agree with a TNF-α-mediated induction of HIV expression, especially as Medrano *et al.* (1998b) found that, 6 months before their VL was diagnosed, their co-infected cases had levels of HIV RNA serum transcripts that were similar to those recorded at the time of VL diagnosis.

Tumour growth factor-β (TGF-β), a potent inhibitor of the immune response, is over-expressed in HIV infection and thus could contribute to the immune defects observed (Kekow *et al.*, 1990). This cytokine, however, is a suppressor of HIV expression in chronically infected promonocytic cells and...
primary monocyte-derived macrophages (Poli et al., 1991). TGF-β is also produced by murine macrophages exposed to L. braziliensis and causes susceptibility to symptomatic leishmaniasis (Barral et al., 1993). In theory, therefore, individuals infected with HIV and Leishmania, who seem to have much lower serum concentrations of TGF-β than those infected with HIV alone (Fig. 8), should have less TGF-β-related suppression of their HIV but also less likelihood of developing symptomatic leishmaniasis. More research is necessary to elucidate the interactions between HIV and Leishmania parasites, at least in monocytes and macrophages. The question that arises is can antileishmanial treatment of the co-infected cases, by inducing a decrease in the serum concentrations of TGF-β, lead to increased viral replication in macrophages (Medrano et al., 1998a)?

In both Leishmania and HIV infection, it has been proposed that susceptibility is associated with a Th2-type response and resistance with a Th1-type response. These associations have been confirmed for leishmaniasis in murine models (Reiner and Locksley, 1995) and in the cutaneous (Kemp et al., 1994) and visceral (Kemp et al., 1993; Miralles et al., 1994) forms of the human disease. In HIV-positives, progression to AIDS may be related to a Th1–Th2 switch (Clerici et al., 1993), although this remains a matter of controversy (Graziosi et al., 1994; Maggi et al., 1994). In HIV-positive patients, a defect in the functioning of antigen-presenting cells would certainly induce the secretion of Th2 cytokines (Borthwick et al., 1994; Chehimi et al., 1994). Since an individual's protective response to infection with Leishmania usually depends on the presence of cytokines during early T-cell activation (Scott, 1991), the immunological status of HIV-infected patients is particularly favourable for the multiplication and spread of leishmanial parasites (Cacopardo et al., 1996).

CONCLUSIONS

Humans co-infected with HIV and L. infantum are able to mount a T-cell response against the parasite after antileishmanial treatment but this response is lost as the viral infection progresses and almost always followed by relapse of the leishmanial infection. The co-infection may induce both an uncontrollable spread of the parasite and an increase in viral replication. Treatments against leishmaniasis that are effective but do not promote HIV replication need to be developed.

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REFERENCES


