Terminal ileitis as a manifestation of immune reconstitution syndrome following HAART

The immune reconstitution inflammatory syndrome (IRIS) constitutes a clinical complication associated to the use of HAART in HIV-infected patients. It is estimated that 10–25% of patients who start HAART experience an IRIS event [1]. This syndrome comprises opportunistic infections that arise during the initial weeks of treatment, mainly in patients with a low CD4 cell count, plus a paradoxical worsening of infections that were previously controlled. The majority of treatments are dermatological, in particular genital herpes and warts [1]. The clinical manifestations are atypical [2–4]. Histological examination of lesions reveals an intense inflammatory response [5]. This phenomenon has also been described in other immunosuppressed patients on withdrawal of chemotherapy or immunosuppressive treatment [6–9].

Case report

A 27-year-old HIV-seropositive woman had presented with symptoms of watery, bloodless diarrhoea, epigastric pain, weight loss and several episodes of candidal oesophagitis during the previous year. Physical examination revealed moderate malnutrition, temperature 38°C and a soft abdomen with pain in the epigastrium and right iliac fossa during deep palpation. On admission, her haemoglobin was 111 g/l, white cell count 6.0 x 10^9 cells/l (neutrophils 68%), platelets 527 x 10^9/l, erythrocyte sedimentation rate 55 mm/h, CD4 cell percentage 10.3%, CD4 cell count 10 cells/μl, CD8 cell percentage 49%; CD8 cell count 209 cells/μl and plasma HIV RNA load 1030 copies/ml. Cryptosporidium spp. were isolated in several faecal samples. Abdominal ultrasound showed terminal ileum wall thickening. Colonoscopy findings included pancolitis and terminal ileitis. Intestinal biopsy showed an ulcerated ileal and colonic mucosa with signs of chronic inflammation. Cultures for cytomegalovirus, herpes virus and mycobacteria were negative. Treatment was established with paromomycin, stavudine, lamivudine and lopinavir/ritonavir. Tolerance was good and both abdominal pain and diarrhoea improved. One month after beginning treatment, the patient presented with abdominal pain, fever, occasional vomiting and intense malnutrition, without diarrhoea. At this point, her major lymphocyte subsets cell counts were CD4 cells 334 cells/μl (7%) and CD8 cells 3254 cells/μl (75%). Abdominal computed tomography (Fig. 1a) showed an oedematous thickening of the terminal ileum, and the small bowel series showed Kantor’s string sign at the terminal ileum level. Cryptosporidium spp. was not isolated in the stools. Symptoms improved after establishment of an empiric antibiotic treatment and digestive rest. Four months after beginning HAART treatment, and following two further admissions for the same symptoms, the patient was once again admitted showing signs of intestinal subocclusion. An exploratory laparotomy revealed a terminal ileum thickening and erythema with several spot-like perforations and mesenteric adenopathies (Fig. 1b). Right hemicolectomy was performed and an erosive enteritis with chronic fibrosis and inflammation was identified (Fig. 1c). Viral and mycobacteria cultures from surgical specimen were negative. The patient recovered after surgery, all symptoms disappeared and immunological and virological response to HAART remained positive.

The institution of HAART in HIV-infected patients restores protective immune responses against a wide variety of pathogens and dramatically decreases mortality. In a subset of patients receiving HAART, however, immune reconstitution is associated with a pathological inflammatory response leading to substantial short-term morbidity and even mortality. IRIS appears to be related not only to an increase in the CD4 cell count but also in the CD8 subset, this considered to be the primary factor contributing to a worsening in hepatitis B and C and herpes zoster infections [10–12].

It has also been suggested that increased cytokine activity contributes towards some forms of IRIS. The use of cytokines such as interleukin–2, once antiretroviral therapy has begun, as well as certain genetic mutations of innate cytokines, can increase the tendency for inflammatory responses [13,14]. This would indicate that symptoms are a consequence of inflammatory damage rather than the infection itself. The onset of IRIS occurs between 12 and 16 weeks after HAART treatment begins and can be associated with infections by mycobacteria, cytomegalovirus, varicella–zoster, hepatitis B or C, cryptococci, Pneumocystis carinii, other less frequent infections and other conditions such as multifocal progressive leukoencephalopathy, Kaposi’s sarcoma and lymphoma [2]. Treatment for IRIS includes continuation of primary therapy against the offending pathogen, continuation of HAART and the use of anti-inflammatory agents [15].

To our knowledge, this is the first report of IRIS following Cryptosporidium infection. In this woman, abdominal symptoms initially improved once HAART
and Cryptosporidium infection-specific treatment were started. However, the institution of an IRIS event following HAART is suggested by the fact that symptoms worsened later, coinciding with a significant early increase of CD4 and CD8 cell counts, the absence in faeces of Cryptosporidium spp. and other opportunistic diseases, and the intense inflammatory activity revealed by the intestinal biopsy.

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Fig. 1. Ileitis. (a) Computed tomographic scan showed thickened distal ileum (arrow) without associated free fluid. (b) Laparotomy showed erythema and grossly limited inflammation in the terminal ileum. It also revealed several spot-like perforations (arrow). (c) Haematoxylin–eosin stain of the specimen showed ulcero-erosive enteritis (×50).
HIV-contaminated syringes are not evidence of transmission

Based on the detection of HIV RNA in syringes recently used in HIV-infected patients Apetrei and colleagues [1] claimed that they ‘provided proof’ of concept that injection practices could account for a significant proportion of new HIV infections’. This is used to counter the conclusions of epidemiological studies from rural Zimbabwe and Uganda, where no association of HIV infection and a self-reported history of injections was found [2,3]. Debate about the role of unsafe injections in the HIV epidemics of sub-Saharan Africa is not about the potential of HIV transmission through injection with contaminated needles but the proportion of infections acquired through this route [4,5]. We agree that ‘the belief that HIV may not be transmitted by re-used needles is dangerous to public health’ [1], but no one has argued that HIV cannot be transmitted in this way.

Through sensitive reverse transcriptase–polymerase chain reaction, HIV-related genetic material could be detected in approximately one in three syringes used for intravenous injections and one in 40 syringes used for intramuscular injections [1], clearly showing some contamination of syringes. However, no evidence is given to support their conclusion of the important role of injections in the epidemic because the study offered no evidence from the field, analysed directly [2,3] and to inform models [8], does not support a major role for unsafe injections in the HIV epidemics in Africa.

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References

Severe vitamin D deficiency diagnosed after introduction of antiretroviral therapy including efavirenz in a patient living at latitude 59°N

HIV-infected individuals have an increased risk of osteopenia, osteoporosis and osteomalacia [1–3]. The aetiology is multifactorial [4,5].

We report the case of a previously healthy Ethiopian man, who had migrated to Sweden in the early 1990s, was HIV-negative in 1996, but was diagnosed with asymptomatic HIV infection in April 2002.

His CD4 cell count was 180 × 10⁶ cells/ml. His plasma viral load was 205 000 HIV-1-RNA copies/ml. His body mass index was 20.5. He was a strict vegetarian. Clinical signs of rickets or osteomalacia were absent.

In June 2002, treatment with zidovudine, lamivudine and efavirenz was initiated. Liver enzymes were within the normal range, and were followed routinely at clinical visits. During autumn, serum alkaline phosphatase (ALP) increased to 11.7 μU/l (reference range 0.8–4.6), whereas serum transaminase levels remained normal. Fractionated ALP analysis revealed a dominance of skeletal isoenzymes. Bone scintigraphy and prostate-specific antigen was unremarkable. Parathyroid hormone (PTH) was elevated to 100 ng/l (reference 10–65) in June 2003 and 205 ng/l in July 2003, in the presence of a normal calcium level (2.32 mmol/l, reference 2.20–2.60) indicating secondary hyperparathyroidism.

The preferred laboratory analysis to evaluate nutritional vitamin D status is 25(OH) vitamin D in serum. In December 2003, this was below the detection limit (<18 nmol/l, reference 25–125). The cause of the elevated PTH and ALP was considered to be vitamin D deficiency. Dual X-ray absorptiometry (Hologic, Bedford, Massachusetts, USA) in February 2004 revealed a bone mineral density with a T-score of −3.13 in the lumbar spine and −3.84 in the left hip, 70% and 64%, respectively, of the mean age-matched values.

In December 2004, after approximately one year of supplementation with vitamin D2 (175 μg/week), vitamin D3 (20 μg/day) and calcium (1000 μg/day) serum ALP and PTH levels were within normal limits. A dual energy X-ray absorptiometry measurement after 9 months of supplementation showed an improvement of bone mineral density by 4% in the lumbar spine and by 11% in the femoral neck.

Bone tissue is constantly turned over. Vitamin D is necessary for maintaining calcium homeostasis and for the mineralization of the bone matrix. Adult vitamin D deficiency is associated with osteomalacia, muscle weakness and lower back pain [6,7].

The main vitamin D source is 7-dehydrocholesterol, which is transformed into vitamin D3 in the epidermis when unprotected skin is exposed to sunlight. Vitamin D-enriched dairy products and fat fish are other sources of vitamin D (Fig. 1).

In Stockholm, at latitude 59° north, virtually no vitamin D is produced in the skin during the dark winter months. At this latitude, highly pigmented skin is a risk factor for vitamin D deficiency [8,9].

Several enzymes have been implied in the 25-hydroxylation of vitamin D, including the mitochondrial CYP27A and the microsomal CYP2R1 [10]. CYP3A4 was recently shown to be a vitamin D 25-hydroxylase [11]. Efavirenz is an inducer of CYP3A4. The inactivation of active vitamin D is catalysed by CYP24, which is inducible through pregnane X receptor activation, the pathway through which efavirenz is presumed to cause its induction of CYP3A4 [12,13]. Efavirenz could thus affect both the availability of substrate for 1α-hydroxylation, and the kinetics of 1,25(OH)2 vitamin D inactivation.

Notably, there are several published reports in which chronic treatment with CYP450 enzyme-inducing enzymes...
antiepileptic agents was associated with decreased levels of 25(OH) vitamin D and elevated serum ALP [14–16]. Controlled studies concerning the effect of efavirenz on vitamin D homeostasis are not available. However, one observational study detected significantly lower 1,25(OH)2 vitamin D levels in patients on protease inhibitors or non-nucleoside reverse transcriptase inhibitors, compared with treatment-naïve HIV-infected controls [17]. Another publication described the in-vitro inhibition of hepatocyte 25-hydroxylation of vitamin D by nefarnavir, indinavir and ritonavir [18], implying that these protease inhibitors might affect vitamin D homeostasis. In our patient ALP elevation developed after the initiation of antiretroviral therapy. PTH rose dramatically despite sun exposure during the summer season. We suggest that CYP450 enzyme induction by efavirenz might have affected vitamin D metabolism, resulting in an aggravation of a pre-existing vitamin D insufficiency caused by nutritional habits and less vitamin D synthesis in the skin.

Further studies on the effect of chronic treatment with CYP enzyme inhibitors and inducers, such as efavirenz and ritonavir, on vitamin D and other endocrine functions are mandated. Risk groups should be screened for subclinical vitamin D deficiency before starting therapy.

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Contribution of platelet activation to plasma IL-18 concentrations in HIV-infected AIDS patients

IL-18, originally discovered and named as the IFN-γ-inducing factor, is a multifunctional and pleiotropic cytokine, which acts in synergy with other cytokines (e.g. IL-12 and IL-15) to induce IFN-γ from T and natural killer cells [1]. It increases the cytolytic potential of cytotoxic T lymphocytes and natural killer cells. The cytokine promotes proliferation and development of T helper type 1 CD4 T cells. However, in the absence of IL-12, it promotes IgE production and the development of T helper type 2 CD4 T cells [2]. We and others have reported increased concentrations of circulating IL-18 in HIV-infected AIDS patients compared with healthy HIV-seronegative individuals [3,4]. Higher concentrations of the cytokine were observed in these patients despite its decreased expression at the protein and messenger RNA levels in the peripheral blood mononuclear cells from
these patients [3,5]. These observations raised the question as to the source of increased concentrations of this cytokine in infected individuals.

We recently found that human platelets contain abundant amounts of IL-18, which they release into medium upon activation [6]. The objective of this study, therefore, was to determine whether platelet activation contributes to plasma IL-18 concentrations in HIV-infected patients.

For this purpose, we determined IL-18 concentrations as well as platelet activation in a group of 17 HIV-infected AIDS patients (after their written informed consent), whose CD4 T-cell counts, viral loads, and other clinical characteristics have been described previously [3]. The plasma IL-18 concentrations were determined using a commercial enzyme-linked immunosorbent assay kit (MBL, Naka-ku, Japan) as described [3]. As a marker of platelet activation, we measured soluble glycoprotein V (sGPV) in the plasma of these patients using an enzyme-linked immunosorbent assay kit (Asserachrom Soluble GPV; a gift from Diagnostica Stago, Gennevilliers, France). The glycoprotein V is an 82 000 M<sub>r</sub> transmembrane glycoprotein, which is non-covalently associated with GP1b (a heterodimer of α and β polypeptides) and GPIX forming the platelet GP1b-V-IX complex [7,8]. Thrombin cleaves the glycoprotein V component of the complex, releasing a 68 000 M<sub>r</sub>, N-terminal fragment in a dose-dependent manner. The released fragment is called sGPV. Stored platelet concentrates also release sGPV spontaneously. The plasma sGPV level, therefore, is a biomarker for in-vivo thrombin-induced platelet activation [8].

As shown in Fig. 1a, the HIV-infected individuals had significantly higher (P < 0.05; two-tailed Student’s t-test) mean sGPV levels in their plasma compared with the HIV-seronegative control samples. To the best of our knowledge, this is the first report to document enhanced circulating sGPV levels in HIV-infected AIDS patients compared with HIV-seronegative control individuals. These results suggest enhanced platelet activation in the infected individuals, and are in accord with earlier studies, which determined platelet activation by measuring the surface expression of a well-known platelet activation marker, CD63 [9].

In order to see whether activated platelets might be contributing to the circulating IL-18 concentrations in these patients, we sought to determine correlation between sGPV and IL-18 concentrations in their plasma samples. As shown in Fig. 1b, a significant correlation (P < 0.01) was found between these two parameters. As activated human platelets release IL-18, these data strongly suggest that platelet activation is contributing towards plasma concentrations of this cytokine in these patients. No significant (P > 0.05) correlation was found between sGPV levels and viral loads (HIV-RNA copies per millilitre of plasma) and between sGPV levels and CD4 T-cell counts in these patients (data not shown).

IL-18 is known to be produced by many cell types and tissues in the human body, e.g. monocyte macrophages, dendritic cells, keratinocytes, adrenal cortex, etc. [1]. A significant correlation between the platelet activation

![Fig. 1. IL-18 and sGPV concentrations in the plasma of HIV-infected persons.](image-url)
marker sGPV and plasma IL-18 concentration indicates a contribution from activated platelets. However, these results do not exclude any contribution from non-platelet sources, e.g. from the adrenal cortex or keratinocytes in these patients.

It is noteworthy that platelets constitute the most numerous physical entities in blood after erythrocytes, and they usually become activated in viral infections. In addition to IL-18, platelets are also rich in other immunologically important cytokines, e.g. transforming growth factor beta. Furthermore, activated platelets express important immune regulatory molecules on their surface e.g. FasL and CD40L [10]. The enhanced platelet activation may be a contributing factor towards increased plasma concentrations not only of IL-18 but also of other platelet-contained cytokines, e.g. transforming growth factor beta, and may cause immune dysregulation in HIV-infected individuals. Our results also have implications for understanding the pathogenesis of other chronic viral infections that are accompanied by platelet activation.

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