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Chapter 6

"Immunopathology as a result of highly active antiretroviral therapy in HIV-1 infected patients"

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Summary

Unusual clinical inflammatory syndromes associated with underlying previously unrecognized opportunistic infections are increasingly being noted shortly after starting highly active antiretroviral therapy (HAART). This study examined the possible relationship between such unexpected disease manifestations and in vitro parameters of microbial antigen-specific immune reactivity in patients infected with HIV-1 who had a Mycobacterium avium intracellulare or Mycobacterium xenopi infection.

In vitro T-cell proliferation experiments were performed after specific stimulation of a patient's peripheral blood mononuclear cells (PBMC) with M. avium and M. xenopi antigen and non-specific stimulation with phytohaemagglutinin (PHA). The results were compared with appropriate controls. Five patients who presented with unusual clinical syndromes associated with M. avium or M. xenopi infection within weeks of experiencing large rises in CD4+ cell counts following the initiation of antiretroviral therapy.

In all patients except one, mycobacteria-specific lymphoproliferative responses rose significantly following HAART; this was temporally associated with elevations in CD4+ cell counts and the occurrence of clinical disease. The patient with M. xenopi infection appeared to clear his infection subsequently without antimycobacterial therapy. In three of the four patients with M. avium infection, antimycobacterial treatment could be stopped without recurrence of infection.

Our findings support the hypothesis that HAART may lead to clinically relevant inflammation as a result of restoration of specific immune reactivity against microbial pathogens that are subclinically present at the time treatment is initiated. Continuation of HAART may subsequently result in protective immunity and clearance of infection.
Pathological effects of immune reconstitution

Introduction

The introduction of potent combination antiretroviral therapy, also known as “highly active antiretroviral therapy” (HAART), has resulted in reduction of HIV replication, increases in CD4+ lymphocyte counts and reductions in HIV-related morbidity and mortality to a degree that was hitherto rarely observed with less potent antiretroviral regimens [1-8]. These findings indicate at least a partial recovery from HIV-induced immunodeficiency. The often impressive rises in CD4+ cell counts seen within weeks to months following the initiation of HAART have been demonstrated to result largely from a rise in CD4+ cells of the memory phenotype, with cells with a naive phenotype showing a much more limited and slower recovery [9-11].

Apart from these changes in CD4+ cell numbers, in vitro improvement both in overall T-lymphocyte function and in lymphoproliferative responses specific for certain microbial antigens has also been demonstrated in response to HAART [10,12].

Clinically, HAART-induced immune recovery may be associated with improvement in specific HIV-related opportunistic disease manifestations such as chronic parasitic diarrhoea [13,14], progressive multifocal leukoencephalopathy [15,16], treatment-refractory oral candidiasis [17-19], molluscum contagiosum [20,21] and Kaposi’s sarcoma [22-24]. However, unusual previously unrecognized syndromes such as focal mycobacterial lymphadenitis [25-28], laryngeal Kaposi’s sarcoma [29] and vitritis (i.e., inflammation of the anterior chamber of the eye) in patients with cytomegalovirus (CMV) retinitis [30-32] have also occurred within weeks to months after the initiation of HAART. It is suggested that these clinical syndromes may represent inflammatory responses that occur as a result of recovery of specific immunity directed against smoldering infections patients happen to harbour at the time of HAART initiation.

We present evidence in support of this hypothesis by demonstrating the recovery of mycobacteria-specific cellular immune responses in five patients presenting with unusual clinical signs of atypical mycobacterial infection shortly after starting HAART.
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Materials and methods

In vitro T-cell proliferation studies were performed to investigate if specific antimycobacterial lymphocytotoxicity had increased during HAART. Cell cultures with $1.5 \times 10^5$ cryopreserved peripheral blood mononuclear cells (PBMC) per well from each of the five patients were set up in 96-well round-bottom microtitre plates in a humidified atmosphere containing 5% CO$_2$ at 37°C. Cells were cultured in RPMI 1640 (Gibco BRL, Gaithersburg, Maryland, USA) supplemented with 10% pooled heat-inactivated human serum (CLB, Amsterdam, The Netherlands) and antibiotics. Cell cultures were stimulated for 7 days with medium alone or 10 μg/mL purified protein derivative (PPD) from *Mycobacterium avium* intracellulare complex (MAC) (patients B-E) or *Mycobacterium xenopi* (Patient A) (Statens Serum Institut, Copenhagen, Denmark). In patient A, cells were also stimulated with 35 μg/mL tetanus toxoid (RIVM, Bilthoven, The Netherlands) or with 1:50 diluted and extensively dialyzed antigen from *Candida albicans* (HAL, Haarlem, The Netherlands). At day 6, cells were pulsed with 0.2 mCi (7.4 kBq) $[^3]$H-thymidine for 18 h before harvesting. Incorporated radioactivity was measured on a beta scintillation counter (Betaplate LKB, Bromma, Sweden). Assays were performed in triplicate and results are reported as stimulation indices [counts per minute (c.p.m.) obtained for (cells + antigen)/(cells + medium)].

HIV-1-negative PBMC from a *Mycobacterium bovis* Bacille Calmette-Guérin (BCG)-vaccinated blood donor was used as a cross-reactive control for proliferation upon stimulation with PPD from *M. tuberculosis*, MAC or *M. xenopi*. PBMC from an HIV-negative not BCG-boosted individual, from an HIV-infected patient with a known MAC infection but without HAART (CD4 T count < 50 x 10$^6$ cells/l) and from an HIV-infected patient without MAC infection were used as controls.

From all patients, 5 x 10$^4$ cells were stimulated with 1 μg/mL phytohaemagglutinin (PHA) (Sigma, St. Louis, Missouri, USA) as a positive control for non-specific T-cell reactivity. Cultures were kept for 3 days and at day 2 $[^3]$H-thymidine was added for 18 h. The laboratory technician was not aware of the patients’ exact case histories when performing the assay but was aware of whether a sample belonged to the patients or to the controls.

Plasma HIV RNA levels were measured in serum using the NASBA assay (Organon Teknika, Oss, The Netherlands) as described [33].

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Results

Patient A
Patient A is a 38-year-old homosexual man who in June 1995 after his CD4+ cell count had dropped to $19 \times 10^6$ cells/l despite the use of nucleoside analogues added ritonavir to his treatment [34]. He was on fluconazole prophylaxis because of recurrent candidiasis, but he did not use MAC prophylaxis. Several months before starting ritonavir, he started complaining of night sweats, weight loss, fever, loose stools and a persistent non-productive cough. A diagnostic work-up including direct smears and culture of sputum and stool for mycobacteria, showed no abnormalities. After starting ritonavir, impressive rises of CD4+ and CD8+ cell counts were observed, with a concomitant 3 log decrease in plasma HIV-1 RNA levels (Figure 1A). One month after starting ritonavir, he again became febrile and developed watery diarrhoea. A chest X-ray and a computed tomographic (CT) scan of the chest showed multiple bilateral nodular changes and some left-sided pleural fluid. A bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsies were performed, but no pathogens, including acid-fast bacilli, were demonstrated. During the following week, the fever disappeared and the diarrhoea improved without specific treatment. At this time, a sputum culture that had been obtained prior to starting ritonavir was reported to grow \textit{M. xenopi}. The same microorganism was later also cultured from stool and sputum specimens obtained both several days before and directly after the BAL. In view of the resolution of clinical symptoms, specific antimycobacterial treatment was withheld. Three months later, the patient’s bowel movements had normalized and his cough lessened. Repeated cultures of sputum, stool, blood and urine remained negative for mycobacteria. A CT scan of the chest was repeated and showed a clear improvement of prior abnormalities. Although CD4+ and CD8+ lymphocyte counts have gradually declined since, he remains free from any signs or symptoms of \textit{M. xenopi} infection. Fluconazol prophylaxis was discontinued without recurrence of oropharyngeal candidiasis.

Patient B
Patient B is a 37-year-old woman who acquired HIV-1 infection in 1993. In June 1995, her CD4+ cell count had declined to $< 10 \times 10^6$ cells/l despite treatment with nucleoside analogues. In December 1995, indinavir 800 mg three times a day was started. Ten days later she was admitted to the hospital because of fever ($39.4 ^\circ$C),
weight loss, nausea and dyspnoea. On examination, the heart and lungs appeared normal, but the liver was palpable 2 cm below the right costal margin. Laboratory tests on admission showed a rise in alkaline phosphatase and aspartate aminotransferase, as well as a prolongation of prothrombin and partial thromboplastin times compared with values prior to starting indinavir. The CD4+ lymphocyte count, which had been \(< 10 \times 10^6\) cells/l at baseline, had risen to \(160 \times 10^6\) cells/l but decreased rapidly after antiretroviral therapy was interrupted on admission (Figure 1B). A radiograph of the chest was normal. Abdominal ultrasound revealed hepatospleno-megaly without focal abnormalities. A liver biopsy was performed and revealed scattered granulomas containing acid-fast bacilli. Cultures of bone marrow and blood drawn on admission as well as a specimen of hepatic tissue later all revealed MAC. Anti-MAC treatment was initiated and 3 weeks after admission the patient was rechallenged with indinavir. This resulted for the second time in a rise of the CD4+ lymphocyte count from \(10 \times 10^6\) cells/l to \(120 \times 10^6\) cells/l. This occurred within 21 days of starting treatment and now was accompanied by impressive mediastinal lymphadenopathy and ulceration of a supraclavicular lymph node, from which MAC could be cultured repeatedly. She recovered fully after 1 year of antimycobacterial therapy. She has been without any signs of MAC infection for 6 months after MAC treatment was discontinued.

Patient C
Patient C, a 34-year-old homosexual male seropositive for HIV-1, started zidovudine and zalcitabine in 1995 after his CD4+ lymphocyte count had declined to \(40 \times 10^6\) cells/l. One month later his CD4+ lymphocyte count had risen to \(200 \times 10^6\) cells/l (Figure 1C). At that time he complained of fever and abdominal discomfort. A CT scan of the abdomen revealed multiple enlarged retroperitoneal lymph nodes. An intraperitoneal necrotic mass was aspirated. Acid-fast bacilli were seen and the culture grew MAC. Blood and stool cultures remained negative. After starting antimycobacterial treatment, he fully recovered and MAC therapy was stopped after 7 months. So far he has remained free of MAC-associated disease for 2 years.

Patient D
Patient D, a 38-year-old HIV-1-seropositive man, was diagnosed with AIDS in 1994 when he developed \(Pneumocystis carinii\) pneumonia. In 1995, lamivudine and
saquinavir were added to his therapy with zidovudine because of a high plasma HIV RNA level. Twelve weeks later, when his CD4+ lymphocyte count was found to have risen from $20 \times 10^6$ cells/l to $100 \times 10^6$ cells/l, he was admitted to hospital because of cough and abdominal discomfort (Figure 1D). A chest X-ray revealed a dense infiltrate in the right lung. A CT scan of the abdomen showed enlarged para-aortic lymphadenopathy. Examination of stool and sputum showed acid-fast bacilli, and MAC was cultured from both specimens. Blood cultures remained negative. Although a positive sputum and stool culture is not definitive proof of MAC-associated disease, this finding in combination with a pulmonary infiltrate and extensive mediastinal and para-aortic lymphadenopathy in the absence of an alternative explanation strongly suggested generalized infection and antimycobacterial therapy was started. The diagnosis was supported by the subsequent course, which showed that 7 months later the patients had fully recovered and MAC treatment could be discontinued without recurrence of clinical signs and symptoms of mycobacterial infection.

**Patient E**

Patient E is a 30-year-old HIV-1-seropositive female who is a former intravenous drug user. She started zidovudine in 1994 after she had developed candidal oesophagitis. In March 1996, she entered hospital with MAC enteritis. She decided to stop all antiretroviral and antibiotic drugs in June 1996. Subsequently, MAC was again cultured from sputum, stool and blood. HAART consisting of saquinavir, stavudine and lamivudine, as well as antimycobacterial treatment, was started at a time when her CD4+ lymphocyte count was $< 10 \times 10^6$ cells/l. Eight days after the start of HAART, she became severely ill with fever, cough and shortness of breath. A chest X-ray showed patchy lesions in the right lower lung and a considerable widening of the superior mediastinum caused by lymphadenopathy (Figure 2). A mediastinal lymph node was aspirated and showed granulomatous inflammation. Culture of the lymph node grew MAC. Plasma HIV RNA levels at that time had decreased to below the detection limit of 1000 copies/ml. CD4+ cell counts increased significantly in the ensuing weeks (Figure 1E), but decreased again during the subsequent months. After ritonavir was added to her regimen, the same clinical syndrome was observed within 4 weeks and MAC was again isolated from blood and stool.
Figure 1A
Pathological effects of immune reconstitution

Figure 1B
Figure 1C
Figure 1 D
Figure 1E
Pathological effects of immune reconstitution

Figure 1A-F
Changes occurring in five patients (A-E) after starting highly active retroviral treatment (HAART). 1. CD4⁺ cell count and plasma HIV RNA; 2. non-specific T-cell proliferation after stimulation with phytohaemagglutinin (PHA) (normal value ≥ 17000 c.p.m.); 3. T-cell proliferation index in patient A after stimulation with \textit{M. xenopi}, candida and tetanus purified antigens and in patients B-E after stimulation with \textit{Mycobacterium avium} complex (MAC) purified antigen (x-fold expansion over background). Timepoint 0 is the first moment a new highly active antiretroviral therapy was started. The arrows indicate the moment of unmasking or paradoxical worsening of disease. † Patient E was not fully compliant to the antiretroviral therapy during this period. AZT, zidovudine, ddl, didanosine, dDC, dideoxycytidine, 3TC, lamivudine, d4T, stavudine. Graph F shows the proliferative responses of peripheral blood mononuclear cells (PBMC) in response to mycobacterial antigens. The stimulation index is expressed as the ratio of T-cell proliferation with the specific antigen to the background (stimulation index values 0-2 are considered to be comparable to the background value). Lane A, high cross-reactive responses are observed after stimulation of PBMC from a healthy BCG vaccinated donor with purified antigen of \textit{M. tuberculosis}, \textit{M. xenopi}, and MAC. Lane B, there is no response after stimulation with \textit{M. xenopi} and MAC in a healthy, not boosted donor. Lane C, stimulation with MAC did not provoke a specific response in a HIV-1-infected patient not receiving HAART with disseminated MAC infection. Lane D, there was no significant proliferation after stimulation with MAC in a HIV-1 infected donor without MAC infection.
Figure 2A
Figure 2B
Chest X-ray of patient IE obtained before (A) and 2 weeks after (B) start of highly active antiretroviral therapy (HAART). Following HAART, the widening of the mediastinum, bilateral hilar lymphadenopathy, and a right middle lobe infiltrate became apparent.
in vitro studies

The in vitro T-cell proliferation studies with MAC and M. xenopi antigens revealed that spontaneous proliferation by cells from an immunocompetent person was extremely low compared of cells from a healthy control who was recently vaccinated with BCG (Figure 1F). The cells from two HIV-1-seropositive control patients likewise showed no response, regardless of whether they had MAC infection or not. All the patients studied had severe HIV-related immunodeficiency and were anergic for both mycobacterial antigens before receiving HAART (Figure 1A-E). After starting HAART, improvement of specific T-cell immunity to mycobacterial antigen, as indicated by an increased stimulation index, was observed in all the patients except patient B. In addition, in patient A an improvement of specific immunity to the recall antigen C. albicans was also observed (Figure 1A). The other patients did not show any change of specific immunity to C. albicans (data not shown).

Although in patient B a measurable increase in specific antimycobacterial immunity was not observed, an increase in non-specific proliferation to PHA could be demonstrated. In patient E the increase in T-cell proliferation following specific stimulation with mycobacterial antigen could only be demonstrated during the second episode of the HAART-associated clinical syndrome, as PBMC samples were not available from the time of the first episode.

In all patients with measurable increases in T-cell proliferation specific for mycobacterial antigens, these changes coincided with increases in CD4+ T-cell counts and the start of clinical signs and symptoms. Although in most patients both the increase in CD4+ cells and the improved in vitro proliferative responses were not sustained, no relapses of their mycobacterial infections have been diagnosed to date.

Discussion

We describe five HIV-1-infected patients who unexpectedly presented with clinically unusual and severe manifestations of an atypical mycobacterial infection shortly after starting effective antiretroviral therapy and coinciding with a major rise in CD4+ cells. The rapidly progressive presentation in most of the patients, with severe pulmonary
symptoms (patients A, D and E), evidence of hepatic decompensation (patient B) and impressive mediastinal lymphadenopathy (patients B and E), was markedly different from that of disseminated MAC infection in the pre-HAART era. The typical presentation at that time was with a prolonged febrile illness accompanied by general malaise, weight loss, anaemia and sometimes chronic diarrhoea in patients with, virtually without exception, very advanced HIV disease and a CD4+ T-cell count of less than 50 x 10^6 cells/l [35]. The occurrence of disease within weeks to a few months after starting HAART, suggested that mycobacterial infection was already present subclinically or only marginally symptomatic at the time of starting HAART. This was definitively proved in two patients. Unmasking or paradoxical worsening of mycobacterial infection may have resulted from intensified inflammatory responses caused by the sudden increase of lymphocytes specifically reacting against mycobacterial antigens. The early rises in CD4+ and CD8+ lymphocytes observed shortly after commencing HAART are mainly explained by rises in memory T cells [9]. The presence of immunological memory specific for mycobacterial antigens is to be excepted in patients with advanced HIV infection, who commonly have subclinical MAC infection [25]. Following HAART, this may result in preferential recruitment of specific T cells towards the site of subclinical infection. These cells are known to consist predominantly of CD4+ T cells, which produce antigen-dependent cytotoxicity as well as macrophage activation [36-39]. In patients with advanced and untreated HIV infection, absence of such cells typically results in poor granuloma formation and a high bacillary load. The finding of granulomas containing only modest numbers of mycobacteria in the liver biopsy of patient B supports our hypothesis of the occurrence of a more normal specific immune response. MAC itself is by and large not cytotoxic and tissue damage in the context of MAC infection is mainly the result of the specific immune response against the organism, leading to the release of various inflammatory cytokines [40,41]. It is understandable why a sudden and much intensified specific immune response following HAART could result in clinically overt signs and symptoms of inflammation. The results in four of the five patients of the ex vivo experiments, which demonstrated an increase in mycobacteria-specific lymphocyte reactivity at the time of presentation with clinical disease shortly after commencing HAART, supports the suggested immunopathogenesis.

The clinical course of patient A not only illustrates immunopathological reactions after HAART but also exemplifies the potential for HAART-associated
immunoprotection, as manifested by the subsequent clearance of *M. xenopi* without the administration of specific antimycobacterial therapy. Spontaneous clearance of *M. xenopi* infection has been reported [42] but seems unlikely in this patient, who had a CD4+ cell count of less than 20 x 10⁶ cells/l prior to treatment with ritonavir. The recent reports of vitritis in conjunction with CMV retinitis in patients who recently started HAART [30,32] probably represent examples of the same immunopathological mechanism, as suggested by ourselves and others for mycobacterial infections [28]. An increase in a specific lymphoproliferative response to CMV following HAART has indeed been reported [12]. The same immunopathology has been suggested concerning the recently reported exacerbations of chronic viral hepatitis following HAART [43,44].

A minimum degree of restoration of HIV-induced immunodeficiency is probably needed for these immunopathological responses to occur. This is to likely be achieved more often with the current HAART regimens. Occasionally, the same phenomenon may be observed with just nucleoside analogue combinations, as was demonstrated in patient C and has previously been demonstrated by French et al. [45].

In conclusion, with the widespread use of HAART sudden profound improvement in cellular immunity is increasingly being achieved. On the one hand, this may be associated with clinical remission of opportunistic diseases that are present at the time and may allow the discontinuation of opportunistic infection-specific prophylactic therapies. On the other hand, clinicians prescribing HAART should also be aware of the increased potential for the occurrence of unusual inflammatory syndromes during the early phases of the treatment, which may be indicative of an immune response against smoldering underlying opportunistic infections. This is especially pertinent when considering HAART in patients with advanced HIV infection. Discriminating between such an expression of specific immunopathology and an adverse reaction to the newly instituted antiretroviral treatment may be difficult but would be important in deciding whether to interrupt or continue HAART. In the former, if clinically acceptable, continuation of HAART would be the preferred option in order for protective immunity against the underlying infection to take precedence ultimately and to help to control the infection.
References


