ORIGINAL RESEARCH

Immune restoration disease after the treatment of immunodeficient HIV-infected patients with highly active antiretroviral therapy

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Background
To determine if infectious disease events in HIV-infected patients treated with highly active antiretroviral therapy (HAART) are a consequence of the restoration of pathogen-specific immune responses, a single-centre retrospective study of all HIV-infected patients commencing HAART prior to 1 July 1997 was undertaken to determine the incidence, characteristics and time of onset of disease episodes in HAART responders (decrease in plasma HIV RNA of > 1 $\log_{10}$ copies/mL).

Methods
Baseline and post-therapy changes in CD4 T-cell counts and HIV RNA were compared in patients with and without disease and delayed-type hypersensitivity responses to mycobacterial antigens were measured in selected patients.

Results
Thirty-three of 132 HAART responders (25%) exhibited one or more disease episodes after HAART, related to a pre-existent or subclinical infection by an opportunistic pathogen. Disease episodes were most often related to infections by mycobacteria or herpesviruses but hepatitis C virus (HCV), molluscum contagiosum virus and human papilloma virus were also implicated. They were most common in patients with a baseline CD4 T-cell count of < 50/uL and occurred most often during the first 2 months of therapy and when CD4 T-cell counts were increasing. Mycobacteria- and HCV-related diseases were associated with restoration of pathogen-specific immune responses.

Conclusions
We conclude that improved immune function in immunodeficient patients treated with HAART may restore pathogen-specific immune responses and cause inflammation in tissues infected by those pathogens.

Key words: HAART, HIV, immune restoration

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Introduction

While the immediate aim of using antiretroviral therapy is suppression of HIV replication, the ultimate aim is restoration or maintenance of protective HIV-specific and pathogen-specific immune responses. Treatment of immunodeficient patients with highly active antiretroviral therapy (HAART) appears to restore pathogen-specific immune responses resulting in prevention or regression of diseases caused by opportunistic pathogens [1]. However, in some patients suppression of HIV replication by antiretroviral therapy and the resultant increase in blood CD4 T-cells is associated with inflammation in tissues infected by those pathogens. Thus, disease related to subclinical or pre-existent infections by *Mycobacterium avium* complex (MAC) [2–4], *M. tuberculosis* (MTB) [5,6], Bacille Calmette–Guerin (BCG) [7], cytomegalovirus (CMV) [8–10], hepatitis B virus (HBV) [11,12], hepatitis C virus (HCV) [13] and *Cryptococcus neoformans* [14] has been reported in patients who had a beneficial virological and

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immunological response to HAART. Many of these disease episodes were associated with the presence of a pathogen-specific immune response and/or more exaggerated inflammatory response than is usually present in patients with opportunistic infections. It has therefore been argued that the disease results from the effects of a restored immune response against the pathogen [2–7,10,11,13,14]. However, Michelet *et al*. have argued that 'opportunistic infections' in patients treated with HAART reflect delayed restoration of immune function [15].

Disease which results from the restoration of a pathogen-specific immune response (immune restoration disease) clearly has a different significance to disease which results from the delayed recovery of immune function in patients treated with HAART. We have therefore undertaken a retrospective study of all patients starting HAART at a single HIV treatment centre to determine the incidence, characteristics, time of onset and immunological and microbiological features of infectious disease events in patients responding to HAART.

Methods

Patients

The study was undertaken on 161 male and 18 female adults who represented all patients commencing HAART at Royal Perth Hospital or associated private clinics between 1 September 1996 and 1 July 1997. Patients were included if they were treatment-naïve or experienced and had been commenced on triple combination antiretroviral therapy, including a protease inhibitor or nevirapine, or treatment-naïve and had been commenced on lamivudine and another nucleoside analogue. Medical records were reviewed to obtain information on infectious disease events after the commencement of HAART and to verify the drugs used and treatment start-dates. CD4 T-cell counts and plasma HIV RNA concentrations were obtained from an HIV patient database [16].

Patients with a decrease in plasma HIV RNA of > 1 $\log_{10}$ copies/mL on at least one occasion during the first 16 weeks of HAART were classified as responders, whereas those with a < 1 $\log_{10}$ decrease in HIV RNA on two or more occasions during the first 16 weeks of therapy were classified as non-responders. Patients who could not be classified in this way or whose medical records contained insufficient information were excluded from the study.

Clinical methods

Data were collected from medical and laboratory records on clinical events reported by the patient and/or observed by a physician up to 30 weeks after commencement of HAART. Retinal examination by fundoscopy and retinal photography was undertaken by an ophthalmologist (M-L T-K) at 1-month intervals in patients being treated for CMV retinitis. Other patients with CD4 T-cell counts < 50/uL had an ophthalmology review at 3-month intervals.

Immunological and microbiological methods

Cutaneous delayed-type hypersensitivity (DTH) responses to tuberculin and *M. avium* antigen (Commonwealth Serum Laboratories, Melbourne, Australia) were measured by the Mantoux method in patients presenting with lymphadenopathy and/or fever with no apparent cause [2]. DTH responses to a panel of 'recall' antigens were also measured using the Multitest CMI^{TM} method (Pasteur Merieux, Lyons, France). Plasma HIV RNA was assayed by the Amplicor method (Roche, Branchburg, USA) and CD4 T-cell counts were measured by standard flow cytometric methods. Hepatitis C virus (HCV) qualitative and quantitative PCR assays were performed using the Amplicor and Amplicor Monitor methods (Roche, Branchburg, USA), respectively. HCV antibodies were detected by an enzyme immunoassay (EIA version 3.0, Abbott, Wiesbaden-Delkenheim, Germany). Cerebrospinal fluid (CSF) was examined for herpes simplex virus (HSV), CMV and *Varicella zoster* virus (VZV) using 'in-house' nested PCR assays and a standard rapid culture method with detection of virus by immunofluorescence. Cultures for mycobacteria were performed using the Bactec 460 system (Becton Dickinson, Maryland, USA) with 12B or 13A bottles for tissue or blood, respectively.

Data analysis and statistics

Baseline CD4 T-cell counts and plasma HIV RNA concentrations were those recorded nearest to and before the commencement of HAART. When these were used to define changes over time, only measurements recorded less than 4 weeks before commencement of therapy were included. Following commencement of HAART, values at 6 weeks and 14 weeks were taken as the average of all measurements recorded within a window of 6 ± 3 weeks and 14 ± 5 weeks, respectively. Due to the limits of accurate detection, values of HIV RNA < 400 copies/mL were regarded as left-censored at 400 copies/mL.

A two-sample *t*-test was used to compare baseline means. Trends over the following 6- or 14-week intervals were compared via a censored-data Mann–Whitney analogue using the individual changes in measurements over the corresponding intervals. Average CD4 T-cell count and log HIV RNA profiles were constructed using mean

measurements at baseline, 6 weeks and 14 weeks, with censorship taken into account via conditional mean imputation. This was achieved by fitting Weibull distributions, which probability plots indicated were appropriate for these data. Risk factors associated with the development of disease following HAART were identified using logistic regression analyses. All reported *P*-values are two-sided.

Results

One hundred and seventy-nine patients commenced HAART, of whom 38 were excluded because of insufficient laboratory data or clinical follow-up. Of the remaining 141 patients, nine exhibited a decrease in plasma HIV RNA of $< 1 \log_{10}$ copies/mL and were classified as non-responders. Thirty-three of the 132 responders (25%) had one or more disease episode after commencing HAART (Table 1). All patients who developed disease had been treated with an HIV protease inhibitor. The baseline CD4 T-cell count was < 50/uL in 19 (58%) of the patients who had a disease episode including all seven (100%) patients with more than one episode. The diseases observed in these patients are described below.

'Relapse' of CMV retinitis

Six patients had a 'relapse' of CMV retinitis. All had a baseline CD4 T-cell count of < 50/uL and were receiving intravenous or intravitreal ganciclovir or foscarnet as secondary prophylaxis for CMV infection. In all patients there was subsequent resolution of the retinitis and five of the six patients ceased prophylactic anti-CMV therapy when CD4 T-cell counts had been > 100/uL for a period of 1–15 months and the plasma HIV RNA concentration was

Table 1 Infectious disease in HAART responders

Patient	Disease no. 1	Time*	Disease no. 2	Time*	Disease no. 3	Time*	CD4 T-cell count (/uL)		Mantoux test (mm)	
							Baseline	Maximum**	Tuberculin	*M. avium*
1	Retinitis/CMV	8					35	154		
2	Retinitis/CMV	5					20	99		
3	Retinitis/CMV	1					33	110		
4	Retinitis/CMV	9	Myelopathy***	11	Peri-anal herpes	23	0	24		
5	Retinitis/CMV	2	TL encephalitis	5			4	120		
6	Retinitis/CMV	7					6	55		
7	Lymphadenopathy/MAC	1	Hepatitis/HCV	12			42	78	0	26
8	Lymphadenopathy/MAC	1					12	57	0	15
9	Lymphadenopathy/MAC	6	Inflamed MC	28			9	12	7	12
10	Lymphadenopathy/MAC	4					91	182	14	24
11	Hepatitis/MAC	1	Naso-labial herpes	1	Myelopathy	1	42	231	ND	ND
12	Hepatitis/HCV	16					266	486		
13	Relapsed pulm. MTB	10					55	320	23	0
14	Focal cerebritis	8					20	122	16	2
15	Dermatomal zoster	6					378	483		
16	Dermatomal zoster	20					90	300		
17	Dermatomal zoster	2					208	325		
18	Dermatomal zoster	17					36	144		
19	Dermatomal zoster	13					119	280		
20	Dermatomal zoster	15					84	66		
21	Dermatomal zoster	26					225	450		
22	Zoster sine zoster	24					170	368		
23	Peri-anal herpes	1	Encephalomyelitis	6			5	18		
24	Penile herpes	4					25	100		
25	Peri-anal herpes	4					35	140		
26	Peri-anal herpes	1					48	304		
27	Oro-genital herpes	4					40	117		
28	Peri-oral herpes	6					459	460		
29	Peri-anal herpes	3					96	280		
30	Inflamed MC	12	Hepatitis/HCV	24			32	180		
31	Inflamed MC & warts	24					156	285		
32	Inflamed warts	4					10	48		
33	Inflamed warts	15					54	164		

*Time to presentation in weeks; **maximum CD4 T-cell count in first 16 weeks; ***also had cerebral mass lesions (see text). MC = molluscum contagiosum; TL = temporal lobe.

< 400 copies/mL. Follow-up for a mean time of 14 months (range 7–21) continues to show inactive retinitis based on careful fundoscopic and photographic examinations. However, patient 2 presented with vitreal inflammation in an eye with inactive retinitis 16 weeks after ceasing anti-CMV prophylaxis and subsequently had a detached retina in that eye.

Acute hepatitis associated with HCV infection

Symptomatic hepatitis occurred in three patients in association with an increase in serum alanine aminotransferase (ALT) to at least five-fold higher than the upper limit of the reference range. Only patient 7 had known HCV infection. Patients 12 and 30 seroconverted from HCV antibody-negative to -positive after commencing HAART and on examination of stored plasma it was demonstrated that HCV RNA had been present for at least 12 months before starting therapy. Both these patients had evidence of chronic hepatitis without eosinophilia or granulomata in a liver biopsy. Further details of these patients are presented elsewhere [13].

'Relapse' of pulmonary tuberculosis

Commencement of HAART following successful treatment of pulmonary tuberculosis in patient 13 resulted in an exacerbation of pulmonary inflammation associated with necrotizing mediastinal lymphadenitis (demonstrated by a CT scan). Mycobacteria were not demonstrated in sputum samples. This was associated with a rise in the blood CD4 T-cell count from 55/uL to 320/uL in the first month and DTH test conversion from anergy to a tuberculin response of 10 mm using the Multitest method (without a response to other antigens) and 23 mm using the Mantoux method. Full details are presented elsewhere [5].

Mucocutaneous herpes

Eight patients had an outbreak of mucocutaneous herpes during the first 8 weeks of HAART. Patient 4 also developed mucocutaneous herpes but only after 23 weeks of HAART following a very slow increase in the CD4 T-cell count after CD4 T cells had been < 1% for over 2 years. This patient and patients 5 and 23 also developed an acute myelopathy (see below). Three patients were taking low-dose acyclovir (600 mg or less/day) at the time they developed the herpes lesions. Most cases were satisfactorily managed with oral acyclovir therapy. However, patient 26, who did not give a history of previous herpes, had extensive perianal lesions with a fever up to 39 °C and required hospital admission for treatment, and patient 11 had severe haemorrhagic naso-labial lesions.

Dermatomal zoster

Eight patients had an outbreak of dermatomal zoster, including one with zoster sine zoster (characterized by pain in a dermatomal distribution which resolved after famciclovir therapy). This occurred up to 30 weeks after commencing antiretroviral therapy (Table 1) and when CD4 T-cell counts were higher than baseline, except in patient 20 (data not shown). All but one patient had a baseline CD4 T-cell count > 50/uL and none of the patients had another disease episode. Three patients were taking low-dose acyclovir at the time the zoster developed. All patients were successfully treated with acyclovir or famciclovir and did not have subsequent herpes zoster lesions.

Inflamed cutaneous warts or molluscum contagiosum

Five patients were noted to have inflammation of warts or molluscum contagiosum lesions that had been present before starting HAART. Biopsy of an inflamed molluscum contagiosum lesion from patient 30 demonstrated a mononuclear cell infiltrate surrounding virus-infected cells.

Localized MAC disease

Five patients had localized MAC disease presenting in a similar manner to that described in patients treated with zidovudine monotherapy [2]. Patients 7–10 had fever and lymphadenopathy (mediastinal, mesenteric or axillary), demonstrated clinically, radiologically or by a Gallium scan. This was associated with a > 10 mm DTH response to *M. avium* antigen but not mycobacteraemia. The lymphadenopathy was painful in three of the four patients. Patient 11 had fever and painful hepatomegaly associated with MAC bacteraemia (DTH responses were not measured). Patient 8 was receiving ciprofloxacin and monthly infusions of amikacin as secondary prophylaxis for previously diagnosed MAC infection, but the other patients had not received prophylactic anti-MAC therapy. All patients were treated with anti-MAC therapy in addition to HAART with subsequent disease resolution. In addition, prednisolone therapy was also used in patients 8 and 9 to control painful lymphadenopathy.

Steroid-responsive relapsing focal cerebritis

Patient 14 developed focal cerebritis complicated by focal seizures and fever associated with an increase in the CD4 T-cell count from 20/uL to 122/uL, and a DTH response to tuberculin of 16 mm by the Mantoux method and 4 mm by the Multitest CMI^{TM} method (without a response to other antigens). DTH responses to tuberculin in the Multitest

CMI^{TM} panel had been absent on each of the five occasions tested over the 5 years prior to starting HAART. Mycobacteria were not demonstrated in CSF by culture or PCR. Following failure to control the lesions by treatment for *Toxoplasma gondii* and MTB infection, dexamethasone therapy was given and the lesions completely resolved. Two subsequent recurrences of focal cerebritis following non-adherence to medication were also associated with a > 10 mm Mantoux test response. On each occasion the cerebritis resolved after recommencing dexamethasone and anti-MTB therapy.

Encephalitis and/or myelopathy

Patient 5 developed a left temporal lobe encephalitis, with findings on clinical examination, magnetic resonance imaging (MRI) scan and electroencephalogram (EEG) strongly suggestive of herpes encephalitis. This resolved completely following intravenous acyclovir therapy. This patient had previously had a 'relapse' of CMV retinitis (Table 1). Both the encephalitis and retinitis occurred while the patient was receiving intravenous ganciclovir therapy as secondary prophylaxis for CMV infection.

A myelopathy was the predominant neurological abnormality in the other three patients. In patient 23 this was associated with clinical evidence of an encephalitis and computerized tomographic (CT) scan changes of a fronto-temporal arachnoiditis, and eventually led to his death. Patient 4 developed a severe spastic paraparesis with evidence of a multifocal myelitis on an MRI scan of the thoraco–lumbar spinal cord. A CT scan of the brain demonstrated lesions in both caudate nuclei and the right frontal lobe, but there was no serological evidence of *Toxoplasma* infection. This patient had also had a previous 'relapse' of CMV retinitis while receiving prophylactic intravenous ganciclovir. Neither the CMV retinitis nor myelitis responded to revision of anti-CMV therapy including intravenous cidofovir. However, the myelitis and cerebral mass lesions resolved following prednisolone therapy. The patient was well with a plasma HIV RNA concentration of < 400 copies/mL and CD4 T-cell count of 144/uL 23 months after the onset of the myelitis, although there was residual spasticity of the lower legs. Patient 11 had an acute dorsal column myelopathy from which he eventually recovered without specific antimicrobial therapy.

All four patients with encephalitis or myelopathy had a baseline CD4 T-cell count of < 50/uL. Examination of CSF from all patients for HSV, CMV, VZV and *Treponema pallidum* infection and by routine culture and microscopy failed to demonstrate a pathogen. However, all the myelopathy patients developed mucocutaneous herpes (with isolation of HSV) after not having had a recorded episode for at least a year.

Time of disease presentation after commencing HAART

Disease episodes occurred within the first 8 weeks of HAART in 22 (67%) patients (Table 1). Dermatomal zoster often presented later and was seen up to 30 weeks after commencing HAART. Symptomatic HCV-related hepatitis and inflamed molluscum contagiosum or warts presented at 12 weeks or later, except for patient 32 who presented with inflamed warts after 4 weeks. Patient 4 developed myelitis and cerebral mass lesions after 11 weeks and perianal herpes after 23 weeks but had a relatively slow increase in CD4 T-cells after having had undetectable CD4 T-cells for over 2 years prior to commencing HAART.

Disease in HAART non-responders

One of nine HAART non-responders had oesophageal candidiasis 2 months after commencing therapy but there were no other infectious disease events. This was despite these patients having a median baseline CD4 T-cell count of 16/uL (range 1–228) compared with 42/uL (range < 1–459) in HAART responders with disease ($P < 0.1$).

CD4 T-cell counts and HIV RNA in HAART responders with or without disease

Average profiles of CD4 T-cell counts and HIV RNA for patients with disease and those remaining disease-free are given in Figs 1 and 2. For CD4 T-cell counts, those patients developing disease had a significantly lower mean count at commencement of HAART (88 vs. 237 cells/uL, $P = 0.0001$). An upward trend over the following weeks was evident

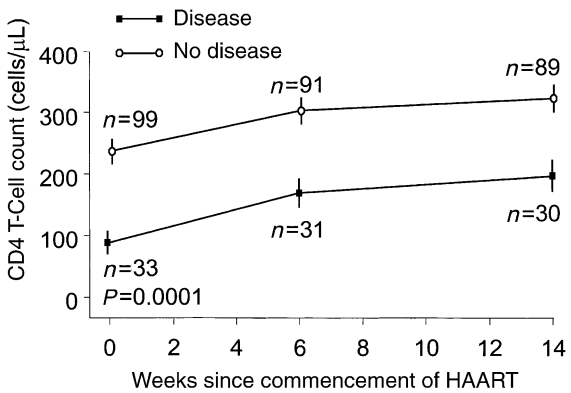

Fig. 1 CD4 T-cell counts at baseline and after HAART in HAART responders with or without disease.

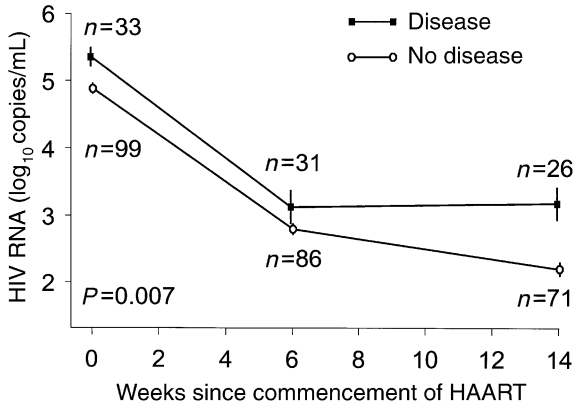

Fig. 2 HIV RNA at baseline and after HAART in HAART responders with or without disease.

both in patients who subsequently developed disease and those remaining disease-free. The changes in observed count were similar in both groups over the 6 weeks following commencement of HAART (median increase of 97 vs. 66 cells/mL, $P = 0.34$) and over the 6–14-week interval (median change of 16 vs. 12 cells/mL, $P = 0.74$). For HIV RNA, there was a significantly higher mean baseline value in the disease group (5.36 vs. 4.88 $\log_{10}$ copies/mL, $P = 0.007$) and relatively little difference in the rate of change over the 6 weeks subsequent to commencement of HAART ($P = 0.39$) with a marked decline in viral load observed in both groups. However, over the following 8-week interval there was a difference between the two groups in the change in HIV RNA ($P = 0.086$), with the viral load of disease-free patients continuing to decline whereas the decrease in patients with disease was less pronounced (Fig. 2).

Logistic regression analysis was used to assess the influence of CD4 T-cell counts and HIV RNA on the development of disease. The baseline CD4 T-cell count was a highly significant predictor of disease ($P = 0.003$), but when this was taken into account we found no additional significant effect of baseline HIV RNA concentration ($P = 0.73$).

Discussion

In this single-centre retrospective study of all patients treated with HAART during a defined period of time, we demonstrated that 25% of HAART responders had one or more infectious disease event. These were associated with pre-existent infections by CMV, MAC, MTB, HCV, HPV or MCV in some patients, but in other patients they were associated with a subclinical infection by these or other pathogens. It is possible that the disease episodes in these patients were 'opportunistic infections' occurring after HAART because of the delayed recovery of immune function [15]. However, as outlined below we would argue that the disease resulted from the restoration of an immune response against opportunistic pathogens, here defined as 'immune restoration disease'.

Patients who had a disease episode after HAART had lower baseline CD4 T-cell counts and higher plasma HIV RNA concentrations than those who did not develop disease, and were therefore more immunodeficient prior to the commencement of HAART. However, the rate of increase in the CD4 T-cell count and rate of decrease in HIV RNA after HAART was similar in both groups, suggesting that disease episodes were not the result of a delayed immunological or virological response to HAART. Furthermore, only one of the patients who did not respond to HAART had an infection after commencing HAART (oesophageal candidiasis) despite these patients having lower baseline CD4 T-cell counts, although it is acknowledged that this group of patients was small. We interpret our findings as indicating that the most immunodeficient patients had persistent or subclinical infections by opportunistic pathogens, and that inflammation was only produced when a pathogen-specific immune response was restored by HAART. We have previously proposed that this occurs in patients with MAC disease after zidovudine monotherapy [2,17].

Disease episodes occurred during the first 2 months of HAART in 67% of patients, which is remarkably similar to the 68% incidence of 'opportunistic infections' during the first 2 months of HAART reported by Michelet *et al.* [15]. Disease related to MAC, CMV and cryptococcal infections after HAART also presented at a similar time in other studies [2–4,8,10,14]. This is a time when numbers of memory CD4 T cells and antigen-specific T cells are increasing [18–20] which might result in restoration of pathogen-specific immune responses. Support for this proposal is provided by our previous observation that localized MAC disease in immunodeficient patients treated with zidovudine monotherapy is associated with the restoration of a DTH response to mycobacterial antigens [2], and the observation in this study that patients with a similar clinical presentation had a cutaneous DTH response to *M. avium* antigens. Similarly, Foudraine *et al.* reported that patients with inflammatory syndromes associated with *M. avium* or *M. xenopi* infection after HAART had increased mycobacteria-specific lymphoproliferative responses [4]. Furthermore, inflammation at sites of previous MTB infection after HAART was also associated with the restoration of a cutaneous DTH response to tuberculin in patients who were anergic to tuberculin prior to commencing HAART [6], as demonstrated in a patient in this study [5].

Hepatitis following the use of HAART may be an hepatotoxic effect of protease inhibitors [21,22] but in most patients it is associated with HCV and occasionally HBV infection [11–13,23–27]. As demonstrated in two of our series of patients [13], liver biopsies from HCV/HIV co-infected patients with a relapse of hepatitis after HAART do not demonstrate changes characteristic of drug-induced hepatitis [26,28]. Also, the hepatitis is unlikely to be a direct effect of increased HCV replication because plasma HCV RNA concentrations are not increased in patients treated with HAART [29,30]. Two patients who developed hepatitis after HAART in our series had subclinical HCV infection and seroconverted to HCV antibody-positive [13], suggesting that the hepatitis resulted from the restoration of an immune response against HCV. Pialoux *et al.*, in a prospective study of 120 patients with HCV/HIV co-infection treated with HAART, demonstrated a relapse of hepatitis in 8% of patients and also argued that the hepatitis reflected enhancement of anti-HCV immune responses [28]. Similarly, hepatitis in HBV/HIV co-infected patients responding to HAART has been attributed to the restoration of HBV-specific immune responses [11,12].

Intra-ocular inflammation after HAART in patients with stable CMV retinitis is associated with increased CD4 T-cell counts and lymphoproliferative responses to CMV antigens [31,32]. It is probable that relapses of CMV retinitis [10] and first presentations of CMV retinitis [8,9,15] associated with increased CD4 T-cell counts after HAART also result from an immune response against persistent or subclinical CMV infection [33]. In agreement with Casado *et al.* [10] we have shown that prophylactic anti-CMV therapy may not prevent relapses of CMV retinitis after HAART. However, we have also confirmed the findings of several groups [10,34,35] that the retinitis subsequently resolves and does not relapse when prophylactic anti-CMV therapy is ceased.

Other disease episodes after HAART were associated with an infection by a pathogen and an increased CD4 T-cell count, and although investigations were not undertaken to demonstrate their association with restoration of pathogen-specific immune responses, we would argue that these were also immune restoration diseases. Mucocutaneous herpes occurred during the first 8 weeks of HAART in all but one patient and two patients with severe disease required hospitalization. Dermatomal zoster presented later than mucocutaneous herpes and most other inflammatory diseases, as demonstrated elsewhere [36,37]. Aldeen *et al.* [36] reported a five-fold increase in herpes zoster in patients on HAART with cases occurring up to 80 weeks after starting therapy, and Martinez *et al.* [37] reported an overall two-fold increase in episodes of herpes zoster with a nine-fold increase between weeks 4 and 16. In the latter study, herpes zoster occurred in patients with a higher baseline and greater increase in the percentage of CD8 T-cells. It was therefore argued that enhancement of CD8 T-cell responses may have caused reactivation of VZV infection. If so, it is difficult to explain why the zoster eruptions occurred much later than other inflammatory diseases, including those related to infections by other herpesviruses such as CMV and HSV. An alternative explanation is that the zoster eruptions were the result of restored anti-VZV immune responses and often occurred later because VZV infection reactivates much less frequently than HSV infection [38] resulting in antigen recognition by T-cells occurring less often.

We also observed central nervous system disease in five patients from whom pathogens were not isolated, but would also argue that these were immune restoration diseases. Relapsing steroid-dependant cerebritis was associated with persistent cutaneous DTH responses to tuberculin in a patient who had been anergic to tuberculin for 5 years prior to commencing HAART. Cerebral mass lesions associated with MTB infection have been described in one other patient treated with HAART [39] but MTB-specific immune responses were not assessed. Cerebral tuberculosis does not usually present as cerebral mass lesions in HIV-infected patients. However, Lesprit *et al.* [40] found that two-thirds of patients who presented in this way had a positive Mantoux test and some patients had granulomatous tissue inflammation, indicating an ability to produce a cellular immune response against MTB. The relationship with antiretroviral therapy was not discussed.

Temporal lobe encephalitis highly suggestive of HSV encephalitis occurred in a single patient. This is a very unusual occurrence in HIV-infected patients and has been associated with relatively conserved cell-mediated immunity [41]. Three other patients developed an encephalomyelitis or myelopathy. A patient with encephalomyelitis died, despite empirical broad spectrum antimicrobial therapy, and one of the patients with a myelopathy developed a severe spastic paraparesis which only resolved after the introduction of steroid therapy. All three patients who developed a myelopathy had an outbreak of mucocutaneous herpes which was the first for over a year and occurred despite increased CD4 T-cell counts. It is therefore possible that restoration of an immune response against HSV caused inflammation at sites of subclinical HSV infection in the spinal cord even though HSV rarely causes myelitis in HIV-infected patients [42]. As all patients with central nervous system disease after HAART had a baseline CD4 T-cell count < 50/uL, severe immunodeficiency may be a risk factor for this complication of successful HAART.

In summary, we have demonstrated that 25% of our cohort of patients who responded to HAART developed

disease episodes related to a pre-existent or subclinical infection by an opportunistic pathogen. We suggest that these disease episodes were a consequence of HAART-induced restoration of pathogen-specific immune responses and should therefore be considered as immune restoration disease rather than immunodeficiency disease. Patients with a CD4 T-cell count < 50/uL treated with a protease inhibitor are most at risk of developing such disease. Measurement of pathogen-specific immune responses in patients at risk of developing disease may differentiate immune restoration disease from immunodeficiency disease but prospective studies are required to test this proposal.

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