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Immune restoration disease in HIV-infected individuals receiving highly active antiretroviral therapy: clinical and immunological characteristics

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ABSTRACT

Background: HIV-infected patients responding to HAART can show a diverse spectrum of symptoms caused by inflammatory reaction. The pathogenesis of this phenomenon, called immune restoration disease (IRD), is unclear. This study describes the spectrum of IRD and analyses the immunological and clinical parameters that could be related to its development.

Methods: In a retrospective, matched case-control study, 17 HIV-infected individuals who developed inflammatory symptoms <12 months after initiation of HAART were included. HIV-infected controls were matched for age, gender and CDC classification. Factors included in the analysis were: CD4+ and CD8+ cell counts, ΔCD4+ and ΔCD8+, CD4/CD8 ratios, HIV-1-RNA load (VL), ΔVL and the number of CDC events prior to HAART.

Results: The median time after initiation of HAART and developing IRD (n=17) was 72 days (range 2-319). In nine cases (53%) a mycobacterial infection was identified as the underlying cause. HAART was started at a mean CD4+ count (± SD) of 55 x 10^6 /l (± 59) and 85 x 10^6 /l (± 78.0) for cases and controls, respectively (p=0.13). After initiation of HAART, the CD4+ count showed a 10.6 fold increase at the onset of IRD in the cases and a 2.7 fold increase in the controls in an equal period of time (p=0.020). The other parameters analysed did not differ significantly between cases and controls.

Conclusion: We conclude that the risk of developing IRD is associated with a high-fold increase in CD4+ lymphocytes. In this study, mycobacteria are the pathogens most frequently associated with IRD.

INTRODUCTION

The future prospects for HIV-infected patients have changed dramatically because of the effectiveness of highly active antiretroviral therapy (HAART). In the majority of patients, plasma viral load decreases and CD4+ lymphocyte count starts rising directly following initiation of HAART, resulting in a lower morbidity and mortality.[1-4] However, over the past five years, the occurrence of opportunistic infections and newly developed inflammatory reactions shortly after initiating HAART was observed.[5,6] The cases reported in the literature include reactivation of hepatitis B virus, cytomegalovirus (CMV) infection and predominantly mycobacterial infection.[7-10] These episodes of opportunistic disease usually occurred in the first months after HAART was started.[7,11] It is hypothesised that the infective

pathogens are present before HAART is initiated. The recovery of the immune system enables the inducement of a local or systemic inflammatory response to these micro-organisms. This phenomenon is generally referred to as the immune restoration syndrome (IRS) or immune restoration disease (IRD).[12-14] Due to its atypical presentation, the immune restoration syndrome may well be overlooked. Here, we describe the spectrum of the immune restoration syndrome in 17 HIV-infected individuals treated with HAART, along with the immunological parameters, in order to detect factors that are associated with the development of IRD. To illustrate various aspects of IRD, we first describe a characteristic case history.

CASE REPORT

A 31-year-old woman recently diagnosed with an asymptomatic HIV infection (CD4+ lymphocyte count of 4 x 10^6/l, HIV-RNA of 267,000 copies/ml), was treated with cotrimoxazole (480 mg once daily) and HAART (consisting of zidovudine, lamivudine and indinavir). There was no history of prior infection with mycobacteria and a chest X-ray was normal. Four months after initiation of therapy she developed night sweats, malaise, fever, weight loss, an enlarged lymph node in her neck and a productive cough. Sputum showed acid-fast rods. The X-ray of the chest and a CT scan revealed extensive mediastinal lymphadenopathy but no intrapulmonary abnormalities (*figure 1*). Although infection with *M. tuberculosis* could not be excluded at that moment, the clinical presentation was compatible with a nontuberculous mycobacterial infection. Initial treatment with isoniazid (INH), clarithromycin, ethambutol and rifabutin was prescribed. Eventually *Mycobacterium avium* complex (MAC) was cultured from the sputum and INH was stopped. At the time of the manifestation of MAC the CD4+ lymphocyte count had increased to 71 x 10^6/l and no HIV-RNA could be detected in the plasma. The emergence of the mycobacterial infection, accompanied with signs of inflammation (clinical symptoms and lymphadenopathy), within months after initiation of HAART points to immune restoration disease (IRD). HAART and antimycobacterial therapy were continued all through this episode. The patient's symptoms subsided within three weeks; eventually the lymphadenopathy resolved. Anti-MAC therapy was continued up to six months after the CD4+ lymphocyte count exceeded 200 x 10^6/l.

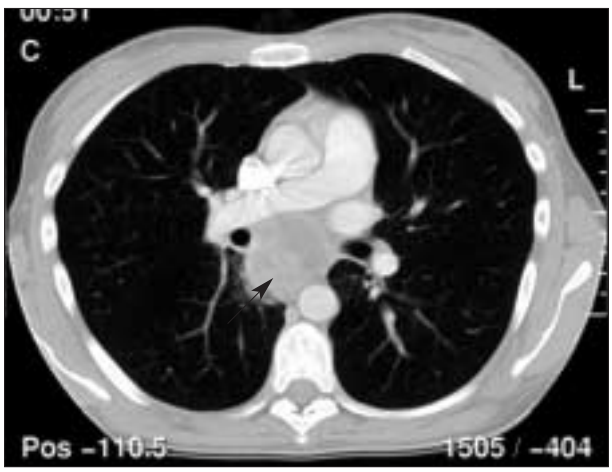

Figure 1
Slide of the thorax showing mediastinal lymphadenopathy (arrow)

PATIENTS AND METHODS

The study was conducted in the Leiden University Medical Centre (LUMC) and two large urban hospitals in The Hague, the Netherlands. In design, the research was a retrospective matched case-control study and the time frame covered the period between 1 January 1995 and 1 March 2001. For the purpose of this study, IRD was defined as the development of any inflammatory symptom, occurring within 12 months after initiation of HAART. As explained in the introduction of this article, it is hypothesised that underlying opportunistic infection causes these symptoms if combined with immune reconstitution. So, the presence of an opportunistic micro-organism also had to be confirmed or considered to be most likely.
Since the study was conducted in retrospect, the clinicians had to review their patient population carefully to detect each suspected case of IRD conform the definition mentioned above.
Exclusion criteria were the evidence of noncompliance to antiretroviral therapeutics or incomplete patient data. The control group was selected from the database of the LUMC of the HIV-infected individuals treated with HAART.
For each individual in the case group, one or two matched controls were included. Cases were matched for age, gender and CDC classification. The controls also received HAART. If two suitable controls were found, the mean outcome of the comparison was used.
Medical history, clinical data, CDC classification, gender, age, past and present treatment conditions were obtained from the patient files. To calculate the 'fold increase', i.e. relative increase in CD4+ lymphocytes for each individual, the number of CD4+ cells at a certain time point after initiation of HAART was divided by the number of CD4+ cells at the start of HAART. HAART was defined as combination antiretroviral therapy containing at least three antiretroviral drugs, which include at least a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI). The diagnosis 'infection' was made on

the basis of the clinical presentation and if the presence of the micro-organism was confirmed by culture, PCR, serology or direct microscopy. The time to onset of opportunistic infection is defined as the time from the moment of initiation of HAART to the first onset of clinical symptoms of IRD.
The CD4+ and CD8+ lymphocyte counts were determined by use of a direct immuno-fluorescence technique (Becton-Dickinson, San José, California, USA and flow cytometry (Facscan, Becton-Dickinson, Immunocytometry Systems). Quantification of HIV1-RNA load in plasma was performed using a quantitative polymerase chain reaction (RT-PCR) assay (AMPLICOR-HIV monitor test, Roche Molecular Systems, Branchburg, USA).
The immunological data are expressed as mean (± SD) and median values. Differences between both groups were statistically evaluated using the Student's t-test for paired samples, Levene's test for equality of variances and the Wilcoxon ranking test.

RESULTS

Seventeen cases out of a population of approximately 700 HIV-infected individuals receiving HAART were included. The main case characteristics are shown in *table 1*. Twenty patients were selected as matched controls by the conditions described above. The median time after initiation of HAART and IRD was 72 days. When HAART was started the mean CD4+ lymphocyte counts (± SD) were 55 x 10^6 /l (± 59) and 85 x 10^6 /l (± 78) for cases and controls respectively (p=0.13). At one month and two months after initiation of HAART there were no significant differences in absolute increase nor in fold increase in CD4+ lymphocytes.
The mean number of CD4+ lymphocytes at the onset of IRD in the case group and, in the comparable period of time, in the control group were 220 x 10^6 /l and 186 x 10^6 /l, respectively.
However, the CD4+ count after initiation of HAART to the onset of IRD showed a 10.6 fold increase (SE mean=3.28) in the cases and a 2.7 fold increase (SE mean=0.91) in the controls in matched period of time (p=0.020).
Neither the increase in CD8+ lymphocytes, the CD4/CD8 ratios nor the decrease in viral load was significantly different between cases and controls. The average number of CDC events prior to starting HAART was 3.2 for the cases and 1.6 for the controls (p=0.14).

DISCUSSION

This study demonstrates that IRD occurred predominantly within three months after initiation of HAART and, in our population of HIV-infected individuals, is most frequently (>50%) associated with mycobacterial infection.

Table 1

CASES	GENDER	AGE*	HAART	CDC#	CD4+ X 10^6 /L AT ONSET OF HAART	DAYS TO IRD	CD4+ X 10^6 /L AT ONSET OF IRD	PATHOGEN
1	M	44	3TC, AZT, indinavir	C2	110	132	210	*M. tuberculosis*€
2	M	42	3TC, d4T, indinavir	C3	30	185	360	*M. tuberculosis*€
3	F	28	d4T, 3TC, saquinavir, ritonavir	C3	21	215	518	*M. tuberculosis*€
4	M	36	3TC, d4T, nelfinavir	C3	2	20	50	*M. avium*€
5	M	33	d4T, 3TC, ritonavir, saquinavir	C3	10	34	180	*M. avium*
6	M	26	3TC + AZT, nelfinavir	C3	40	51	90	*M. avium*
7	M	31	3TC, d4T, ritonavir, saquinavir	C3	43	58	533	*M. avium*€
8	F	30	AZT, 3TC, indinavir	C2	43	150	54	*M. avium*
9	M	26	Ritonavir, amprenavir, 3TC, efavirenz, abacavir	C3	19	86	105	*M. xenopi*€
10	F	51	3TC, d4T, ddI, ritonavir	C3	104	8	141	*P. carinii*
11	M	57	3TC + AZT, nelfinavir	C3	220	17	250	*P. carinii*
12	M	36	AZT, 3TC, indinavir	C3	43	35	590	Toxoplasma
13	M	54	AZT, 3TC, indinavir	C3	6	137	59	HZV (disseminated)
14	M	62	3TC, AZT, ritonavir, saquinavir	C3	3	319	151	HZV (disseminated)
15	M	55	3TC, d4T, nelfinavir	C3	74	72	196	HBV
16	M	48	ddI, AZT, ritonavir	B3	20	82	96	HBV
17	M	32	3TC, d4T, ritonavir, saquinavir	C3	144	24	162	Viral meningitis‡

**Age at the moment of starting HAART, #CDC classification 1993, €extrapulmonary, ‡evident clinical picture, no pathogen identified, M = male, F = female, 3TC = lamivudine, AZT = zidovudine, ddI = didanosine, d4T = stavudine, 3TC + AZT = Combivir. HZV = herpes zoster virus, HBV = hepatitis B virus.*

Furthermore, the IRD cases demonstrated a significantly higher fold increase in CD4+ lymphocytes compared with controls. There was no association with baseline or absolute increase of CD4+ lymphocytes, CD8+ lymphocytes, or decrease in HIV1-RNA load.

The principal idea regarding the development of IRD is that the recovery of the immune system enables a response to a residing, but clinically silent, opportunistic infection. The significantly higher increase in CD4+ lymphocytes in the cases compared with the controls, observed in our study, suggests a relation with the occurrence of IRD. The exact nature of this relation now remains unclear. Restoration of pathogen-specific lymphoproliferative response (LPR) to recall antigens in the first months following HAART has been described earlier.[3,15-17] This increase in potency of an immunological response to recall antigens evidently correlated with the increase in CD4+ T lymphocytes.[3] However, it is also advocated that IRD reflects a pathogen-specific immune response but that a specific (Th2) cytokine environment is a critical factor in the development of IRD and a large redistribution of antigen specific T cells is not.[18-21]

The precise role of interleukin 6 and of associated HLA haplotypes are currently under investigation.[22,23]

Our results confirm the observation that mycobacterial infection is frequently associated with IRD.[8,11,24] *In vitro* experiments show that, in mycobacterial-related IRD, mycobacterial-specific lymphocyte reactivity can be induced by the presence of antigen.[14] In our study *M. avium*-related IRD occurred on average at a lower CD4+ cell count compared with *M. tuberculosis*-related IRD, 63 and 177 x 10^6/l respectively. In progressive HIV-1 infection with decreasing CD4+ T-cell numbers, *M. avium* is (on average) more frequently found at lower CD4+ T-cell counts than *M. tuberculosis*. The opposite is true for the situation where the CD4+ T-cell counts are rising, likely reflecting a difference between the complexity of the immune response to both pathogens.

The difference in the number of CDC events and the slightly lower mean number of CD4+ lymphocytes, prior to effective HAART in the IRD cases compared with the controls, probably merely reflects that a longer period of immunodeficency also predisposes to the development of IRD.

This finding could reflect a higher cumulative risk, or a higher antigen load, when the period of severe immune deficiency is longer.

Given the above and presuming that, in IRD, present asymptomatic opportunistic infection is unmasked by restoring immunity, both the restoration of immune reactivity (specific T-cell response and/or primary cytokine related) and antigen are the main interacting components. In future research the restoration of specific immune responses in combination with host-antigen interaction during and before starting HAART have to be studied in order to elucidate the exact pathogenesis of IRD.

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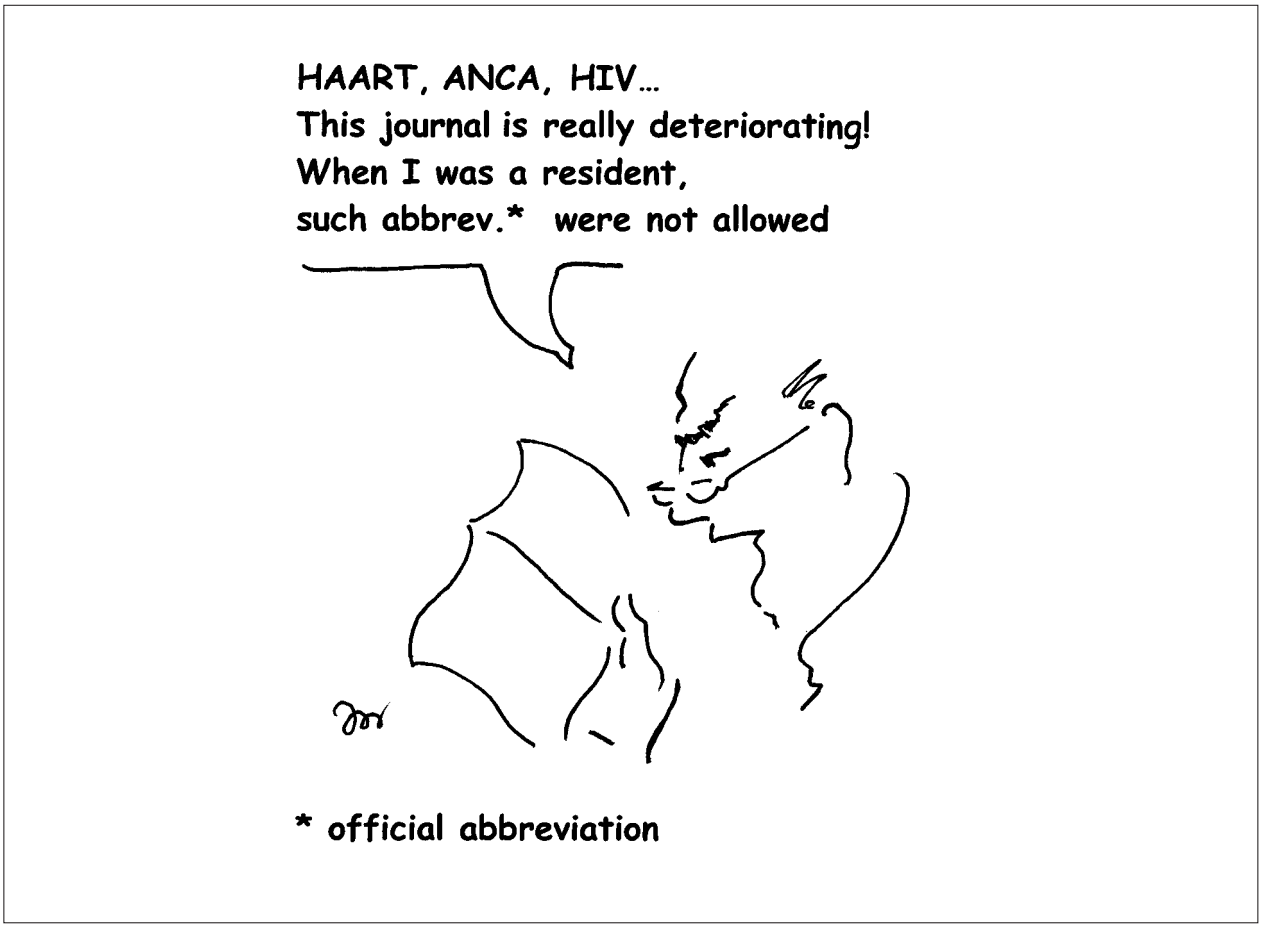

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