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Article *in* Clinical Immunology · November 2010

DOI: 10.1016/j.clim.2010.07.003 · Source: PubMed

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Clinical Immunology (2010) 137, 199–208

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Extremely prolonged HIV seroconversion associated with an MHC haplotype carrying disease susceptibility genes for antibody deficiency disorders

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Received 11 May 2010; accepted with revision 16 July 2010
Available online 8 August 2010

KEYWORDS
HIV;
Seroconversion;
CVID;
Pneumocystis jirovecii

Abstract Severe immunodeficiency during primary human immunodeficiency virus (HIV) infection is unusual. Here, we characterized viral and immunological parameters in a subject presenting with *Pneumocystis jirovecii* pneumonia in the setting of prolonged primary HIV illness and delayed seroconversion. HIV antibody was only detected by enzyme-linked immunosorbent assay 12 months after presentation, and Western blot profiles remain indeterminate. Isolated virus was of R5 phenotype, exhibited poor viral fitness, but was otherwise unremarkable. Analysis of HIV antibody isotypes showed failure to mount a detectable HIV IgG response over nearly 2 years of infection, in particular IgG_1- and IgG_3-specific responses, despite normal responses to common infections and vaccines. Genetic analysis demonstrated homozygosity for part of an MHC haplotype containing susceptibility genes for common variable immunodeficiency (CVID) syndrome and other antibody deficiency disorders. Thus, a primary disorder of specific antibody production may explain exceptionally slow antibody development in an otherwise severe

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1521-6616/$ – see front matter
doi:10.1016/j.clim.2010.07.003

seroconversion illness. This highlights the need for multiparameter testing, in particular use of a fourth generation HIV test, for confirming HIV infection and underscores the importance of host factors in HIV pathogenesis.

Introduction

Infection with human immunodeficiency virus (HIV) frequently causes seroconversion illness characterized by fever, rash and lymphadenopathy, usually lasting less than 2 weeks although durations of up to 10 weeks have been reported [1]. Modest declines in CD4+ T-cell count during seroconversion are common but only 7% of seroconverters have CD4+ T-cell counts below 300 cells/μl [1], and severe immunosuppression is unusual. Consequently, AIDS defining illnesses (ADIs) are rare during seroconversion. However, approximately 2% of patients experience esophageal candidiasis [2], and ADIs including *Pneumocystis jirovecii* pneumonia [3], cryptosporidiosis [4], herpes oesophagitis [5] and cytomegalovirus colitis [6] have been reported during seroconversion. In addition, HIV-related complications can be present such as retinopathy [7], nephropathy [8] and neurological problems including meningoencephalitis, Guillain–Barre syndrome and cranial nerve palsies [1].

Antibody tests for HIV are extremely sensitive, detecting most infections within 4 weeks of infection [9] and essentially all by 3 months [10]. We previously described a subject with Kaposi's sarcoma who remained HIV seronegative for >4 years by conventional testing despite seropositivity to HIV gp41 and Nef by enzyme-linked immunosorbent assay (EIA) [11]. We now report a 45-year-old man who experienced atypical seroconversion illness with *P. jirovecii* pneumonia and exceptionally slow HIV seroconversion. We undertook a series of virological, immunological and genetic studies to better understand the role of the virus and host contributing to rapid disease progression and atypical seroconversion in this subject.

Materials and methods

Ethics statement

Written informed consent was obtained from the subject, and experiments were conducted according to guidelines endorsed by the Monash Medical Center institutional review board which approved this study, and guidelines described in the Australian National Statement on Ethical Conduct in Research Involving Humans. The study subject has read the manuscript and gave written consent for publication.

Western blot and isotype-specific EIA

Isotype-specific Western blots and anti-p24 and anti-gp120 isotype-specific EIA's were performed as described previously [12]. For the anti-gp120 EIA, highly purified HIV_{SF2} gp120 derived from CHO cells was used [13].

Viral load testing

Viral load testing was performed on fresh and stored (−70 °C) plasma using the COBAS Amplicor HIV-1 Monitor assay™ version 1.5 (Roche Diagnostics, Branchburg, NJ, U.S.A.) according to manufacturer's instructions or the ExaVirLoad assay version 2 (Cavidi Tech, AB) as described previously [14].

Isolation of HIV

HIV was only isolated from the patients peripheral blood mononuclear cells (PBMC) from blood taken in January 2006 (Tables 1 and 2), using standard coculture techniques [15,16]. Both PBMC and plasma were tested. Virus stocks were quantified by determining the 50% tissue culture infectious dose ($TCID_{50}$) [17] in donor PBMC using HIV p24 as a measure of virus replication [18], and stored at −70 °C.

Virus neutralization assay

The primary virus isolate and a reference CCR5-using (R5) subtype B strain HIV-1_{MBC925} [19] were tested for sensitivity to neutralization by HIV envelope monoclonal antibodies IgG1b12, 2G12, 4E10 and 2F5, polyclonal HIV Ig and autologous patient sera or plasma, as described previously [20] with minor modifications. The modifications were that HIV p24 antigen production was used to titrate virus stocks and measure virus production in neutralization assays, the viral inoculum was between 10 and 100 $TCID_{50}$ and cells were washed with phosphate buffered saline following infection. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis of virus neutralization curves.

Viral fitness assay

Viral fitness was tested by measuring the kinetics of total HIV DNA production (Arnott et al., in preparation). Briefly, 2×10^5 PHA-activated PBMCs were inoculated with equivalent amounts of HIV (600 pg p24 antigen). Virus stocks were harvested during exponential growth to minimize the contribution of soluble CD4. Samples were harvested at the indicated times post-infection, DNA extracted, and total HIV DNA production was measured using a quantitative real-time PCR assay targeting the HIV long-terminal repeat (LTR). Reference strains HIV-1_{MBC925} and HIV-1_{D36} [19,20] were used as controls.

IgG isotype testing

Total IgG was measured by rate immunoturbidimetry (Abbott Architect, Abbott Park, IL) and IgG subclasses by rate immunonephelometry (The Binding Site, San Diego, CA).

Table 1 Western blot and p24 antigen EIA results over time.

Date of bleed	HIV EIA antibody testing	HIV WB antibody testing	HIV EIA antigen testing	Viral load (Log_{10} RNA copies/ml)
29 July 2005	Not detected—HIV-1/2 assay [a]	Not tested	Not tested	
18 August 2005	Not detected—HIV-1/2 assay [b]	Not tested	Positive [e,f] (S/CO 21.0)	
1 October 2005	Not detected—HIV-1/2 assay [b]	Not tested	Positive [e] (S/CO 10.2)	
8 October 2005	Not detected—HIV-1/2 assay [b]	Indeterminate—GPIII (1+, p24 band only) [c,d]	Positive [e] (S/CO 11.2)	>5
29 November 2005	Not detected—HIV-1/2 assay [b]	Indeterminate—GPIII (1+, p24 band only) [c,d]	Positive [e] (S/CO 21.0)	5.0
25 January 2006	Not detected—HIV-1/2 assay [b]	Negative (no p24 band) [c]	Not tested	3.6
28 March 2006	Detected—HIV-1/2 assay (S/CO 4.0) [b]	Indeterminate—GP IV (1+ gp160 band) [c]	Not tested	2.6
25 June 2006	Detected—HIV-1/2 assay (S/CO 11.9) [b]	Indeterminate—GPIV (2+ p24, 1+ gp120, 3+ gp160 bands) [c]	Not tested	3.0
15 August 2006	Detected—HIV-1/2 assay (S/CO 12.6) [b]	Indeterminate—GP IV (1+ p17, 2+ p24, 1+ gp120, 3+ gp160 bands) [c]	Not tested	1.7
5 November 2006	Detected—HIV-1/2 assay (S/CO 16.8) [b]	Indeterminate—GP IV (1+ p17, 3+ p24, 2+ gp120, 4+ gp160 bands) [c]	Not tested	<1.7
1 February 2007	Detected—HIV-1/2 assay (S/CO 14.3) [b]	Indeterminate—GP IV (1+ p17, 3+ p24, 2+ gp120, 4+ gp160 bands) [c]	Not tested	2.2
4 December 2007	Detected—HIV-1/2 assay (S/CO 13.1) [b]	Indeterminate—GP IV (2+ p17, 3+ p24, 2+ gp120, 3+ gp160 bands) [c]	Not tested	<1.7
7 September 2008	Detected—HIV-1/2 assay (S/CO 13.3) [b]	Indeterminate—GP IV (2+ p17, 3+ p24, 2+ gp120, 3+ gp160 bands) [c]	Not tested	<1.7

S/CO: specimen-to-cutoff ratio
Antiretroviral therapy was commenced 22 October 2005.
HIV-1 was isolated from blood taken on 25 January 2006.
[a] Abbott AxSym assay.
[b] Murex Ab assay.
[c] MP Diagnostics HIV blot.
[d] p24 band considered non-specific reactivity.
[e] Genetic systems HIV-1 Ag.
[f] Tested retrospectively.

HIV genotyping

HIV genotype analysis was performed on plasma using the Viroseq® HIV-1 Genotyping system version 2.6 [21].

Humoral response to influenza and hepatitis A and B vaccines

The subject was injected subcutaneously with 0.5 ml of Vaxigrip influenza vaccine (Sanofi Pasteur), and antibody response determined using an in-house complement fixation test at 0, 1 and 5 weeks post-vaccination. The subject was injected intramuscularly with 1 ml of Twinrix inactivated hepatitis A vaccine (720 ELISA units) and recombinant hepatitis B surface antigen (20 g) (Glaxo SmithKline) at 0, 3 and 13 months, and antibody production (Abbott, Architect) determined at 3 months after the 3rd dose.

CCR5 and Class I and II MHC genotyping

Chemokine receptor gene polymorphisms were determined using multiplex PCR as described previously [22]. HLA typing was undertaken by DNA sequencing as described previously [23].

Results

Clinical description

The subject is a 45-year-old man who presented initially in March 2005 with fever, sweats and severe sore throat and was prescribed amoxicillin/clavulanic acid, then amoxicillin, without benefit. Additional symptoms of lethargy and nausea ensued. Blood tests in June 2005 showed mild neutropenia (1.6×10^9/l), lymphopenia (0.8×10^9/l) and thrombocytopenia (141×10^9/l) and mild transaminitis (ALT 72 U/l, AST 54 U/l). Over 4 months of illness the subject experienced weight loss of

Table 2 Virus Neutralization results over time compared with control antibodies.

Sample type	Date of bleed	CD4+ T-cell count (cells/μl) [a]	Viral load (Log_{10} RNA copies/ml) [b]	Antiretroviral therapy [c]	Virus tested [d]	Neutralisation Titer (IC_{50}) [e]
Serum	8/10/05	29 (ex 3/10/05)	>5	None	HIV-1_{MBC925}	0 (ADE [f])
Plasma	25/1/06	429	3.6	AZT, 3TC, EFV	HIV-1_{MBC925}	150
Serum	24/5/07	392 (ex 1/2/07)	2.2 (ex 1/2/07)	AZT, 3TC, EFV	HIV-1_{MBC925}	150
					HIV-1_{FRC}	>400
HIV Ig	NA [g]	NA	NA	NA	HIV-1_{FRC}	40 μg/ml
2G12	NA	NA	NA	NA	HIV-1_{FRC}	2.5 μg/ml
IgG_1b12	NA	NA	NA	NA	HIV-1_{FRC}	0 (ADE)
4E10	NA	NA	NA	NA	HIV-1_{FRC}	2.5 μg/ml
HIV neg	NA	NA	NA	NA	HIV-1_{FRC}	0 (ADE)

Dates shown are day/month/year.
[a] CD4+ T-cell levels were measured by flow cytometry.
[b] Plasma HIV-1 RNA was measured using the COBAS AMPLICOR monitor version 1.0 (Roche Molecular Diagnostic Systems, Branchburg, NJ, USA) prior to July 1999 and version 1.5 after July 1999. HIV-1 RNA levels of <400 copies/ml (Version 1.0) or <50 copies/ml (Version 1.5) were considered below detection, BD.
[c] AZT = zidovudine, 3TC = lamivudine and EFV = Efavirenz.
[d] Reference clade B strain and FRC represents the patient isolate cultured from PBMC taken on 25/1/06 as described in the "Materials and methods".
[e] IC_{50} values of patient samples or reference antibodies are expressed as the reciprocal of sample dilution or concentration required to inhibit 50%, respectively.
[f] Antibody dependent enhancement, ADE.
[g] NA = not applicable.

25 kg. Examination by a gastroenterologist in July 2005 revealed an emaciated man with dry skin and no rash, mild hypotension and left axillary lymphadenopathy. There was no evidence of oral candidiasis or hepatosplenomegaly. At this time mild pancytopaenia was again noted, there was evidence of a generalized inflammatory reaction on serum protein electrophoresis and ferritin was raised (3330 μg/l). The subject denied HIV risk factors, and serological testing for HIV antibodies by EIA was negative in July 2005.

In August 2005, chest X-ray (CXR) showed minor changes consistent with bronchitis. Gastroscopy showed patatal ulcers, and biopsies of these were non-diagnostic on histology. Bone marrow biopsy showed non-specific changes consistent with reactive haematopoiesis, although flow cytometry of bone marrow cells showed an inverted CD4: CD8 ratio of 1:3.

In September 2005, with further 5 kg weight loss, fever, night sweats, dry cough and mild delirium, CXR revealed bilateral interstitial infiltrates, and *P. jirovecii* pneumonia was confirmed by microscopy on bronchoscopy samples. He responded well to treatment with 3 weeks of high dose oral co-trimoxazole and prednisolone.

In October 2005, HIV antibody testing was again negative, but a positive qualitative p24 antigen EIA (Table 1) and plasma viral load of >100,000 copies/ml confirmed HIV infection. CD4+ T-cell count was 52 cells/μl. The subject subsequently tested negative for hepatitis B surface antigen and antibody and was negative for hepatitis C antibody. CMV IgG was positive but IgM was negative. He commenced antiretroviral therapy (ART) consisting of zidovudine, lamivudine and efavirenz in October 2005. A rapid response to therapy was achieved, but the patient's viral load rarely reached undetectable levels (<50 RNA copies/ml) over the next 2 years, despite self-reported compliance (Fig. 1).

The subjects only prior history was of a tonsillectomy as a child. He had ceased smoking 20 years prior, and reported occasional excessive alcohol intake, but denied any illicit drug use. He lives with a female partner who remains well, has no identifiable risk factors and who has subsequently tested negative for HIV. The subject later admitted his likely source of infection was infrequent sexual contact with men.

Prolonged and indeterminate antibody responses by EIA and WB confirmed by p24 antigen testing

Routine HIV antibody testing (EIA) was negative on 6 occasions between July 2005 and January 2006, and first became positive in March 2006, approximately 12 months after presentation and 6 months after *P. jirovecii* pneumonia diagnosis (Table 1). Antibody reactivity by Western blot was extremely weak and showed very slow development, from a single p24 band in October 2005 to 4 bands (p18, p24, gp120, gp160) but still indeterminate according to the stringent criteria used in Australia [24], at the most recent Western blot, 42 months after presentation (Table 1). Similar reactivity was observed using in-house Western blots with total IgG secondary antibodies, but IgG_1 and IgG_3 HIV antibodies were absent at all times (data not shown).

To further investigate the lack of IgG_1 and IgG_3 antibody reactivity, we next determined early antibody responses by IgG isotype reactivity to p24 and gp120 as described previously [12]. Anti-p24 and anti-gp120 antibody responses were detected by total IgG, IgM and IgA initially, but diminished within 3 months (Fig. 2). This may have been due to the subject commencing therapy in October 2005, shortly after testing commenced. In contrast, anti-p24 and anti-gp120 IgG_1 and IgG_3 responses were not detected over

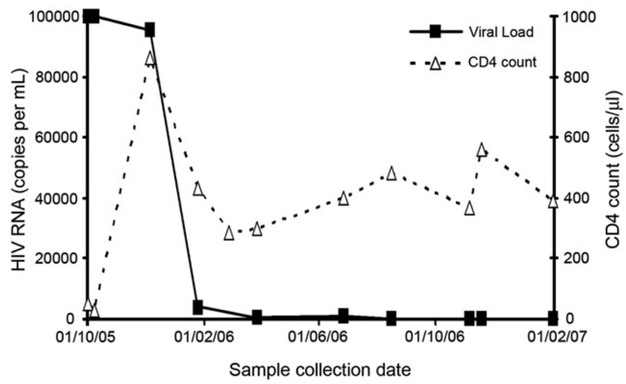

Figure 1 Changes in viral and immunologic parameters over time. Time course from presentation with *P. jirovecii* pneumonia (1/10/2005), showing plasma viral load (HIV RNA copies/ml) (solid line) and CD4 cell counts (dotted line). Viral load testing was performed on fresh and stored (−70 °C) plasma using the COBAS Amplicor HIV-1 Monitor assay™ version 1.5 (Roche Diagnostics, Branchburg, NJ, U.S.A.) according to manufacturer's instructions, or the ExaVirLoad assay version 2 (Cavidi Tech, AB) as described previously [14]. The subject commenced ART 4 weeks later with a regime of AZT, 3TC and Efavirenz. The dates shown are day/month/year.

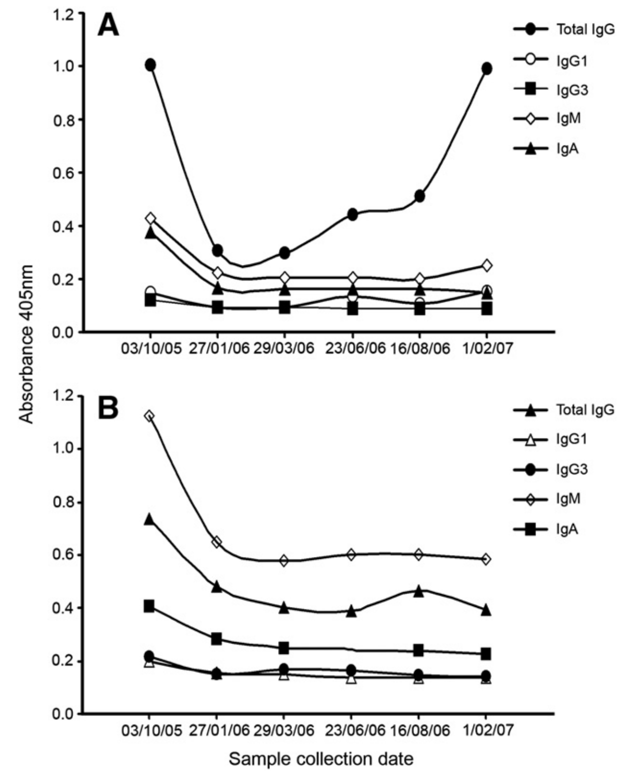

Figure 2 Detection of anti-p24 and anti-gp120 IgG, IgG_1, IgG_3, IgM and IgA antibodies by EIA over time. Antibody responses against purified HIV p24 (A) and gp120 (B) antigens were measured by EIA as described in "Materials and methods". The absorbance values obtained at 405 nm were plotted against the date on which plasma samples were taken. The dates shown are day/month/year.

the period tested (Fig. 2), confirming the Western blot data (data not shown). From the anti-p24-specific antibodies total IgG but not IgG_1 was detected by February 2007, as for the Western blot reactivity. Interestingly, anti-p24 and anti-gp120 total IgG antibodies were detected for the first time point tested along with IgM responses, but this was not reflected in the Western blot data (data not shown). Overall, the antibody responses were very weak compared with typical seroconversion to HIV [12]. HIV EIA p24 antigen testing, both retrospective (where samples were available) and coincident, confirmed infection (Table 1).

HIV sequence analysis

To better understand the nature of the viral quasispecies, phylogenetic analysis of concatenated *env* and *gag* sequences derived from each of the patient samples was conducted which formed a distinct monophyletic cluster (data not shown). Furthermore, genescan analysis [25] on the same samples showed homogeneity based on V1V2 length polymorphisms (data not shown). Thus, minimal virus evolution occurred between August 2005 and June 2006. HIV genotyping performed on virus present in January 2006, after commencement of ART (using the Viroseq® HIV-1 Genotyping system version 2.6 [21]), displayed minor NRTI (L210F) and PI (A71V) mutations.

Neutralization of the patient isolate by reference antibodies and patient sera

Despite the weak antibody responses overall, the ability of patient sera and reference monoclonal antibodies to neutralize a reference strain and a virus strain isolated from the subject (using standard coculture techniques [15,16]) was measured using a PBMC-based neutralization assay based on a method described previously [20]. Analysis of coreceptor usage in Cf2-Luc indicator cells [16,26] showed that the patient isolate was CCR5-restricted (data not shown). Patient sera from selected time points pre- and post-ART were tested against the reference clade B strain HIV-1_{MBC925} (Table 2). The pre-ART sera enhanced viral replication by 2- to3-fold. It is generally non-neutralizing antibodies that enhance infection [27]. However, both plasma/sera collected post-ART neutralized viral replication with IC_{50} dilutions of 1:150. Serum collected 22 months after initial presentation demonstrated homologous neutralizing antibody responses with an $IC_{50} > 1{:}400$. While unlikely, AZT, 3TC or Efavirenz could not be excluded as contributing to the inhibition observed at 1:400 (Ho, J. and McPhee, D., unpublished data).

Results of the preceding studies were surprising given the overall antibody responses to HIV were extremely weak. This prompted further study of neutralization of the primary virus by reference antibodies. Monoclonal antibodies 4E10 (IC_{50} = 2.5 µg/ml) and 2G12 (IC_{50} = 2.5 µg/ml) and polyclonal HIV Ig (IC_{50} = ~ 40 µg/ml) were able to neutralize replication of the patient isolate (Table 2). However, IgG_1b12 did not demonstrate significant neutralization and in fact showed enhanced viral replication at some concentrations tested. Collectively, these results indicate that the patient developed a neutralizing antibody response against HIV-1 and that there were homologous neutralizing antibodies present.

The patient isolate has poor replication fitness

In the neutralization assays it was apparent that the patient virus was able to replicate only weakly in PBMC (data not shown). Thus, we next tested viral fitness by assessing the time to viral DNA production, and relative amounts of HIV DNA produced. Testing in parallel was designed to detect even the most attenuated virus infections. Replication of the primary virus was compared with a reference wild-type virus (HIV-1_{MBC925}) and a Nef-defective attenuated virus (HIV-1_{D36}) [20]. Infection with the reference wild-type virus showed a very low DNA copy number initially post-infection but this expanded by 5 $\log_{10}$ over the 158 h followed (Fig. 3). Direct comparison of viral DNA production shows the patient isolate is markedly attenuated relative to the reference strain tested and had delayed replication kinetics compared to a well-characterized attenuated viral strain (Fig. 3).

Immunoglobulin analysis

Given the impaired HIV-specific IgG_1 and IgG_3 antibody responses, we next tested measured total IgG and IgG subclass levels in blood drawn in October 2005, using rate immunoturbidity (Abbott Architect, Abbott Park, IL) and rate immunonephelometry (The Binding Site, San Diego, CA), respectively. Total IgG and IgG subclasses were all within normal ranges (NR). Total IgG was 7.7 g/l (NR 7.0-16.5), IgG_1 was 4.6 g/l (NR 3.8-9.3), IgG_2 was 2.8 g/l (NR 2.4 – 7.0), IgG_3 was 0.46 g/l (NR 0.22 – 1.76) and IgG_4 was 0.04 g/l (NR 0.04–0.86). However, given the subject was HIV positive at the time of testing, these levels could be considered low as serum IgG levels typically increase with HIV infection (17.2 g/l compared with 9.45 g/l in HIV negative subjects) [28].

Antibody responses to influenza, hepatitis A and hepatitis B vaccines

The subject was injected subcutaneously with 0.5 ml of Vaxigrip influenza vaccine (Sanofi Pasteur), and antibody response determined using an in-house complement fixation test at 0, 1 and 5 weeks post-vaccination. The subject was also injected intramuscularly with 1 ml of Twinrix inactivated hepatitis A vaccine (720 ELISA units) and recombinant hepatitis B surface antigen (20 g) (Glaxo SmithKline) at 0, 3 and 13 months, and antibody production (Abbott, Architect) determined at 3 months after the 3rd dose. Influenza A and B antibody titres were undetectable prior to vaccination, but low levels (titer of 20) were detectable at 1 and 5 weeks post-vaccination, which is typical in subjects receiving influenza vaccine. After 3 doses of combined hepatitis A and B vaccine the patient had detectable hepatitis A total antibody, and a hepatitis B surface antibody titer of 258.7 IU. Thus, the subject was able to mount humoral responses to these viral antigens.

CCR5 and HLA typing

We next undertook genetic analyses to better understand host factors which may have contributed to the subject's atypical seroconversion and rapid progression, as described previously [22,23]. Genetic studies revealed homozygosity for the CCR5 wt/wt genotype, and that he carried human leucocyte antigen (HLA) alleles HLA-A*02, -B*44, -C*05, -DRB1*04, -DRB1*15 and -DQ*0602 alleles. Other alleles were excluded, indicating homozygosity for a major histocompatibility complex (MHC) haplotype that contains susceptibility genes for common variable immunodeficiency disorder (CVID) and other antibody deficiency disorders [29].

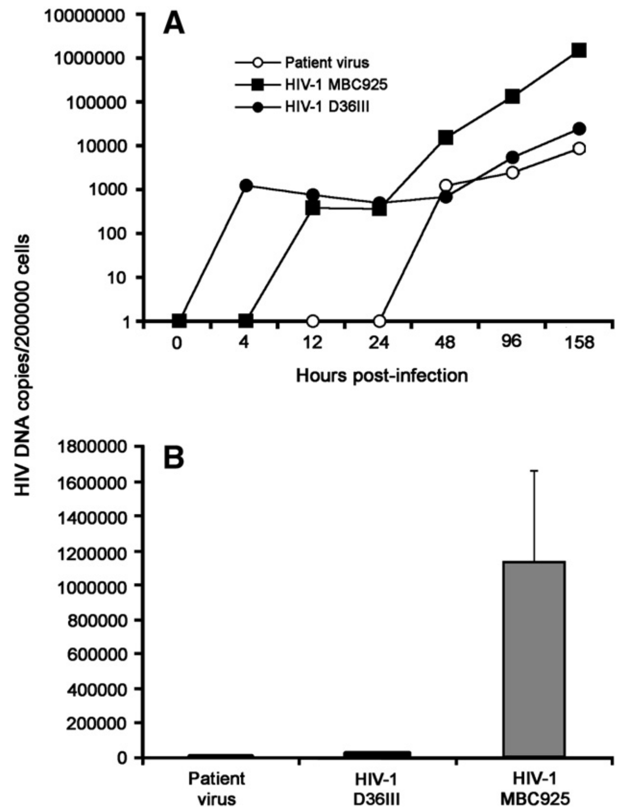

Figure 3 Testing viral fitness relative to wild-type and attenuated strains. Viral fitness was tested by measuring the kinetics of total HIV DNA production (Arnott et al., in preparation). Briefly, 2×10^5 PHA-activated PBMCs were inoculated with equivalent amounts of HIV (600 pg p24 antigen). Virus stocks were harvested during exponential growth to minimize the contribution of soluble CD4. Samples were harvested at the indicated times post-infection, DNA extracted, and total HIV DNA production was measured using a quantitative real-time PCR assay targeting the HIV long-terminal repeat (LTR). Reference strains HIV-1_{MBC925} and HIV-1_{D36} [19,20] were used as controls. (A) Replication in PHA-activated PBMC was measured by HIV DNA production by the reference isolate HIV-1_{MBC925}, the attenuated (*nef*-deleted) isolate HIV-1_{D36} and virus isolate from the subject at the indicated times post-infection. (B) HIV DNA production at 158 h post-infection. Minor background levels of HIV DNA at time zero, representing products of intravirion reverse transcription, were subtracted from values obtained from subsequent time points. Error bars represent SEM.

Discussion

We describe a patient with *P. jirovecii* pneumonia in the setting of a prolonged primary HIV illness and an exceptionally long HIV seroconversion (over 12 months), who was homozygous for part of an MHC haplotype that contains susceptibility genes for CVID. More prompt p24 antigen and viral load testing would have diagnosed this infection earlier, in particular the sensitive 4th generation HIV tests currently available for screening [30,31]. HIV WB reactivity showed very slow development, with a single p24 band in October 2005 to 4 bands (p18, p24, gp120 and gp160) but still indeterminate 42 months later. Collectively, the CD4 cell counts, WB reactivity, VL data and p24 antigen results all point to an early infection as opposed to a late stage infection [32] or seroreversion [33]. Additionally, serodiagnosis from chronic or late stage infection almost always includes reactivity against an immunodominant gp41 region, originally described by Wang et al. [34]. This highly conserved region of gp41 is included in any current 3rd or 4th generation EIAs. Hence, the results from longitudinal testing showing negative to clearly positive EIA reactivity (Table 1) would preclude this individual being in the chronic or late stage of infection. Virus was isolated on one occasion during ART and showed poor replicating fitness. The presence of anti-HIV neutralizing antibodies following commencement of ART was unexpected given the overall weak antibody responses. The presence of antiretrovirals cannot be excluded from contributing to inhibition of viral replication at high dilutions of plasma. Purification of IgG antibodies would exclude this.

A number of causes of false negative HIV antibody results have been described. These include testing within the window period [35], non-clade B virus infection [10,36], HIV-2 infection [10], end-stage AIDS [37] and seroreversion on early ART [38] as well as defects in humoral immunity like agammaglobulinemia and CVID [39]. The individual studied does not fall into any of these categories and may represent a rare case where multiple factors could be contributing; specifically, an unusual genetic background, a highly pathogenic virus with low *ex vivo* fitness and commencing ART prior to seroconversion. Given that the patient's total IgG levels after infection were low and IgG levels are higher than normal in HIV-infected individuals [28,40], this suggests low IgG levels but not agammaglobulinemia (7.7 compared with 2.1 g/l) [41].

The extremely slow antibody development in an otherwise severe seroconversion illness may have been due to a primary disorder of antibody production. HLA typing showed the patient was homozygous for part of an MHC haplotype that contains susceptibility genes for CVID, a condition associated with false negative HIV testing [39]. Our patient did not have the typical immunological findings of CVID [41], as there was a normal initial immunoglobulin profile, including IgG subclasses, and he was able to produce antibodies to CMV and to influenza and hepatitis A and B vaccines. However, carriage of HLA-B44 has been associated with a broader range of antibody deficiency disorders including IgG subclass deficiency and recurrent sinopulmonary infections with normal serum immunoglobulin levels [29,42]. Homozygosity for one or more alleles on the MHC haplotype containing HLA-B44 and -DR7 might therefore have resulted in a blunted antibody response to HIV, with failure to mount a specific detectable IgG_1 and IgG_3 response over nearly 2 years of illness despite detectable neutralizing antibody responses. This was complicated by the introduction of ART which may have further delayed immune responses.

It is also well recognized that patients with primary immune deficiencies have heterogeneous clinical and immunologic features that may reflect specific immune deficiencies within these conditions. While antibody responses to vaccines and pathogens can be variable and unpredictable among individuals with primary immune deficiencies such as CVID, these patients often have severely blunted antibody responses to polysaccharide antigens (such as pneumococcal vaccine) while maintaining responses to protein-peptide antigens (such as hepatitis and influenza vaccines). Supporting this, 83% of individuals with a humoral primary immune deficiency developed four-fold or greater rises in antibody titre following influenza A/H1N1 vaccination compared with 100% of healthy controls. In addition, the low immunogenicity of an antigen, such as the 23 polyvalent pneumococcal polysaccharide ("Pneumovax") vaccine antigen, can also contribute to the failure to elicit protective antibody responses in individuals with CVID [43]. Thus it may not be surprising that the subject reported here was able to mount detectable responses to both influenza and hepatitis A and B vaccination, while having an attenuated response to HIV, which *in vitro* demonstrated impaired fitness.

Early reports that some patients have prolonged viremia before antibody production [44,45] led to concerns that testing strategies may be inadequate to ensure safety of the blood supply. However, these findings were probably related to the testing methodology, were not verified in later studies [46] and were later withdrawn by the authors. First generation serological tests for HIV were less sensitive [47]; even so, most patients had detectable antibodies by three months [48]. Current 3rd generation assays generally detect infection by 30 days post-exposure [9]. All EIA tests in use at the time our patient presented were 3rd generation assays that did not incorporate p24 Ag testing. Fourth generation assays, detecting antibodies and p24 antigen, have further reduced the window period for detecting virus infection [49,50]. These "combo" tests became routine at the testing laboratories involved late in 2005. Retrospective testing of samples available from our patient revealed p24 antigen was present earlier and would have been detected by 4th generation assays (Table 1). In addition, our patient had a high viral load that would have been detected by nucleic acid testing strategies routinely employed to ensure safety of the blood supply. Nucleic acid testing or viral load testing can detect infection earlier than p24 antigen production (approximately 7 days) [32]. This highlights the need for HIV testing using multiple parameters in addition to antibody responses, including viral antigen and nucleic acid testing when an infection is suspected. This may benefit cases where little or no antibodies have been observed [11,51].

The viral isolate characterized from the patient had reduced fitness compared with wild-type HIV, a somewhat surprising result given the severity of the individual's seroconversion illness. Infection with a highly attenuated strain of HIV-1 with reduced viral fitness has been shown to result in a much delayed seroconversion (Kondo, M et al., 2005). However, typically highly attenuated HIV-1 infection has been associated with minimal symptoms, at least in the early stages of infection [52–54]. The virus was not unusual such as subtype O, found mainly in Gabon and Cameroon, which replicate poorly and have been associated with false negative HIV testing [55]. Hence, while a prolonged seroconversion may have been attributed to a poorly replicating clade B strain it does not explain the rapid viral pathogenesis. We have characterized viral fitness of multiple HIV strains isolated from patients very early during HIV-1 infection and unlike strains characterized from later in infection [56], found no correlation with viral load (Arnott, Gorry, McPhee et al., submitted). This highlights the fact that viral fitness may not be directly related to virulence and the pathology associated with the infection process [51,57]. Hence, the virus infection in this case may be resulting in disease due to multiple factors, including perhaps a compromised immune response. Of note, virus replication persisted for many months after the introduction of ART as measured by viral load. This may have been exacerbated by the low levels of circulating antibodies, including neutralizing antibodies, being unable to block all viral entry. Nonetheless, we cannot exclude the possibility that viral fitness may have been compromised due to an effect of antiretroviral therapy.

Could the delay in antibody production also be an effect of early treatment? Early ART has been shown to blunt evolution of HIV-specific antibody responses, and can alter the expected kinetics of this response in subjects discontinuing therapy [33]. Hare et al. [58] describe 12 patients treated during seroconversion. All ultimately developed HIV antibodies but one patient remained antibody negative on all 2nd generation assays, although this patient was positive on a 3rd generation assay. Interestingly, this patient was WB positive at 16 weeks, but then indeterminate at 24 weeks. Of 75 patients treated during "early infection" (<12 months), five "lost antibody" at some point (62–250 weeks). HIV-specific antibodies reappeared in all when therapy stopped [58]. Early treatment in conjunction with a poorly replicating clade B virus might explain slow development of HIV antibodies.

AIDS defining illnesses during HIV seroconversion are well described but uncommon. Our patient differs from other cases of *P. jirovecii* pneumonia presenting during primary HIV infection. Vento et al. [3] describe three such cases: all were extremely immunosuppressed at diagnosis (CD4 count 62–91 cells/μl). However, all three had short duration of symptoms, presenting with *P. jirovecii* pneumonia within 2 weeks of onset of seroconversion symptoms, and with very precipitous drops in CD4 cell count in the weeks prior to presentation. Unlike our patient all three developed normal HIV antibody responses (at 30, 30 and 45 days after symptom onset, respectively) [3].

Conclusions

In summary, our patient's presentation had two unusual aspects: he developed *P. jirovecii* pneumonia in the setting of a prolonged primary HIV illness, and had an exceptionally delayed seroconversion. Viral studies revealed a poorly replicating virus but with few other unusual characteristics. His failure to mount detectable IgG_1 and IgG_3 responses over nearly two years of illness probably reflected a primary disorder of antibody production, and HLA typing showed that he was homozygous for part of an MHC haplotype that contains susceptibility genes for CVID syndrome. The introduction of ART may have further contributed to the extremely slow antibody development.

Acknowledgments

Highly purified HIV-1_{SF2} gp120 derived from CHO cells, used for the anti-gp120 EIA, was kindly provided by Dr. N Haigwood (Seattle Biomedical Research Institute, Seattle, WA, U.S.A.). This study was supported, in part, by a program grant from the Australian National Health and Medical Research Council (NHMRC) to SMC (358399), and grants from the Australian NHMRC to PRG (433915) and DAM (502617). PRG is the recipient of an Australian NHMRC Level 2 Biomedical Career Development Award. The authors gratefully acknowledge the contribution to this work of the Victorian Operational Infrastructure Support Program received by the Burnet Institute.

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