INTRODUCTION
Autoantibodies have been described in samples from HIV positive patients, but the effects of antiretroviral therapy (ART) remain unclear. In a retrospective longitudinal study, we applied clinical assays for autoantibodies to sera collected from 13 HIV positive patients as they began ART with <210 CD4 T-cells/μL and over 2 years on treatment.

Twelve of the 13 patients had at least one autoantibody. The frequency peaked before ART (21 from 156 assays) and declined to 8/143 positive reactions after 2 years. As anti-smooth muscle (ASM) antibodies remained common, these assays were applied to HIV patients (n = 67) who had <50 copies HIV RNA/mL plasma after 13 (2–17) years on ART, and healthy controls (n = 55). The frequency of ASM was high in these patients and correlated with levels of total IgG. Hence the high frequency of autoantibodies before ART declined, but did not disappear, with successful therapy. Autoantibody levels may reflect B-cell hyperactivity in patients stable on ART.

Key words: Autoantibodies; B-cell expansion; HIV; antiretroviral therapy.

MATERIALS AND METHODS

Samples and assays
Lithium heparin plasma was stored at ~20°C or ~80°C. Aliquots were clotted using thrombin (Siemens, Germany) when serum was required. Total IgG was quantified using plates coated with polyvalent goat anti-human IgG (Innovigen, USA). Binding was detected using goat anti-human IgG conjugated HRP (Sigma-Aldrich USA) followed by TMB (tetramethylbenzene; Sigma-Aldrich). Eight autoantibodies were assayed using commercial enzyme-linked immunosorbent assay (ELISA) kits: anti-thyroid peroxidase (TPO); anti-citrullinated peptide (CCP; EliA, Sweden). Reference values (RV) used in clinical practice are defined by the manufacturer for the commercial assays. Our RV for CCP was ≤10 U/mL.

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Autoantibodies assayed by immunofluorescence included ANA on a sub-strate of Hep-2000 cells (Immunocorex, USA) detected with a FITC-conjugated anti-human IgG (H+L chains; Immunocorex), anti-neutrophil cytoplasmic antibodies (ANCA; in-house preparation) and tissue-specific antibodies, including anti-parietal cell (APC) and smooth muscle (ASM) autoantibodies (t-AB; IgA, IgM and IgG; detected on rat liver, stomach kidney and mouse stomach; MediCa, USA). These were detected with FITC-conjugated goat anti-human IgG, IgM and IgA (Millipore, Germany). ANCA antibodies were further characterised as reactive with vessels (V), glomeruli (G) and kidney tubular (T) tissue.3

**Statistical analyses**

Data were analysed using GraphPad Prism v5.04 software (Graphpad Software, USA) and STATA 12 (StataCorp, USA). Significance was set at $p < 0.05$. Mann–Whitney U tests were used to compare controls and patients. All correlations were non-parametric (Spearmann’s) and used values from a specified time point. Median (range) are cited throughout.

**RESULTS AND DISCUSSION**

The frequency of 12 autoantibodies was assessed in 13 HIV positive individuals prior to ART and after approximately 1 and 2 years on ART (Fig. 1). The frequency of autoantibodies was highest before ART, with 21/156 positive results. The frequency declined to 7/144 positive results after 2 years of ART ($\chi^2, p = 0.03$). When the frequency of autoantibodies was compared with our earlier study of healthy controls ($n = 198$) representing the Caucasian Australian population,9 the frequency of autoantibody positivity was higher among HIV positive patients before treatment ($p < 0.001$), with a small effect after 1 year of ART ($p = 0.07$). The frequency of aCL remained elevated in the patients after 2 years on ART ($p = 0.014$).

Twelve of the thirteen patients were positive for at least one autoantibody at some time. ASM and aCL were found in six patients before ART, and remained the most common autoantibodies in patients on ART (Fig. 1). Two patients retained aCL antibody throughout. Three patients were consistently positive for ASM, whilst one remained weakly B2A positive and a single patient was positive for t-TGA at each time point, declining from 45 U/mL at baseline to 27 U/mL after 2 years.

One patient had a low level positive ANA with a nucleolar pattern of 7IU/mL (RV < 7IU/mL), but no samples had ENA identified by immunoblot. ANA is a screening test for systemic autoimmune disease and low levels are seen in the healthy population.7 Iordache et al.10 describe the autoantibodies in a distinct cohort of HIV infected patients ($n = 92$, 45% females, 55% sub-Saharan background, 74% unetectable HIV RNA, 78% on ART). They report an ANA frequency of 33% at a titre of 1:80 and 6% at 1:160. These titres correspond to 4 and 7 IU/mL, respectively, where our RV is 7 IU/mL. As age, gender and ethnicity may affect ANA frequencies,11–13 it is notable that the frequency of a detectable ANA reported by Iordache et al. at the higher dilution matches our results.

Iordache et al.10 reported a frequency of c-ANCA of 10% and p-ANCA of 3%. Here ANCA was identified by IF in a single patient (7.6%) with a 5 U/mL cytoplasmic ANCA (RV < 3) at baseline. This declined to 3 U/mL after 1 year on ART. Secondary testing was not performed on this sample.

Antiphospholipid antibodies can be found in patients with infections, including HIV, as well as in antiphospholipid syndrome.14 Several studies have reported the presence of aCL and anti-β2-glycoprotein 1 antibodies among HIV patients with variable estimates of prevalence. Variability might relate to differences in assay technique, reference ranges and patient health and demographics. The significance of antiphospholipid antibodies in the context of HIV infection is unclear, but they are less often associated with thrombotic events than antiphospholipid antibodies in patients with connective tissue diseases.15 A single patient in our study group had low level aCL and B2A positive results at baseline and after 1 year on ART, with hypergammaglobulinaemia at all timepoints. A further patient had aCL and B2A at baseline only. The remaining four patients with aCL were negative for B2A and B2G.

A single patient was consistently positive for t-TGA (2-fold above the RV), but had no history of coeliac disease. One patient was positive by IF in the first year on ART, but dropped below the RV in the second year, with no evidence of pernicious anaemia when the patient died several years later. High levels of t-TGA antibodies are associated with coeliac disease. However tissue transglutaminase also breaks down components of apoptotic cells. AIDS patients have higher levels of the enzyme in circulation due to high rates of CD4 T-cell apoptosis,16 with levels likely to decline on ART. Hence high levels of t-TGA antibodies in this context may reflect sensitisation due to circulating enzyme, rather than nascent coeliac disease.

Overall the increased frequency of autoantibodies at the pre-ART timepoint is consistent with the increased levels reported by Meng et al.17 and Baroncelli et al.18 but Meng et al. included antibodies that are not routinely reported in diagnostic laboratories.

Here levels of total Ig were high, with hypergammaglobulinaemia evident in 10/13 patients before ART.8 Of the four patients with hypergammaglobulinaemia after 2 years, only two still had autoantibodies. No clear trends emerged when levels of autoantibodies assayed as a continuous variable were correlated with levels of total Ig or soluble BAFF (data not shown), but the $n$ values are very low. For example at baseline, comparisons between total Ig and autoantibody levels yielded $r$ values between −0.27 and 0.35, whilst sBAFF and autoantibodies yielded $r$ values between −0.47 and 0.10 with no significant associations. However high levels of Ig correlated with an increased number of autoantibodies when results from all timepoints were pooled ($r = 0.34, p = 0.035$).

ASM and aCL remained common in HIV positive patients after 2 years of ART (Fig. 1). To determine whether this trend was stable on ART, these assays were applied to a larger HIV positive cohort ($n = 69$) recruited in Sydney after a median (range) period of 13 (2–17) years on ART.8,17 We selected 55 persons from the Busselton cohort who were matched by age and gender to the HIV positive individuals (67 males, 2 females; Table 1), as older age and female gender are risk factors for autoantibody production.24

Only one HIV positive individual stable on ART was low level positive for aCL (12 U/mL). This is not significantly different from the two aCL positive individuals in the matched control cohort ($n = 55$, $p = 0.58$). In contrast, the frequency of ASM was higher in HIV positive individuals. High titre ASM VGT staining is associated with autoimmune hepatitis type I, whilst ASM VG has a range of disease associations including viral infection. Only ASM VG antibodies were detected here. Levels of total IgG were slightly higher in people with ASM $\geq 1$ than those negative for this
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