Understanding the high incidence of autoantibodies in HIV patients

Christine BUNDELL[1], Samantha J. BRUNT[2], Lucette A. CYSIQUE[3,4,5,] Anna BRUSCH[1], Bruce J. BREW[3,4,5], Patricia PRICE [6].

1. Clinical Immunology, PathWest Laboratory Medicine, QEII Medical Centre, Nedlands, Australia
2. Pathology and Laboratory Medicine, University of Western Australia, Nedlands, Australia
3. Peter Duncan Neurosciences Unit, St. Vincent's Applied Medical Research Centre, Sydney, Australia
4. Neuroscience Research Australia, Sydney, Australia
5. Neurology Department, St Vincent's Hospital, Sydney, Australia
6. School of Biomedical Science, Curtin University, Perth, Australia

Corresponding Author:
A/Professor Patricia Price
School of Biomedical Science,
Curtin University
Bentley,
Western Australia
Tel 618-92669716
Email: patricia.price@curtin.edu.au

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Short title: Autoantibodies in HIV patients

Abstract

Autoantibodies have been described in samples from HIV$^+$ 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$^+$ patients as they began ART with <210 CD4 T-cells/µl and over 2 years on treatment. All 13 patients had at least one autoantibody. The incidence 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 incidence of ASM was high in these patients and correlated with levels of total IgG. Hence the high incidence of autoantibodies before ART declined, but did not disappear, with successful therapy. Autoantibody levels may reflect B-cell hyperactivity in patients stable on ART.

150 words

Key words:

Autoantibodies, B-cell expansion, HIV, antiretroviral therapy

1. Introduction

Autoimmunity is described as immune recognition and reaction against self-antigens, and reflects a breakdown of immune tolerance. Autoantigens include nuclear, cytoplasmic and surface membrane components of host cells [1]. Autoantibody secretion has been linked with B-cell hyperplasia during untreated HIV infection [2]. Navarta *et al* [3] reported a broad range of autoantibody reactivities in HIV-infected children. These included antibodies recognising smooth muscle (ASM), neutrophil cytoplasmic antigens (ANCA) and nuclear antigens (ANA). Plasma markers of B-cell activation are elevated and B-cell populations are depleted in HIV$^+$ patients [4]. Markers of B-cell activation that are high in untreated HIV infection decline with commencement of antiretroviral therapy (ART) and numbers of circulating B-cells increase. However levels of B-cell activation markers do not normalize after 2 years of ART [5] [6], and the persistence of autoantibodies over this period has not been investigated systematically. Here we identify autoantibodies in HIV$^+$ patients and assess their relationship to B-cell activation assessed via levels of total IgG. We assess HIV patients beginning ART and patients stable after several years on ART [7].

2. Materials and Methods

2.1 Patients:

HIV patients [10 males, 3 females, aged 45(28-66) years] were selected from the HIV database of Royal Perth Hospital (Western Australia) on the basis of achieving <50 copies HIV RNA/mL within 6 months of commencing ART, baseline CD4 T-cell counts <210 cells/µl, no evidence of HCV co-infection and archived plasma samples available for analysis collected at baseline and after approximately 1 and 2 years on ART. All patients showed steady increases in CD4 T-cell counts on ART and 12/13 achieved plasma HIV RNA levels below <400 copies/ µl [6]. The second HIV^+ cohort comprised 69 participants treated at St Vincent's Hospital, Sydney, Australia. These individuals were aged >45 years with nadir CD4 T-cell counts <500 cells/µl, <50 copies HIV RNA/mL plasma and on ART for >2 years. They were tested at a single timepoint [8]. Control samples (n=67) were drawn from the 1994 Busselton Health Study collection on the basis of Caucasian ethnicity, age 50(18 – 88) years and only one participant per family. Ethics approval was obtained from the Busselton Medical Research Foundation (Inc.), Sir Charles Gairdner Hospital Human Ethics Committee (SCGH) (Approval Number 2008-042) and Human Research Ethics Committees of St. Vincent's Hospital (08/SVH/90) and University of New South Wales (08380-08/SVH/90).

2.2 Samples and assays:

Lithium heparin plasma was stored at -20 or -80°C. Aliquots were clotted using thrombin (Siemans, Marburg, Germany) when serum was required. Total IgG was quantified using plates coated with polyvalent goat anti-human IgG (Invitrogen; Carlsbad, CA) [6]. Binding was detected using goat anti-human IgG conjugated HRP (Sigma-Aldrich; St Louis, MI) followed by TMB (tetramethylbenzene, Sigma-Aldrich).

Eight autoantibodies were assayed using commercial ELISA kits - anti-thyroid peroxidase (TPO; ORGENTEC, Mainz, Germany), anti-cardiolipin (aCL; ORGENTEC, Mainz, Germany), anti-tissue transglutaminase (IgA, t-TGA; AEKSULA, Wendelsheim, Germany), extractable nuclear antigen antibody (ENE) screen (QUANTA Lite; INOVA Diagnostics, San Diego, CA), anti-mitochondrial dehydrogenase complex (MIT-3; QUANTA Lite), anti-β2-glycoprotein I IgG (B2G) and IgA (B2A) (REAADS Corgenix; Broomfield, CO), anti-intrinsic factor (IF; GENESIS; Croyden, UK) and anti-cyclic citrullinated peptide (CCP; EliA; Uppsala, Sweden). Reference Values (RV) used in clinical practice are defined by the manufacturer for the commercial assays [9]. Our RV for CCP was ≤ 10 U/ml.

Autoantibodies assayed by immunofluorescence included ANA on a substrate of HEp-2000 cells (Immunoconcepts, Sacramento, CA) detected with a FITC-conjugated anti-human IgG (H+L chains; Immunoconcepts), anti-neutrophil cytoplasmic antibodies (ANCA; in-house preparation) and tissue-specific autoantibodies (t-Ab; IgA, IgM and IgG; detected on rat liver, stomach kidney and mouse stomach; MeDiCa; Sacramento, CA). These were detected with FITC-conjugated goat anti-human IgG, M and A immunoglobulin (Millipore; Darmstadt, Germany). ASM antibodies were further characterised as reactive with vessels (V), glomeruli (G) and kidney tubular (T) tissue [9].

2.3 Statistical analyses:

Data were analysed using GraphPad Prism v5.04 software (Graphpad software; La Jolla, CA) and STATA 12 (StataCorp; College Station, TX). Significance was set at $p<0.05$. Mann Whitney U tests were used to compare controls and patients. All correlations were non-parametric (Spearmans) and used values from a specified timepoint.

3. Results and Discussion

The incidence of 12 autoantibodies was assessed in 13 HIV^+ persons 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 incidence declined to 7/144 positive results after 2 years of ART (Chi-square test, $p=0.03$). When the incidence of autoantibodies was compared with our earlier study of healthy controls (n=198) representing the Caucasian Australian population [9], the incidence of autoantibody positivity was higher among HIV^+ patients before treatment ($p<0.001$), with a small effect after 1 year of ART ($p=0.07$). The incidence of aCL remained elevated in the patients after 2 years on ART ($p=0.014$).

All 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 45U/ml at baseline to 27U/ml after 2 years.

aCL antibodies seen with β2-glycoprotein I antibodies (particularly B2G) [10] are associated with anti-phospholipid syndrome. A single patient had low level aCL and B2A positive at baseline and after 1 year on ART, with hypergammaglobulinemia 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 for 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 [11], with levels likely to decline on ART. Hence high levels of t-TGA antibodies in this context may reflect sensitization due to circulating enzyme, rather than nascent coeliac disease.

Levels of total Ig were high, with hypergammaglobulinemia evident in 10/13 patients before ART [6]. Of the four patients with hypergammaglubulinaemia 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 IgG or soluble BAFF (data not shown), but the n values are very low. For example at baseline; comparisons between total IgG 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 IgG correlated with an increased number of autoantibodies ($r = 0.34$, $p = 0.035$).

ASM and aCL remained common in HIV^+ 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^+ cohort (n=69) recruited in Sydney after a median (range) period of 13(2-17) years on ART [8, 12]. We selected 55 persons from the Busselton cohort who were matched by age and gender to the HIV^+ individuals (67 males, 2 females; Table 1), as older age and female gender are risk factors for autoantibody production [13].

Only one HIV^+ individual stable on ART was low level positive for aCL (12 Units/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 incidence of ASM was higher in HIV^+ 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 ≥1 than those negative for this antibody [11(7-19) *vs* 9(5-22) mg/ml; p=0.02] and ASM scores correlated with total Ig (r=0.32, p=0.009).

4. Conclusions

The incidence of autoantibodies is high in untreated HIV infection and does not normalise on ART – with notable persistence of ASM that should be considered in the interrelation of autoantibody findings in persons living with HIV. Increased total immunoglobulin levels may contribute to the maintenance of these autoantibodies when patients become stable on ART.

5. References

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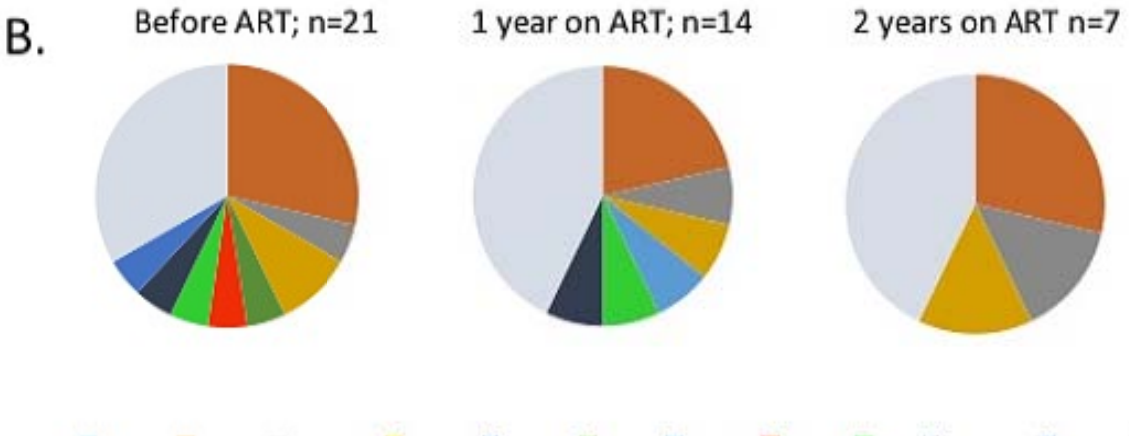
A.

Number of Autoantibodies	Pre treatment (n=13)	Year 1 (n=12)	Year 2 (n=12)
0	2	2	6
1	5	6	5
2	3	4	1
3	2		
4	1		
% Positive (n)	85% (11)	83% (10)	58% (6)

B.



C.