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IMMUNOPATHOLOGY

Low level autoantibodies can be frequently detected in the general Australian population

Pooja Deshpande[1], Michaela Lucas[2,3,4], Samantha Brunt[5], Andrew Lucas[4,6], Peter Hollingsworth[2,5] and Christine Bundell[2,5]

[1]School of Anatomy, Physiology and Human Biology, University of Western Australia, [2]Department of Clinical Immunology, PathWest Laboratory Medicine, QE II Medical Centre, [3]Institute for Immunology and Infectious Diseases, Murdoch University, [4]School of Medicine and Pharmacology, Harry Perkins Building, University of Western Australia, [5]School of Pathology and Laboratory Medicine, University of Western Australia, and [6]Institute for Respiratory Health, Harry Perkins Building, QEII Medical Centre, Nedlands, WA, Australia

Summary

The aim of this study was to determine the prevalence and type of autoantibodies in a general Australian population cohort.

Samples collected from 198 individuals included in a cross sectional Busselton Health Study were tested using autoantibody assays routinely performed at Clinical Immunology, PathWest Laboratory Medicine, Western Australia. At least one autoantibody was detected in 51.5% of individuals (males = 45.1%, females = 58.3%). The most frequently detected serum autoantibodies were anti-beta-2-glycoprotein I (12.1%) followed by anti-smooth muscle (11.6%) and anti-thyroid peroxidase (8.6%). Vasculitis associated anti-neutrophil cytoplasmic antibodies were present in 5.1%, while anti-nuclear antibodies were detected in 8.6% of individuals. Notably, 65% of positive results were detected at low levels with the exception of anti-myeloperoxidase and anti-beta 2 glycoprotein I IgG antibodies.

Autoantibodies are commonly detected at low levels in a predominantly Australian or European population cohort. No large Australian study has yet provided these data for contemporary routine tests. This paper gives important information on the background frequency of autoantibodies in the general population. Due to the nature of this study we are unaware of whether these individuals have subsequently developed an autoimmune disease, however this was not clinically diagnosed at the time of sample collection.

Key words: Autoantibody; general Australian population; Busselton Health Study; background frequency.

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INTRODUCTION

Autoimmune diseases affect approximately 5–6% of the population in Western countries.[1,2] The resulting pathology is the effect of a complex interplay between humoral and cellular immunity directed against host tissues.[3,4] Susceptibility to the development of autoimmunity is likely to be multifactorial and consistent with this are studies that implicate both genetic[5,6] and environmental[1,7] factors. The presence of serum autoantibodies (AAbs) targeted against various self-antigens remains an important characteristic of a number of autoimmune diseases. Detection of these AAbs is a key diagnostic element of clinical immunology.

Several international studies have reported prevalence of serum AAbs in their corresponding general population.[8–14] Ethnicity, gender, age and geographical location may influence prevalence of autoimmunity.[1,2,15,16] Along with these differences, autoimmunity prevalence may also be influenced by differences in methods of testing and reporting of results across different jurisdictions.[17–19] Therefore, current recommendations for laboratories are to generate reference data relevant to the local population.[20] However, sample availability, associated assay costs and laboratory workflow do not necessarily facilitate ongoing generation of these reference data. Laboratories are often reliant on manufacturer's data for reference values (RVs); or commonly, reference data are generated from blood donors who inherently are more likely to be male and younger than seen in the general population.[11,12,21] We take advantage of access to the Busselton Health Study which involves a series of ongoing health surveys combined with sample collections conducted over four decades in a south-west coastal community of Western Australia (WA) of predominantly Caucasian citizens.[22–26] Here, we present AAb data obtained from this cohort using current National Association of Testing Authorities (NATA) accredited commercial or in house laboratory assays. Previous studies have either investigated a single AAb only or they have been cross-sectional but focused on estimating the prevalence of AAbs relevant to certain diseases in control populations.[24,27,28] So far, there is only one other report on the prevalence of serum AAbs in the general Australian population, published in 1972,[26] and more contemporary studies are urgently needed.

MATERIALS AND METHODS

Participants

A total of 198 samples, which were a subset of the Busselton Health Study collection in 1994, were utilised for this study performed at the Clinical Immunology Department, PathWest Laboratory Medicine, Queen Elizabeth II Medical Centre. Samples were selected from donors with predominantly

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Australian or European ethnicity, approximately a similar proportion of males and females, random ages and with not more than one participant per family (demographics of the population are indicated in Table 1). Informed consent was obtained from all participants at the time of sample collection. Ethics approval for AAb testing was obtained from the Busselton Medical Research Foundation (Inc.) and Sir Charles Gairdner Hospital Human Ethics Committee (SCGH).

Samples

The primary serum samples were stored at a maximum of −20°C from the time of collection. At the time of the study the primary serum tube was rapidly thawed, mixed and dispensed into four 60 µL aliquots. Aliquots were stored at −80°C and thawed for testing in batches. Repeat freeze thawing therefore was kept to a minimum (limited to up to four times). Samples were stored at 4°C between assays. A number of clinical samples collected between 1998 and 2006 were retested in the anti-nuclear antibody (ANA) assay in 2015, consistent results were obtained between the original and repeated test (data not shown).

Detection of anti-neutrophil cytoplasmic antibodies

Anti-neutrophil cytoplasmic antibodies (ANCA) were detected by indirect immunofluorescence (IIF) using serum samples diluted 1:20 in serum diluent (Immunoconcepts, USA). Samples were tested on ethanol fixed human neutrophils (in house preparation) and bound antibody detected with fluorescein isothiocyanate (FITC) conjugated goat anti-human IgG (Silenus, Australia). A washing step was carried out after each incubation using phosphate buffered saline (PBS) pH 7.6. Antibody levels were measured against a calibration curve ranging from 0–7 arbitrary U/mL (prepared using 2-fold serial dilutions of positive control serum from a patient with known granulomatosis with polyangiitis and high level ANCA). ANCA was defined as positive if the intensity score was ≥3 U/mL. The ANCA RV has been determined in the laboratory correlating MPO and PR3 AAb enzyme-linked immunosorbent assay (ELISA), clinical and IIF data (data not shown). ANCA were considered low positive if the score was equal to RV+1 (Tables 2 and 3). The inhouse ANCA slides are directly comparable to commercially available slides (unpublished data) and approved for use in the NATA accredited laboratory.

Detection of ANA and antibodies to extractable nuclear antigens

ANA were measured by incubating serum samples diluted 1:40 in serum diluent with HEp-2000 cells (human epithelioid cells; Immunoconcepts, USA) and FITC conjugated anti-human IgG (H+L chains; Immunoconcepts, USA) according to the manufacturer's instructions. The levels were expressed in IU/mL, measuring fluorescence intensities using a calibration curve standardised against World Health Organization 66/223 reference serum. A PBS (pH 7.6) washing step was included after each of the incubations. Samples were not titrated, however equivalent conversion values have been determined in the laboratory (data not shown) and are as follows: 7 IU/mL = 1:160, 10 IU/mL = 1:320, 15–20 IU/mL = 1:640, 25–30 IU/mL = 1:1280.

ANA ≥7 IU/mL (1:160) were considered positive, as described previously.[29] Along with homogenous and speckled patterns, other patterns such as centromere, multiple nuclear dots and nuclear sub matrix pattern were reported. In this study all samples were tested for ANA and screened for AAbs to extractable nuclear antigens (ENA) by ELISA (QUANTA Lite ENA 6 ELISA; Inova, USA). Samples positive in the ENA AAb screen were further characterised by immunoblot (INNO-LIA ANA Update; Fujirebio Europe, Belgium). Samples were tested according to the manufacturer's instructions for the ELISA and the immunoblot, using the manufacturer's RVs to determine a positive and negative result (Inova ELISA RV ≤20 Units, Table 2). ANA and ELISA results were categorised as a low positive if the values were >RV and ≤2 RV (Tables 2 and 3).

Table 1 Demographics of the Busselton cohort in this study

	Males	Females
Total number, *n* (%)	102 (51.5)	96 (48.5)
Mean age ± SD, years	51 ± 17	50 ± 17
Age group, years, *n* (%)		
<30	14 (7.1)	11 (5.5)
30–50	33 (16.7)	38 (19.2)
50–70	40 (20.2)	30 (15.2)
>70	15 (7.6)	17 (8.6)
Country of birth, *n* (%)		
Australia	79 (39.9)	83 (41.9)
Northwest Europe	17 (8.6)	10 (5.1)
Other	2 (1.0)	2 (1.0)
Not stated	4 (2.0)	1 (0.5)

Detection of tissue autoantibodies

AAbs directed against liver kidney microsomal (LKM) antigens and antigens expressed by smooth muscle (ASM), parietal cells (APC) and mitochondria (AMC) were assessed by IIF using serum diluted 1:10 in PBS pH 7.6 and incubated on slides with fixed rat liver and kidney, and rat and mouse stomach tissue sections (MeDiCa, USA). Bound antibody was detected with FITC conjugated anti-human IgG, A and M (H+L chains) (Millipore, USA). A PBS pH 7.6 washing step was included after each of the incubations. Staining pattern and fluorescence intensity were determined by fluorescence microscopy using a quartz iodide lamp. ASM AAb patterns were further characterised as ASM vessels (V), glomeruli (G), tubular (T).[30] A positive control for each specified AAb and a negative control were included in each assay run. Tissue AAbs measured by IIF were defined as positive if the intensity score was >0 (range 0–3; RV = 0). Tissue AAbs were considered low positive if the score was equal to RV+1 (Tables 2 and 3).

Autoantibodies detected by ELISA technology

AAbs detected by ELISA were tested according to the manufacturer's specifications; manufacturer's details and RV used to define a positive result for the ELISA assays are shown in Table 2. ELISA results were categorised as low positive if the values were >RV and ≤2 RV (Tables 2 and 3).

Statistical analysis

Data were analysed by applying the Fisher's exact test using GraphPad PRISM version 5.02 (GraphPad Software, USA); group comparisons have been expressed as odds ratios (OR) and confidence intervals (CI) and statistical significance expressed as p values. Gender and age group distribution cross tabulation with number of AAbs present was performed using the statistical package for the social sciences (SPSS version 21; IBM, USA). p values <0.05 were considered statistically significant.

RESULTS

Of the 198 individuals tested in this study, one or more AAbs were detected in 51.5% of individuals, of which 69.6% had a single AAb, 16.7% had two AAbs and 13.7% had ≥3 AAbs. A higher proportion of females (n = 56, 58.3%) were positive for ≥1 AAb compared to males (n = 46, 45.1%), but this difference was not significant (Fig. 1). The frequency of each AAb detected in this study population is indicated in Table 4. The most frequently detected AAbs were β2GPI (IgG/M/A inclusive 12.1%) followed by ASM (11.6 %) and TPO (8.6%).

ANCA

ANCA was present in 5.1% of individuals, of which five samples showed cytoplasmic ANCA pattern (c-ANCA), one showed a perinuclear ANCA pattern (p-ANCA) and four showed an atypical staining pattern. All samples were tested for MPO and PR3 AAbs. Only one individual was positive for MPO AAb (14.0 U/mL) in the cohort but this was not associated with the typical p-ANCA pattern. A positive c-ANCA with a positive PR3 AAb (7.2 U/mL) was seen in only one of the five c-ANCA positive individuals. A low level (24 U/mL) of GBM AAb was detected in one female.

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