Identification of ovarian antibodies by immunofluorescence, enzyme-linked immunosorbent assay or immunoblotting in premature ovarian failure

N.J.Wheatcroft1, C.Salt2, A.Milford-Ward2, I.D.Cooke3 and A.P.Weetman1,4

1Department of Medicine, Clinical Sciences Centre, Northern General Hospital, University of Sheffield. 2Department of Clinical Immunology, Northern General Hospital, Sheffield S5 7AU and 3University Department of Obstetrics and Gynaecology, Jessop Hospital for Women, Sheffield, S3 7RE, UK
4To whom correspondence should be addressed

The development of new techniques for the detection of ovarian antibodies has challenged early concepts about the rarity of ovarian antibodies in idiopathic premature ovarian failure (POF), but few attempts have been made to compare results between assays. We have sought to define the prevalence of ovarian autoimmunity in a group of 30 idiopathic POF patients compared to a group of 12 patients with POF plus an associated autoimmune disease and a group of 38 controls, using an enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IFL). Ovarian antibodies were detected in 27% of idiopathic POF patients by ELISA (not significantly different compared to POF patients with associated autoimmune disease; P < 0.0003 compared to controls) but only 7% of these patients were positive by IFL. In a further, pre-selected group of individuals, all positive for ovarian antibodies by IFL, 53% had measurable antibodies by ELISA. Some overlap was therefore demonstrated between the two techniques but many POF patients had ovarian antibodies detectable by only one method. Immunoblotting studies revealed that no consistent pattern of binding could be demonstrated for these patients. These results call into question the specificity of ovarian antibodies as a marker for autoimmune POF. Key words: enzyme-linked immunosorbent assay/immunoblotting/immunofluorescence/ovarian antibodies/ovarian failure

Introduction

Premature ovarian failure (POF) is characterized by hypergonadotrophic amenorrhoea occurring before the age of 40 years (LaBarbera et al., 1988). The known causes include genetic abnormalities, viral infections, radiation damage and chemical toxicity but in the majority of patients the aetiology is poorly understood (Fox, 1992). POF is frequently associated with autoimmune disorders, particularly autoimmune thyroid disease (LaBarbera et al., 1988). In the absence of clinically overt disease, some patients have serological evidence of autoimmunity, mainly against thyroid and gastric antigens and more rarely against the adrenal cortex (Betterle et al., 1993). These observations suggest that autoimmunity may be an important cause of ‘idiopathic’ POF.

One of the main elements in the analysis of autoimmune mechanisms in POF has been the identification of ovarian antibodies. Early reports focused on the detection of antibodies in patients with concurrent Addison’s disease that cross-reacted with adrenal, ovary, testis and placenta and were therefore termed steroid cell antibodies (SCA). The target for these antibodies has been defined in patients with autoimmune polyglanular syndrome (APS) type I as the cytochrome P450 steroid synthesizing enzymes, steroid 17α hydroxylase (P450c17) and side chain cleavage enzyme (P450scC) found in the theca interna (Uibo et al., 1994; Winqvist et al., 1995). SCA are significant markers for autoimmune POF, but are rarely present in those patients without associated Addison’s disease (Mignot et al., 1989). Ovarian antibodies distinct from SCA have been detected in POF patients without any other evidence of autoimmune disease (Danwood et al., 1986; Luborsky et al., 1990), but their exact prevalence and role in pathogenesis remain uncertain, partly due to differences in the populations studied and in the techniques used.

Indirect immunofluorescence (IFL) has been the most commonly used technique to identify ovarian antibodies, but variations in the source of ovarian tissue (human, primate and subprimate) and its maturation state have produced wide differences in the reported frequency. In particular, the clinical usefulness of primate tissue for the detection of human antibodies has been questioned (Kirsop et al., 1991). An enzyme-linked immunosorbent assay (ELISA) has been developed as a simplified and more rapid method for the detection of ovarian antibodies (Luborsky et al., 1990; Wheatcroft et al., 1994), but there have been few attempts to compare the results obtained in ELISA and IFL assays (Moncayo et al., 1989; Gobert et al., 1990). We have therefore sought to define the prevalence of autoimmunity in a group of women with idiopathic POF using both of these techniques. To establish the diagnostic relevance of the ovarian antibodies detected by the two techniques, immunoblotting was used to identify any discrete autoantigens against which the antibodies were directed.

Materials and methods

Experimental subjects

Serum was collected from 42 women with POF who were attending an infertility clinic sequentially. All the women had a cessation of menses before the age of 40 years in association with raised serum levels of follicle-stimulating hormone (FSH; >10 IU/l). Thirty of the women had isolated ovarian failure and were termed idiopathic POF.
All were screened for thyroid autoimmunity with negative results and none had clinical or biochemical evidence of Addison’s disease. Five of these women had previously been fertile; six pregnancies resulted in three live births. Two of the patients with idiopathic POF presented post-pill. The remaining 12 women had associated autoimmune diseases. Nine of the women had Addison’s disease (one also had Hashimoto’s thyroiditis), two had autoimmune hypothyroidism and one had Evan’s syndrome. Serum samples were obtained 0.5–15 years after diagnosis.

In addition, ovarian antibody-positive sera from 19 women were supplied by the Department of Clinical Immunology (referred to below as the positive IFL group). These sera were identified by IFL on commercial four organ section slides which contained samples of ovary, adrenal, testis and pituitary of monkey origin (Biodiagnostics, Upton-upon-Severn, UK). Three of the sera were sent to this department to ascertain a diagnosis of autoimmunity as a cause for POF; in the remainder, the diagnosis was suspected Addison’s disease or hypophysitis. Further clinical details were not available as the sera originated outside Sheffield, UK. Heterogeneous staining patterns in the ovary were observed in this group and 13 of the 19 sera contained a combination of antibodies to Sertoli or Leydig cells of testis, pituitary and adrenal organs as well as ovarian antibodies by IFL.

The control group consisted of 38 selected sera from a larger pool of women who had samples sent to the Department of Clinical Chemistry, Northern General Hospital, Sheffield, UK. Twenty-nine of the women were pre-menopausal, confirmed by progesterone measurements >20 nmol/l on day 21, indicating that ovulation was occurring. Nine of the women were post-menopausal, and had mean luteinizing hormone (LH) levels (± standard deviation, SD) of 33 ± 15 IU/l and FSH concentrations of 66 ± 25 IU/l.

Local Ethical Committee approval was received for this study.

Preparation of antigens

Ovarian specimens were obtained from three women undergoing hysterectomy.

Ovary 1
This woman was 51 years (para 2, blood group O, Rhesus positive). She had a total hysterectomy and salpingoophorectomy for low abdominal pain caused by bulkyuterine fibroids. Before surgery, she had regular periods on hormone replacement therapy (HRT) for 1 year. As a result of progestogen side effects of HRT, the patient was later given oestrogen-only HRT following a prophylactic hysterectomy. Uterine and cervical adenomyosis was evident pathologically.

Ovary 2
This 40 year old woman (para 2, blood group A, Rhesus positive) had an abdominal hysterectomy and bilateral bilateral salpingoophorectomy for menorrhagia, dysmenorrhoea and premenstrual syndrome. Her periods had been irregular prior to surgery, but she had been intolerant of HRT and was not taking any medication at the time of surgery. The uterus showed no evidence of malignancy. A corpus luteum was found in the ovarian specimen.

Ovary 3
This 51 year old woman (para 2, blood group A, Rhesus negative) had a 1 year history of irregular periods and prolapse before hysterectomy and pelvic floor repair. She was not on medication at the time of surgery. Histology demonstrated keratosis, no dysplasia and two benign endometrial polyps. An ovarian biopsy was obtained with informed consent from the patient.

The skeletal muscle used in immunoblotting experiments was obtained from a patient undergoing a leg amputation. This patient was blood group O.

Microsomal antigens and 2000 g fractions were prepared from all specimens as described in detail previously (Wheatcroft et al., 1994).

ELISA
Ovarian antigens were used at a coating concentration of 25 µg/ml. The ELISA method was performed as described previously (Wheatcroft et al., 1994). Sera were tested batchwise with a positive POF patient control, defined from preliminary experiments, and a set of at least eight control sera on each plate. The intra-assay coefficient of variation was 0.1–15% (mean 5.8%). The inter-assay coefficient of variation ranged from 0.2–18% between plates (mean 11%).

All samples were assayed in duplicate and the mean calculated. A background control in which the serum was omitted was used in each plate. This provided a measure of background which could be subtracted from the sera under test.

IFL for ovarian antibodies
Commercial slide preparations of monkey ovary (Biodiagnostics) were used to test for ovarian antibodies in sera from 30 of the women not previously tested or selected for antibodies by IFL. Serum samples were diluted 1:10 in phosphate buffered saline (PBS) and 50 µl was pipetted on to the tissue section. Slides were then incubated for 30 min at room temperature in a moist box. The slides were washed in PBS for 15 min after excess serum from each slide had been rinsed off. Fluorescein isothiocyanate (FITC)-conjugated anti-human IgG diluted 1:30 (Sigma, Poole, Dorset, UK) was added at room temperature for 30 min and then the slides were washed in PBS before being mounted with a glycerine/PBS mixture. Slides were examined on a Leitz Labourlux 5 microscope, at ×400 magnification.

A positive control and a no serum negative were included in each experiment and the slides examined without prior knowledge of the sera under test. Each positive sample was tested twice to confirm reactivity.

Immunofluorescence was also carried out using sections of human ovary. Briefly, the tissue was transported from the operating theatre on ice and frozen in liquid nitrogen within 1 h of its removal. This was performed by placing dissected pieces of tissue on cork in Tissue Tek II OCT compound embedding medium (Raymond and Lamb, London, UK) and lowering into liquid nitrogen-cooled isopentane until frozen. The tissue blocks were then stored in liquid nitrogen until required. Sections of 5 µm thickness were cut at –20°C, mounted on 3-(triethoxysilyl) propylalanine (APES)-coated slides (Raymond and Lamb) and allowed to air dry. Sections were used immediately. Before use, the slides were fixed in acetone for 5 min at room temperature.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)
Electrophoresis of the microsomal and 2000 g fraction subfractions of ovary and skeletal muscle was carried out using 9% polyacrylamide bis-crosslinked gels. Antigen (40 µg/ml) was added to each well diluted in loading buffer containing 2-mercaptoethanol. The gel was run at 35 mA for 4 h. The separated proteins were then transferred onto nitrocellulose membrane (Bio-Rad Laboratories, Richmond, CA, USA) using a semi-dry electroblotting apparatus (Genetic Research Instrumentation, Essex, UK) at 30 mA overnight. The nitrocellulose membrane was incubated for 30 min in transfer buffer (25 mM Tris–HCl, 192 mM glycine, 20% methanol) to ensure thorough wetting before blotting.

After blotting, the nitrocellulose was stained with 0.5% amido black/10% acetic acid and destained in 40% methanol/10% acetic acid. The nitrocellulose was then blocked for 1 h at room temperature with 1% casein in PBS. After blocking, the membrane was cut into
antigen bearing strips and stored at –20°C. Acrylamide gels were also run under non-reducing conditions. This involved eliminating 2-mercaptoethanol from the loading buffer.

**Immunoblotting**

Membrane strips were incubated in 3 ml of 1% casein/0.1% fetal calf serum in PBS (PBS-C) containing a 1:60 dilution of the test serum for 2 h at room temperature. The strips were then washed four times with 2% Tween 20 (Sigma) in PBS (PBS-T), rinsed with PBS-C, and incubated in 3 ml of PBS-C containing anti-human IgG peroxidase conjugate (Sigma), 1:300 dilution, for 90 min at room temperature. After this incubation, the strips were washed four times with PBS-T. For detection of antibody, the strips were rinsed in 100 mM Tris–HCl pH 8.0 (TB), then incubated in TB plus 1.5 mg luminol (5-amino-2,3-dihydro-4-phthalazinedione; Sigma) and 7.5 µl 30% H₂O₂ (Sigma). After reaction, the strips were removed without rinsing and aligned on an acetate sheet to avoid drying. Strips were then covered with Saran wrap and exposed to Fuji RX X-ray film for 15–60 s.

A second antibody control strip, in which the serum was omitted in the first step, was included in each assay in order to identify background band signals. A positive control strip was also included in each assay which had been incubated with a serum sample from a patient with SLE. This sample produced multiple binding patterns on immunoblotting against the ovary and skeletal muscle.

**Statistics**

In the ELISA, values greater than the mean control absorbance ± 2 SD were taken as positive binding, a cut-off value previously used by ourselves and other authors using the ELISA to detect ovarian antibodies (Luborsky et al., 1990; Wheatcroft et al., 1994). The χ² test on 2×2 contingency tables was used for statistical analysis unless otherwise stated in the text.

**Results**

**ELISA for ovarian antibodies**

Nine (27%) of the 30 patients with idiopathic POF had ovarian antibodies detectable by ELISA, using an antigen preparation from a group O human ovary (ovary 1; Figure 1), compared to four (31%) of the 12 patients with an associated autoimmune disease (NS; Figure 1). The number of POF patients with ovarian antibodies was significantly higher than the 38 controls, of whom none were ovarian antibody positive (idiopathic POF patients versus controls, P < 0.0003; POF with an associated autoimmune disease versus controls, P < 0.002).

Ten (53%) of the 19 patients pre-selected by the Department of Clinical Immunology for the presence of ovarian antibodies by IFL were also positive by ELISA (Figure 1). The frequency of antibody positivity by ELISA in this group was not significantly higher compared with the idiopathic POF patient or autoimmune POF patient groups. The mean absorbance (reflecting antibody level) determined by ELISA was significantly higher in the IFL positive group compared to idiopathic POF patient group, however (P < 0.01, Mann–Whitney U test). No difference in the mean absorbances could be determined between the POF patients with associated autoimmune disease and the IFL positive group of patients.

**IFL for ovarian antibodies**

Of the 30 patients with idiopathic POF tested by IFL on monkey ovary substrates, two (7%) were positive. Of these patients demonstrated binding to the ova cytoplasm but the other had an immunofluorescent pattern which was complicated by nuclear staining. This made interpretation of the region of the follicle which was being stained difficult. Neither patient was positive for antibodies to ovary 1 by ELISA.

Three (27%) of the 12 POF patients with an associated autoimmune disease had ovarian antibodies detectable by IFL. Serum from one patient with autoimmune hypothyroidism showed intense staining located to the theca interna but the remaining two sera exhibited a nuclear staining pattern. None of these sera were positive for ovarian antibodies by ELISA.

As expected, none of the 10 controls, randomly selected from the larger control group of 38 women, demonstrated ovarian antibodies by IFL.

**Immunoblotting using microsomal fraction of ovary**

Twenty-one of the 42 POF patient sera (12 idiopathic, nine with an associated autoimmune disease) and 10 of the 38 healthy control sera, described in the Materials and Methods section, were randomly selected for immunoblotting against the microsomal fraction of ovary 2 (because of biopsy size, no material was available from ovary 1). Of the 10 control sera screened against this ovary, two (20%) demonstrated binding. One control serum produced bands at 29, 33 and 40 kDa and one showed 29 and 33 kDa bands only. Eight of the
Table I. Summary of premature ovarian failure (POF) patient serum antibody reactivity to ovary 2, ovary 3 and skeletal muscle. Figures are the sizes of the bands recognized (in kDa) in each preparation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Associated autoimmune disease</th>
<th>2000 g fraction Ovary 2</th>
<th>Ovary 3</th>
<th>Skeletal muscle</th>
<th>Microsomal fraction Ovary 2</th>
<th>Ovary 3</th>
<th>Skeletal muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>26</td>
<td>32</td>
<td>59</td>
<td>64</td>
<td>64,60,36</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Addison’s disease</td>
<td>none</td>
<td>33,27</td>
<td>none</td>
<td>66</td>
<td>none</td>
<td>66,39,37</td>
</tr>
<tr>
<td>3</td>
<td>Hashimoto’s &amp; Addison’s disease</td>
<td>80,30</td>
<td>82</td>
<td>84,32</td>
<td>84</td>
<td>none</td>
<td>66,59</td>
</tr>
<tr>
<td>4</td>
<td>Addison’s disease</td>
<td>100,86,82,72,41</td>
<td>none</td>
<td>none</td>
<td>100,86,82,74–72</td>
<td>none</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>86</td>
<td>82</td>
<td>84</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>none</td>
<td>none</td>
<td>90,37</td>
<td>none</td>
<td>94</td>
<td>none</td>
<td>66</td>
</tr>
<tr>
<td>7</td>
<td>Addison’s disease</td>
<td>48</td>
<td>none</td>
<td>30</td>
<td>none</td>
<td>none</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>78</td>
<td>NT</td>
<td>none</td>
</tr>
<tr>
<td>9</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>86,82</td>
<td>NT</td>
<td>NT</td>
<td>none</td>
</tr>
<tr>
<td>10</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>40</td>
<td>NT</td>
<td>NT</td>
<td>none</td>
</tr>
</tbody>
</table>

NT, not tested.

21 POF sera (38%) demonstrated reactivity and the results are summarized in Table I.

**Immunoblotting using 2000 g fraction of ovary**

Sufficient serum was available from 12 of the 21 POF sera described above (six idiopathic, six autoimmune) and 10 controls to test for reactivity against the 2000 g fraction of ovary 2. Of the 10 control sera screened against this ovary, two demonstrated 80 and 41 kDa bands and one control produced a 74 kDa band. Four (33%) of the 12 POF sera demonstrated antibody reactivity to this antigen fraction (Table I). An example blot is shown in Figure 2.

**Immunoblotting using skeletal muscle**

To identify whether the binding observed was specific for the ovary the sera were also tested for binding to the 2000 g and microsomal fractions of skeletal muscle. The results are shown in Table I. Using this comparison, five patients had antibody binding which was specific for ovary 2 (Table I; patients 4, 6, 8, 9 and 10).

**Immunoblotting using different ovarian preparations**

We have previously demonstrated that there is variation in antibody reactivity to different ovarian samples using the ELISA (Wheatcroft et al., 1994). To examine whether the binding patterns observed above with ovary 2 were consistently detected in a second ovarian antigen preparation, 10 of the POF sera tested against ovary 2 (including sera from patients 1–7) were also tested against the microsomal antigen and 2000 g fractions of a further ovary (ovary 3). The results are also shown in Table I, where they are compared with results from ovary 2.

Only one patient (patient 1) demonstrated binding to the microsomal fraction of ovary 3 and the same binding pattern was also observed to skeletal muscle, indicating non-specific reactivity. Five patients (50%) showed antibody reactivity to the 2000 g fraction of this ovary, although in three there was similar binding to a 2000 g fraction of skeletal muscle, again suggesting non-specific reactivity. Overall, there was no similarity in the immunoblotting results using the two different ovarian preparations.

**Figure 2.** Immunoblot using 2000 g fraction of ovary 2. Lane 1 = patient 4, lane 2 = control, lane 3 = control, lane 4 = patient X (not included in the text), lane 5 = patient 5, lane N = no serum control. The left hand margin indicates the position of the molecular weight (kDa) markers. The bands observed in lanes 2, 3 and 4 are outside the range of measurement using these markers, >116 kDa.

**Discussion**

Indirect immunofluorescence has most commonly been used for the detection of ovarian antibodies in POF but the source of ovarian tissue (human, primate, subprimate) and its maturation state has varied (Moncayo and Moncayo, 1993). Even using human ovarian tissue the incidence of ovarian antibodies determined by IFL has ranged from 2 (Ho et al., 1988) to
50% (Damewood et al., 1986). The maturation state of the ovary is particularly important, since the development of ovarian structures is influenced by the menstrual cycle. We have previously demonstrated variations in antigenicity between ovaries using an ELISA (Wheatcroft et al., 1994).

Sotsiou et al. (1980) compared human and animal substrates for IFL to detect SCA and found that human and monkey gonads gave better results than those from rat or rabbit. Rat testis, for instance, gave a ‘pseudo-Leydig pattern’ with sera which were negative on adrenal, and negative on sections of human and monkey testes. More recently, the clinical usefulness of primate tissue for the detection of human antibodies has been questioned in a comparison of ovarian antibody detection by IFL using monkey ovary substrates from two different commercial sources (Kirsop et al., 1991). Ovarian antibodies were detected in only one of the 30 patients with POF and one of 19 controls. Both of these women were also positive for nuclear antibodies. No significant binding was detected with slides from one commercial source due to a lack of follicles in the sections.

The most sensitive method for laboratory diagnosis of ovarian failure with an autoimmune cause remains to be identified. There have been few studies, however, to compare the results obtained using the current methodologies available, the ELISA, recently developed by Luborsky et al. (1990) and the IFL technique routinely used in clinical laboratories. We have therefore examined the detection of ovarian antibodies using both these techniques in three groups of women, one group with idiopathic POF, one POF patient group with associated autoimmune disease and another group of patients chosen for IFL positivity with ovary sections, three of whom actually had POF.

Only five of 42 patients in the first two groups were antibody positive by IFL on monkey ovary sections and even then the antibodies detected were in part nuclear. Furthermore, none of these five were positive in the ELISA. In contrast, the frequency of antibody positivity by ELISA for the third group of IFL positive patients was 53%. The mean absorbance level in the ELISA was also significantly higher for the IFL positive group compared to the idiopathic, but not the autoimmune, POF groups. Previous authors (Gobert et al., 1990) described a good agreement between positivity in IFL and ELISA (94%) for a group of patients selected for IFL positivity, although the source of tissue used for both of these techniques was human. Our attempts to use human ovaries for the detection of antibodies was not successful due to a large amount of autofluorescence.

Our previous studies using the ELISA have indicated that the disease specificity of ovarian antibodies is uncertain as they are frequently detected in Turner’s syndrome and iatrogenic ovarian failure (Wheatcroft et al., 1994). The acceptance of the clinical usefulness of autoantibody determination by IFL has also been hampered by the presence of autoantibodies in some asymptomatic or unaffected individuals (Riley, 1995). In order to determine the diagnostic relevance of ovarian antibodies in idiopathic and autoimmune POF, therefore, further characterization is required, which might also help to explain the different results obtained by IFL and ELISA. We therefore performed immunoblotting studies to establish whether there is any discrete autoantigen against which the antibodies in these patient sera are directed. Immunoblotting has previously been used to demonstrate the presence of autoantibodies to the recombinant cytochrome P450 enzymes, P450c17 and P450sc in APS-I patients with associated ovarian failure (Uibo et al., 1994; Winqvist et al., 1995), but do not occur in idiopathic POF (Wheatcroft et al., 1994).

Immunoblotting has also been used to identify the sizes of the target autoantigens in an experimental model of autoimmune oophoritis: two independent monoclonal antibodies derived from neonatally thymectomized mice bound to proteins of 80/85 kDa and 82 kDa in mouse, pig and rat ovaries (Xun et al., 1993). Immunofluorescence using mouse ovary sections showed that these monoclonals stained the interstitial region around the follicles. Interstitial cells are derived from cells of the theca interna during atresia of the follicle, physiologically resemble the theca interna and contain LH receptors (Erickson and Yen, 1984). These antibodies may therefore recognize proteins involved in steroid synthesis.

Our immunoblotting studies utilizing two human ovarian microsomal and 2000 g antigen preparations were unable to demonstrate a consistent pattern of binding either for the idiopathic or the autoimmune groups of patients. A doublet pattern of binding similar to that observed by Xun et al., (1993) was produced by two idiopathic POF patient sera, both recognizing an 82.86 kDa doublet of protein bands in the microsomal fraction of the ovary 2 homogenate. This pattern was not reproducible using another ovarian antigen preparation, however. A large IgG heavy chain band was produced in the 52–63 kDa region using this sensitive ECL technique. This could mask underlying protein bands particularly in the 2000 g fraction where binding to the cytochrome P450 enzymes might be expected. Attempts to delete the protein fractions of IgG by incubation with protein-A coated Sepharose beads were unable to remove this non-specific binding.

The absence of serum antibodies to the ovary in a large proportion of POF patients using the ELISA and IFL suggests that antibodies are not initial mediators of the disease; T cells are more likely to be crucial to pathogenesis. Experimental studies of animal models have previously shown that zona pellucida 3 (ZP3)-specific T helper cells are sufficient for the induction of oophoritis in recipient mice (Rhim et al., 1992) while, more recently, antibodies to the ZP without the concomitant T cell response were shown to be insufficient for the induction of the disease (Lou et al., 1995). T cell-mediated cytotoxicity and damage resulting from the local release of cytokines could therefore be the initiating cause of ovarian destruction in POF resulting in the release of intracellular antigens. In turn this could lead to an antibody response to a variety of antigens. In support of this hypothesis, Lou and Tung (1993) demonstrated that immunization of mice with a pure T cell epitope for ZP3 resulted in the production of autoantibodies against not only the self protein but also to other determinants on the ZP antigen. The amplified ZP antibodies were directed mainly to conformational determinants on the native ZP. In the human disorder of POF the involvement
of T cells remains speculative and further work to examine the role of T cells is warranted.

In summary, comparisons between the ELISA and IFL methods for the detection of ovarian antibodies demonstrate that neither is a robust test for the presence of these antibodies. Although there is a some overlap between the two techniques, there were also clear discrepancies, perhaps due to species differences in substrates. A characterized target antigen or group of antigens for idiopathic POF would allow a reliable diagnostic test for autoimmune for these patients to be developed. Our immunoblotting studies were unable to demonstrate a consistent pattern of binding using the sera of these patients on two ovarian antigen preparations however. Until the specific ovarian antigens have been identified and included in one assay, it seems likely that the IFL assay will remain the standard as a detection method for ovarian antibodies in the diagnosis of autoimmune POF.

Acknowledgements

We are grateful to Dr S.Odukoya and Dr J.Jenkins for help obtaining ovarian tissues and some serum samples, Mrs M.Fish for technical advice regarding some of the immunofluorescence studies and Mr Alun Price, Department of Clinical Chemistry, for the provision of the control serum samples. This work was supported by Wellbeing.

References


