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Detection of antibodies to ovarian antigens in women with premature ovarian failure

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SUMMARY

Premature ovarian failure is a common condition of uncertain aetiology in most cases, although autoimmunity is thought to play a role in a proportion of cases. The frequency of ovarian antibodies, which may be markers for an autoimmune aetiology in this condition, remains unclear. To define this further, we have examined the sera of 45 women with premature ovarian failure (five with iatrogenic ovarian failure, nine with an associated autoimmune disease, and 27 with idiopathic ovarian failure), as well as four women with infertility due to Turner's syndrome and 41 pre- and post-menopausal controls. Using two human ovarian antigen preparations, 24% and 60% of the ovarian failure patients reacted in an ELISA ($P < 0.05$ and $P < 0.001$ compared with controls), but frequent cross-reactivity was found with fallopian tube antigens. The apparent aetiology of ovarian failure did not correlate with the presence of ovarian antibodies. Using bovine ovary as an antigen, there was a significant overall increase in binding by the ovarian failure patients, but this was almost identical to binding in an ELISA with bovine fallopian tube. In contrast to a previous report, there was no significant increase of binding to soluble or Triton-extracted membrane fractions of bovine corpora lutea containing the LH/hCG receptor by the patients with ovarian failure. These results suggest that ovarian antibodies are common in premature ovarian failure, but their specificity and pathogenic role are questionable.

Keywords autoimmunity ovarian failure ovarian antibodies

INTRODUCTION

Premature ovarian failure (POF) is a common condition affecting 1% of women. It is defined as secondary amenorrhoea accompanied by gonadotrophin measurements in the menopausal range, occurring before the age of 40 years [1]. Once chromosomal, developmental and idiopathic causes have been excluded, autoimmunity is thought to account for up to 20% of the remaining cases, although the diagnosis is usually based on clinical grounds, such as an association with other autoimmune endocrinopathies. In particular, there is a strong association with Addison's disease. For instance, in one series of 157 women with idiopathic Addison's disease, 6% had oligomenorrhoea and 24% had amenorrhoea [2]. However, the absence of other endocrinopathies in the majority of cases of POF, the so-called 'idiopathic' group, leaves a large group of women in whom the aetiology is unclear.

In 1966, antibodies to ova cytoplasm were described in a woman with POF [3]. Since then, ovarian antibodies have been found in such women with POF with varying degrees of success. One study of 19 patients with hypergonadotrophic ovarian failure found 14 with histologically proven POF characterized by an absence of primordial follicles, while three showed histology characteristic of 'resistant ovary syndrome' with the presence of primordial follicles but little or no follicular development: of the 13 patients whose sera were screened for autoantibodies, only one showed circulating antibodies against ovary [4]. By contrast, in a group of 40 women with chronic vaginal candidiasis and menstrual irregularities, 27 had ovarian antibodies, and, of these, five had POF. In addition, a strong correlation was found between the presence of ovarian and *Candida* antibodies [5]. More recently, Moncayo *et al.* developed an ELISA using microsomes prepared from bovine corporal lutea as the antigen, and detected significant levels of ovarian antibodies in patients undergoing treatment for sterility, although all had normal gonadotrophin levels [6]. Furthermore, immunoglobulins which inhibit follicle-stimulating hormone (FSH)-mediated ovarian granulosa cell DNA synthesis were found in 21 of 26 women with POF using Feulgen densitometry, but these did not inhibit luteinizing hormone (LH)-stimulated steroidogenesis *in vitro* in the mouse Leydig cell assay [7].

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A reliable assay for ovarian antibodies would provide a useful serological marker in POF, enabling patients with autoimmunity to be identified early in the course of the disease, as they may benefit from the use of disease-modifying therapy (in particular steroids) which has led to a number of successful pregnancies [8]. We have therefore studied the sera of women with POF due to a variety of causes by ELISA to compare the prevalence and specificity of antibodies against human and bovine ovarian antigens.

PATIENTS AND METHODS

Patients and controls

Serum from 45 sequential patients with infertility attending an infertility clinic was stored at −80°C until required. Four had Turner's syndrome and five had iatrogenic POF; since autoimmunity seemed to be excluded with these diagnoses, they were considered together. The aetiology was unclear in the remainder. However, nine of these 36 women were diagnosed as having a probable autoimmune aetiology for their POF by the gynaecologist, based on the concurrence of Addison's disease ($n=6$; one patient also had Hashimoto's thyroiditis), hypothyroidism ($n=2$) or rheumatoid arthritis ($n=1$). These patients were therefore distinguished from the other 27 women who were regarded as having idiopathic POF. Controls ($n=41$) were provided by the Department of Clinical Chemistry from a bank of normal serum from the general population sent for lipid screening: 16 were premenopausal and 28 were post-menopausal. Women with untreated Graves' disease ($n=23$) served as a second control group.

Source of antigens

Paired ovarian and fallopian tube specimens were obtained from two women undergoing hysterectomy. The first patient was a 43-year-old (blood group A, Rhesus negative) with dysmenorrhoea and menorrhagia but an otherwise normal menstrual cycle. The operation was performed on the twelfth day of the cycle; the other ovary contained a corpus luteum. The fallopian tube was histologically normal. The second specimen was from a 43-year-old (blood group A, Rhesus positive) with dysfunctional uterine bleeding with menorrhagia and no regular menstrual cycle. The patient was taking the oral contraceptive pill. The fallopian tube was again histologically normal. To rule out possible reactivity with isoagglutinins, reacting with blood group A substance, a third specimen was obtained from a 44-year-old (blood group O, Rhesus positive) patient undergoing hysterectomy. Her menstrual cycle had been halted pharmacologically before the operation. A fallopian tube was not obtained from this patient. In addition, bovine ovary, kidney and uterus were obtained from the local abbatoir. As further control antigens, we used human skeletal muscle and thyroid to prepare membranes and human thyroglobulin (TG) prepared as described elsewhere [9].

Preparation of antigens

Tissue was diced and stored in liquid nitrogen immediately after removal until required. The material was thawed on ice, and then dispersed, using a Polytron tissue homogenizer, in PBS containing the protease inhibitors N-tosyl-L-lysine chloromethyl ketone 100 μM (Novabiochem, Nottingham, UK), N-tosyl-phenylalanine chloromethyl ketone 100 μM (Sigma Chemical Co., Poole, UK), phenylmethyl sulphonyl fluoride 1 μM (Sigma) and pepstatin A 10 μM (Novabiochem). The resulting suspension was centrifuged at 2000 *g* for 10 min and the supernatant then centrifuged at 100 000 *g* for 60 min. The pellet was washed once in PBS before being resuspended in 2 ml PBS. All the above stages were carried out at 4°C. The suspensions were depleted of IgG by incubation with protein A-coated Sepharose beads (Pharmacia, Hounslow, UK) according to the manufacturer's instructions. The protein content of the final suspension was determined using a dye binding colorimetric method [10]. The membrane preparations were sonicated immediately before use.

Bovine corpora lutea were prepared and the antigens given the same designation as described elsewhere [6]. Briefly, after carefully excising the connective and fibrous tissue, the corpora lutea were dissected and homogenized using a Polytron homogenizer. The resulting filtered homogenate was centrifuged at 3000 *g* for 30 min. The 3000 *g* supernatant was then centrifuged for 60 min each at 20 000 *g*, 80 000 *g* (the pellet being kept and termed P4) and 288 000 *g* (the supernatant being termed S5). The 20 000 *g* pellet was solubilized using 1% Triton in PBS during 60 min at room temperature followed by centrifugation at 288 000 *g*. The supernatant and pellet from this were termed S3T and P3T, respectively.

ELISA

A 96-well microtitre plate (Costar, Cambridge, MA) was coated with antigen by the addition of 100 μl homogenate (25 μg/ml in carbonate buffer pH 9·2) to each well and storing at 4°C overnight. After antigen coating, excess homogenate was removed and non-specific sites blocked with 3% bovine serum albumin (BSA) in wash buffer (1 h at 37°C). Wells were incubated with POF serum or control serum (1:12 dilution for 2 h at 37°C unless stated) followed by goat anti-human IgG conjugated to biotin (Sigma; 1:1000 dilution for 2 h at room temperature) and with streptavidin-alkaline phosphatase (Sigma; 1:1000 dilution for 1 h at room temperature). The reaction was then developed with alkaline phosphatase substrate (Sigma). The plates were read in a Dynatech UK 5000 automatic plate reader at 410 nm. All samples were assayed in duplicate and the mean calculated. A second antibody control in which the serum was omitted was used in each ELISA plate. This provided a measure of background which could be subtracted from the sera under test. Each assay included a positive and negative control serum defined by preliminary experiments. Plates were coated with the four bovine corpora lutea antigens (P3T, S3T, P4, S5) at 25 μg/ml and the ELISA performed as described, except that sera were used at 1:100 dilution.

Statistical analysis

Values greater than the mean control value + 2 s.d. were taken as positive binding, a cut-off point for positivity used by previous authors [8] using the ELISA to detect ovarian antibodies. The χ^2 test on 2×2 contingency tables was used for statistical analyses.

RESULTS

Binding to human antigens

Forty POF sera were tested on the first ovarian antigen preparation and 41 on the second. The results are shown in Table 1 and Figure 1. The number of POF patients reacting with

Table 1. Number of subjects in each group positive for binding to human ovary and fallopian tube

Group	Antigen preparation: Ovary (donor 1) (%)	Fallopian tube (donor 1) (%)
Controls ($n=33$)	2 (6)	3 (9)
Autoimmune POF ($n=6$)	2 (33)*	3 (50)*
Idiopathic POF ($n=26$)	4 (15)	2 (8)
Turner's syndrome and iatrogenic POF ($n=8$)	3 (38)*	1 (13)
	Ovary (donor 2) (%)	**Fallopian tube (donor 2) (%)**
Controls ($n=41$)	2 (5)	1 (2)
Graves' disease ($n=23$)	4 (17)	1 (4)
Autoimmune POF ($n=6$)	2 (33)*	2 (33)
Idiopathic POF ($n=26$)	16 (62)†	4 (15)*
Turner's syndrome and iatrogenic POF ($n=9$)	7 (78)†	3 (33)†

* $P<0.05$ compared with controls; † $P<0.001$ compared with controls (χ^2 test).

Positivity defined as an absorbance in the ELISA > 2 s.d. above the control group mean.

POF, Premature ovarian failure.

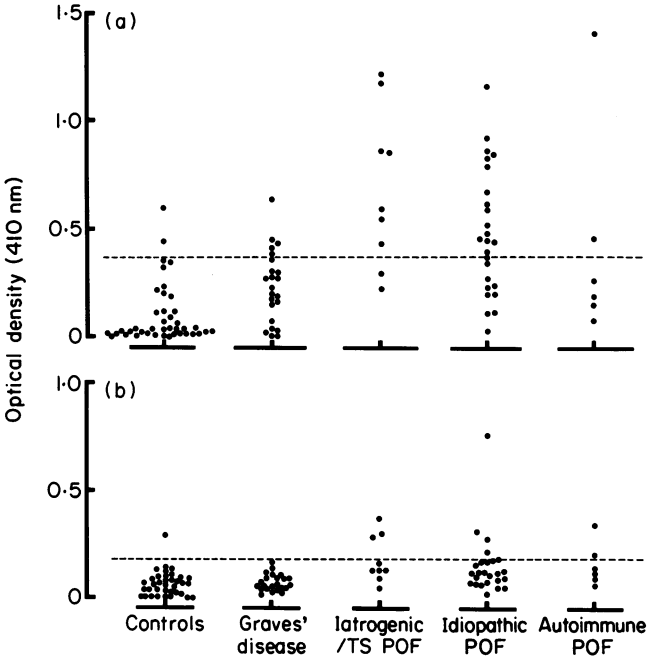

Fig. 1. Dot plots showing patient and control reactivity against (a) human ovary 2, (b) human fallopian tube 2. The dotted line represents the mean+2 s.d. of the reactivity produced by normal control sera. POF, Premature ovarian failure.

ovary was significantly greater than controls ($\chi^2=3.81$; $P<0.05$ for donor 1 and $\chi^2=29.9$; $P<0.001$ for donor 2). Significant binding was also observed for the whole POF patient group compared with Graves' patients when tested on human ovary 2 ($\chi^2=10.7$; $P<0.001$). Overall, 10 POF patients (24%) reacted with the first ovarian antigen preparation and 24 (60%) with the second (i.e. greater than mean+2 s.d. above control values). There was no significant difference in binding between the three subgroups of patients with either ovarian antigen. A significant but incomplete correlation existed between binding of POF sera to the two ovarian antigen preparations ($r=0.51$, $P<0.001$). One iatrogenic POF patient was recognized by donor ovarian antigen 1 and not by ovarian antigen 2, but all other patients who were positive with ovary 1 were positive with ovary 2. Reactivity diluted out in a log-linear fashion in four sera tested, but did not differ significantly from controls at a 1:48 dilution in three of these samples: the remaining serum was still giving a higher absorbance than controls at 1:96 dilution (data not shown).

Sufficient sample remained to test 22 of the 26 sera that gave positive ovarian reactivity with either source of antigen for rheumatoid factor by latex agglutination: five samples were positive. In addition, sufficient sera remained from 37 patients to test on a group O Rhesus-positive ovary, to eliminate any reactivity due to high titre isoagglutinins which react with blood group antigens, interfering with autoantibody determination. Positivity was observed for 17% of the patient sera tested. This value would probably be an underestimate due to the elimination of four patient sera which had tested positive with the donor ovaries described previously, as well as three which had shown a positive result with donor ovary 2 only.

The POF and control sera were tested concurrently for binding to fallopian tube membranes, taken from the same donors to serve as a control antigen. As shown in Table 1, significant binding was observed in some POF subgroups with both fallopian tube antigens. The correlation coefficient between binding to ovarian and fallopian tube antigens was $r=0.69$ ($P<0.001$) using donor 1 and $r=0.41$ ($P<0.01$) for donor 2. Generally, antibodies in POF patient sera which reacted with donor fallopian tube antigen 2 only occurred when ovarian antibodies were detected, although one autoimmune POF patient had fallopian tube antibodies only. One Graves' patient and one control had only fallopian tube antibodies. With fallopian tube 1, one POF patient and three controls had antibodies in the absence of ovarian antibodies. Considering the entire POF patient group, binding was not significant compared with controls for human fallopian tube 1 ($\chi^2=0.58$; $P>0.25$) but significant binding was observed against fallopian tube 2 ($\chi^2=7.3$; $P<0.01$ compared with controls, $\chi^2=3.5$; $P>0.05$ compared with Graves' disease). Only five patients (12%) reacted exclusively with ovarian antigen from donor 1, while 16 (40%) reacted exclusively with ovarian antigens from donor 2.

The POF sera generally reacted less frequently with other control antigens than with ovarian membranes: seven (18%) gave binding greater than 2 s.d. above the control mean with human skeletal muscle membranes, and 10 (25%) bound to human thyroid microsomal antigen. Nine patients (21%) had antibodies against TG (two patients with Turner's syndrome, two with Addison's disease and four patients with idiopathic POF). One patient with idiopathic POF reacted with TG only, and two patients with idiopathic POF had only thyroid microsomal antibodies. One control serum had antibodies

Table 2. Percentage inhibition of antibody binding to fallopian tube antigen (donor 1) by added antigen

	Added antigen					
	Fallopian tube, %		Ovary, %		Skeletal muscle, %	
	500 μg/ml	50 μg/ml	500 μg/ml	50 μg/ml	500 μg/ml	50 μg/ml
POF serum 1	89*	60	41	20	14	9
POF serum 2	95	50	39	5	NT	NT
POF serum 3	86	45	31	11	34	2

* Values represent the percentage reduction in absorbance produced by serum in the presence of added antigen compared with serum without added antigen.
POF, Premature ovarian failure; NT, not tested.

Table 3. Number of subjects in each group positive for antibodies binding to bovine ovary and fallopian tube

Group	Antigen preparation	
	Bovine ovary (%)	Bovine fallopian tube (%)
Controls ($n=30$)	2 (7)	2 (7)
Autoimmune POF ($n=9$)	4 (44)*	4 (44)*
Idiopathic POF ($n=26$)	3 (12)	4 (15)
Turner's syndrome and iatrogenic POF ($n=9$)	4 (44)*	4 (44)*

	P3T (%)	P4 (%)	S5 (%)	S3T (%)
Controls ($n=44$)	2 (5)	4 (9)	4 (9)	2 (5)
Autoimmune POF ($n=9$)	2 (22)	3 (33)*	2 (22)	1 (11)
Idiopathic POF ($n=26$)	3 (12)	6 (24)	4 (15)	3 (12)
Turner's syndrome and iatrogenic POF ($n=8$)	4 (50)*	4 (50)*	3 (38)*	2 (25)

* $P<0.05$ compared with controls (χ^2 test).
Positivity defined as an absorbance in the ELISA >2 s.d. above the control mean.
POF, Premature ovarian failure.

against both TG and thyroid microsomal antigen. POF patient reactivity to skeletal muscle was not significantly different from controls, but there was greater binding to TG and thyroid microsomal antigen ($\chi^2=4.38$ and 5.82, respectively; both $P<0.05$).

The ability of various antigens to inhibit serum binding to fallopian tube antigen was tested by incubating serum with antigens for 1 h at room temperature before addition to the fallopian tube ELISA. As shown in Table 2, almost complete inhibition of binding was produced by added fallopian tube antigen at 500 μg/ml, but significant inhibition of 31–41% also occurred with ovarian antigens at this concentration, with slightly less inhibition in the presence of skeletal muscle antigen. Insufficient ovarian antigen remained after these experiments to assess the effect of added fallopian tube antigen on binding in the ovarian antigen ELISA.

Binding to bovine antigens

Of 44 POF sera tested, 11 (25%) gave positive binding to bovine ovarian membranes ($\chi^2=4.1$; $P<0.05$ compared with controls). The results are shown in Table 3 and Fig. 2a. Within the subgroups, there was a significant difference from controls in the autoimmune and Turner's syndrome/iatrogenic groups of patients ($\chi^2=7.59$; $P<0.01$ for both). The same subgroups showed significant binding against bovine fallopian tube ($\chi^2=7.59$; $P<0.05$ for both). There was a close relationship between the binding to bovine ovarian and fallopian tube antigens ($r=0.81$, $P<0.001$); only one POF serum bound significantly to ovarian but not fallopian tube membranes. Some sera also bound to bovine uterus (21%) and kidney (17%): these were significantly different from controls in the case of bovine uterus ($\chi^2=4.83$; $P<0.05$) but not for bovine kidney ($\chi^2=2.5$; $P>0.05$), and there was a significant correlation with binding to bovine ovary ($r=0.63$; $P<0.001$ and $r=0.48$; $P<0.001$, respectively). The correlation between the binding of POF sera to human and bovine ovarian antigens was not significant ($r=0.32$ for human donor 1 and 0.29 for donor 2). In an attempt to determine if patient reactivity (and cross-reactivity) with bovine antigens was due to species-specific determinants, absorption experiments, which have previously

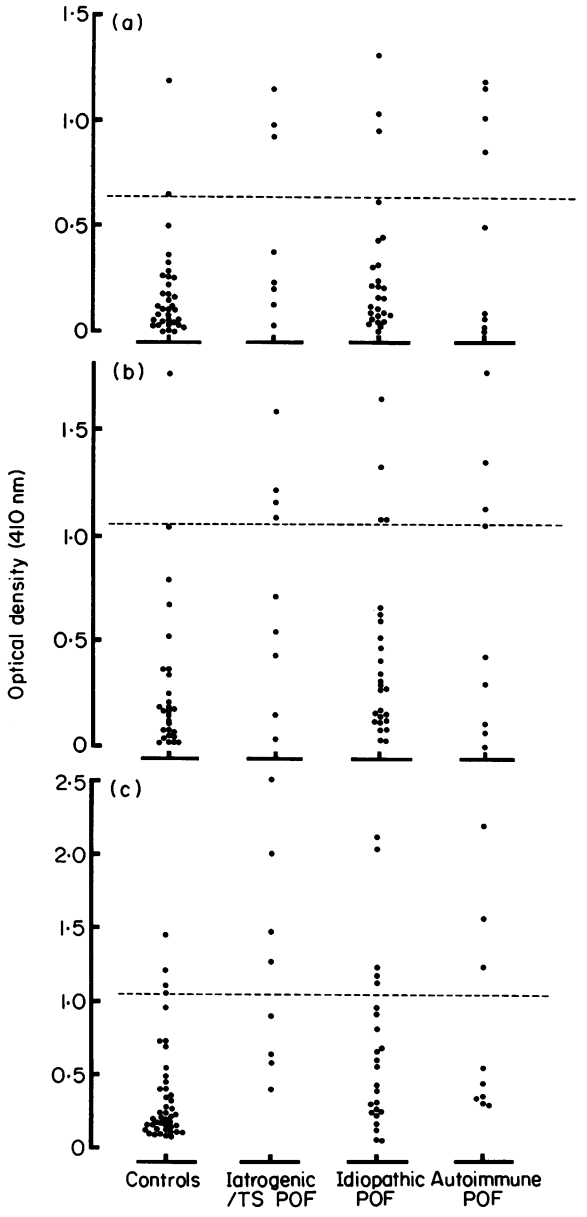

Fig. 2. Dot plots showing patient and control reactivity against (a) bovine ovary, (b) bovine fallopian tube and (c) corpus luteum fraction P4, as determined by ELISA. The dotted line represents the mean+2 s.d. of the normal reactivity produced by control sera. POF, Premature ovarian failure.

been described by others [6], were performed using eight of the sera which had shown positive binding with bovine ovary. Sera were preabsorbed with bovine liver powder before being used in a conventional ovarian ELISA. None of the sera tested showed a decrease in signal after preabsorption (data not shown).

Using the nomenclature of Moncayo *et al.* [6], there was no significant difference between POF patients (together or in subgroups) and controls in their binding to the S3T fraction of bovine corpora lutea and with S5, only the Turner's syndrome/iatrogenic POF subgroup showing significant binding ($\chi^2 = 4{\cdot}69$; $P < 0{\cdot}05$). The Turner's syndrome/iatrogenic subgroup showed significant binding against P4, the 80000 *g* pellet ($\chi^2 = 8{\cdot}70$; $P < 0{\cdot}01$) and P3T, the 288000 *g* pellet ($\chi^2 = 13{\cdot}7$; $P < 0{\cdot}001$). Higher binding was seen with P4 in all POF subgroups, with 24–50% of patients positive compared wiith 9% of control sera (Fig. 2c). The autoimmune and Turner's syndrome/iatrogenic subgroups both showed significant binding to P4 compared with controls ($\chi^2 = 3{\cdot}83$; $P < 0{\cdot}05$ and $\chi^2 = 8{\cdot}7$; $P < 0{\cdot}01$, respectively).

DISCUSSION

Several reports have indicated that premature ovarian failure is reversible in some patients. Return of ovarian function in two patients with myasthenia gravis followed immunosuppressive therapy [11,12], and short courses of corticosteroid treatment have also been used to produce temporary resumption of menstruation in two patients, one with ovarian antibodies [13] and the other with evidence of ovarian lymphocytic infiltration [14]. More recently, it has been suggested that the presence of non-ovarian antibodies and abnormal complement levels may identify those patients who are good candidates for ovulation induction and conception [15]. However, reliable markers for the presence of autoimmune ovarian disease have not been established.

Our results show that ovarian antibodies occur in a significant number of patients with POF. Luborsky *et al.* [8], using an ELISA, showed that about 70% of sera from POF patients contained antibodies against human ovary and oocytes. Earlier data using immunohistochemistry suggested that ovarian antibodies occurred in 18–60% of patients with POF [16–18], compatible with our study in which serum from 60% of POF patients bound to human ovary 2, although with another antigen donor only 24% were positive. This variation in reactivity between ovary preparations could be due to a cyclical variation in antigenic proteins, and indicates the need to screen ovarian preparations for reactivity.

Significant binding of POF sera was observed with both human fallopian tube antigens which were used as controls in this ELISA, but binding to skeletal and thyroid antigens was less prevalent. Inhibition experiments using two POF patients (POF 1 and POF 3) which reacted with both donor ovaries and fallopian tubes also confirmed that there was partial cross-reactivity of patient sera between the ovary and fallopian tube antigens. It appears, therefore, that the POF sera react against multiple ovarian antigens, some of which are cross-reactive with fallopian tube. Unfractionated bovine ovarian membranes appear to be less suitable than human material as an antigen in ELISA, because of the lack of correlation in the binding to the two sources of antigen: the close correspondence between binding to bovine ovarian and fallopian tube suggests reactivity to species-specific determinants, although absorption studies with bovine liver powder using a method described by Moncayo *et al.* [6] showed no decrease in reactivity.

Only five of the sera reacting with ovarian antigens were positive for rheumatoid factor, and the results with the blood group O ovary suggest that reactivity of isoagglutinins with blood group substances does not account for the results. The likelihood that positive ELISA reactivity in these patients could be due to anti-HLA activity is small, as this would only be observed in patients who had previously been pregnant (unlikely in POF patients), or if the patient had been transfused. Also, the normal subjects should control for any of these non-specific reactions. Finally, 50% of the patients positive with

ovarian antigen from donor 1 were not positive with fallopian tube antigen from the same donor, and 67% of the patients positive with ovarian antigen from donor 2 reacted exclusively with this antigen. As the fallopian tube was prepared in the same way to the ovary as a source of antigen, and should contain the same blood group substances, HLA antigens and immunoglobulins, it serves as control for serological reactivity with these antigens, and the dichotomy between the two preparations indicates binding (of a proportion of sera) to specific ovarian antigens.

Although two of six POF patients with an associated autoimmune disease tested had antibodies reacting with human ovary 1 (Addison's disease and rheumatoid arthritis) and against human ovary 2 (both Addison's disease), the remaining patients were negative. A proportion of women with autoimmune adrenal failure have circulating anti-steroid cell antibodies which react with steroid-synthesising cells in the adrenal cortex and in the ovary [2,19–21], and this may be the explanation for the reactivity in the Addison's patients tested in this study. However, not all Addison's patients with POF had detectable antibodies. This could be due to the stage of the disease, as not all antibodies can be detected at a single time point in the disease process [22], or assay insensitivity. Alternatively, the POF could have a non-antibody-dependent cause in these patients. Immunofluorescence assays on green monkey ovary sections (performed by Dr A. M. Ward, Department of Immunology) for both the POF patients described above were negative, and a more extensive comparison between immunofluorescence assays (using human and monkey ovary) and ELISA seems warranted.

As reviewed elsewhere [23], many cases of presumed autoimmune oophoritis, occurring in patients with conditions such as hypothyroidism, hypoparathyroidism, rheumatoid arthritis or pernicious anaemia, lack demonstrable anti-ovarian antibodies, and have had no lymphocytic infiltrate of their ovaries. Whether such patients have a non-immunological cause for POF, occurring by coincidence with an autoimmune disorder, remains to be determined. While a high percentage of patients with Addison's disease have steroid cell antibodies, these are only rarely found in POF patients without adrenal failure [21,24,25], making reactivity with this poorly defined antigen unlikely in our non-Addisonian patients. One possible shared antigen in steroid cells is 17α-hydroxylase [26], but the seven patients who were most strongly positive in our assay system were negative when tested for such antibodies. These seven patients were also tested for antibodies to 21α-hydroxylase and side chain cleavage enzyme and were negative (assays were kindly performed by Professor K. Krohn and colleagues, University of Tampere, Finland).

As shown here and previously [27], ovarian antibodies can be observed in patients with XO gonadal dysgenesis, and the presence of ovarian antibodies therefore does not necessarily indicate an autoimmune aetiology for ovarian failure. We have also observed ovarian antibodies in patients with an iatrogenic cause for POF (either due to chemotherapy or total body irradiation). This could be due to the trauma and ovarian antigen release associated with these procedures.

The hormone receptors for FSH and LH could be the targets in autoimmune POF by analogy with other autoimmune endocrine disorders. Immunofluorescent techniques showing specific localization of antibody binding to the theca interna first suggested that the LH receptors may be involved in the pathogenesis of POF [24,28]. However, others have been unable to demonstrate the presence of antibodies against gonadotrophin hormone receptors [26,29]. Moncayo *et al.* [6] used an ELISA to detect antibodies to the hormone receptor complex of bovine corpus luteum of a group of patients with primary and secondary sterility but with normal gonadotrophin levels. They demonstrated that 35% of patients with infertility present with serum autoantibodies that are directed against the corpus luteum. They found that these patients exhibited similar reactivity to the soluble (S4) and extractable (S3T) antigen preparations of the corpus luteum. In contrast, we did not observe any reactivity of our group of patients with either the S5 (used rather than S4) or the S3T fractions. This discrepancy in results could be due to differences in the patient group being examined, as our group consisted of premature ovarian failure patients with elevated gonadotrophin levels and was similar to previous studies [28,29] which also could not detect anti-gonadotrophin receptor antibodies. The disease process in these two groups could be different.

Chromatofocusing of the extractable S3T membrane fraction by Moncayo *et al.* [6] resulted in two main peaks. Reactivity was observed against both these peaks, showing that binding was not just to the hormone (LH/hCG) receptor present in this fraction, but also to other components. Our ELISA showed binding predominantly to the P4 fraction, suggesting that one autoantigen could be in the ovarian microsomal fraction. The presumed autoimmune POF patients had antibodies only against P4, whereas those with iatrogenic POF or Turner's syndrome had antibodies against P4 and P3T, suggesting that antibodies in these groups could be recognizing separate components of the corpora lutea. Further studies using Western blotting should resolve these issues.

In conclusion, we have found ovarian autoantibodies in POF by ELISA which are separate from steroid cell antibodies, but have been unable to confirm the presence of antibodies against fractionated bovine corpora lutea in these patients. Ovarian preparations vary greatly in their capacity to bind these antibodies, and at least a proportion of ovarian antibodies detected by this method are not specific, as they cross-react with other antigens such as those in the fallopian tube. Furthermore, ovarian antibodies are not exclusive to patients with autoimmune Addison's disease, occurring in patients with idiopathic ovarian failure, chromosomal abnormalities, and in patients who have had total body irradiation and chemotherapy. These factors may explain the divergent results obtained by other groups using radioimmunoassay, immunofluorescence and ELISA, and also suggest that these antibodies are unlikely to have diagnostic or pathogenic significance. Rather, they may arise as a result of non-specific tissue injury. Nonetheless, molecular characterization of the autoantigen(s) recognized seems worthwhile, as this approach could provide important clues to the identity of the target for T cell-mediated autoimmune responses in POF.

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