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Association between thyroid microsomal antibodies of subclass IgG-1 and hypothyroidism in autoimmune postpartum thyroiditis

ROLF JANSSON,* PHILIPPA M. THOMPSON, F. CLARK & SANDRA M. McLACHLAN, **Department of Internal Medicine, University Hospital, Uppsala, Sweden and Departments of Medicine and Pathology, University of Newcastle upon Tyne, UK*

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SUMMARY

The potential role of thyroid microsomal (Mic) antibodies in the development of postpartum hypothyroidism was investigated in 34 euthyroid women, whose sera were found to contain Mic antibodies in pregnancy. Additional serum samples were obtained 2, 5 and 10–12 months after delivery and analysed for IgG class and IgG subclass levels of Mic antibodies by ELISA techniques. Characteristically, Mic antibodies decreased from early pregnancy to 2 months postpartum, increased two-fold 5 months postpartum and had returned 10–12 months postpartum to the early pregnancy level. Mic antibodies were predominantly subclass IgG-1 or IgG-4 with only minor contributions from IgG-2 and IgG-3. In each individual the percentage contribution made by each IgG subclass to Mic antibody was essentially similar in early pregnancy and the postpartum period despite changes in total IgG class Mic antibody.

During the year following delivery, thyrotoxicosis alone (Graves' disease) developed in 5 women. In the remaining 29 patients the absolute levels of Mic antibodies of IgG-4 subclass were similar 5 months postpartum in women with maximal serum thyrotropin (TSH) >20 mU/l (mean optical density in ELISA $\pm$ s.d.; $0{\cdot}84 \pm 0{\cdot}538$; $n=13$) and in women with maximal TSH <10 mU/l ($0{\cdot}69 \pm 0{\cdot}457$; $n=16$). In contrast, significantly higher values were observed for Mic antibody of IgG-1 subclass in patients with TSH >20 mU/l ($1{\cdot}14 \pm 0{\cdot}440$) compared with women with maximal TSH <10 mU/l ($0{\cdot}65 \pm 0{\cdot}289$) ($P<0{\cdot}001$ by t-test for groups).

These results imply that the magnitude of Mic antibody levels of subclass IgG-1 but not IgG-4 is associated with the development of postpartum hypothyroidism and possibly with tissue destruction in autoimmune thyroid disease in general.

Keywords thyroid microsomal (Mic) antibodies postpartum hypothyroidism (ELISA) enzyme linked immunosorbent assay

INTRODUCTION

Transient postpartum hypothyroidism develops frequently in clinically euthyroid women with serum thyroid microsomal (Mic) autoantibodies (Amino *et al.*, 1982; Jansson *et al.*, 1984a). The antibody titres, which characteristically decrease during pregnancy and increase again after delivery, reach maximal levels between 5–7 months postpartum and at the same time some of the

Correspondence: Dr Rolf Jansson, Department of Internal Medicine, University Hospital, S-751 85 Uppsala, Sweden.

women become hypothyroid (Jansson *et al.*, 1984a). Subsequently, the Mic antibody titres fall, reaching the early pregnancy values around 10–12 months postpartum by which time the hypothyroidism has subsided. These changes in the postpartum period seem to reflect a transient immunological 'rebound' following pregnancy-induced immunosuppression (Amino & Miyai, 1983; Jansson, 1984).

Previously we reported that hypothyroidism was more pronounced in women with high Mic antibody titres than in women with low titres (Jansson *et al.*, 1984a; Jansson, 1984). However, we observed that this phenomenon was not absolute since some high titre patients did not develop the expected degree of hypothyroidism. Consequently, we have analysed the Mic antibodies further using a recently established ELISA system to determine their distribution among the IgG subclasses (Parkes *et al.*, 1984). Interestingly, the development of postpartum hypothyroidism was found to be associated predominantly with Mic antibodies of subclass IgG-1.

MATERIALS AND METHODS

Patients and serum samples. Sera were obtained around 2, 5 and 10–12 months after delivery from 34 healthy women (mean age 28 years, range 19–38 years), participating in two prospective studies of autoimmune thyroid dysfunction in the postpartum period (Jansson *et al.*, 1984a; Jansson, 1984). In 15 of the women a serum sample from the first trimester of pregnancy was included as well. All women had Mic antibody titres of 1:100 or greater as determined by haemagglutination tests (Thymune-M, Wellcome Ltd, Beckenham, UK) in a serum sample taken in the first trimester of pregnancy. Two women had a previous history of surgically treated Graves' thyrotoxicosis and three reported a symptomless adolescent goitre. All women were HLA-A, HLA-B, and HLA-DR typed using methods previously reported (Jansson, Säfwenberg & Dahlberg, 1985). Nineteen women (54%) were HLA-DR4 positive. In all women serum thyroxine, triiodothyronine, T3-resin uptake and thyrotropin (TSH) were determined regularly during the first year after delivery by methods in routine clinical use (Jansson *et al.*, 1984a; Jansson, 1984). The upper limit of TSH for normal sera in this study was 4 mU/l (Jansson *et al.*, 1984).

Analysis of thyroid autoantibodies by ELISA. Thyroglobulin (Tg) and thyroid microsomes (the gift of Dr B. Rees Smith, Welsh National School of Medicine, Cardiff, UK) were prepared from Graves' thyroid tissue obtained at operation using methods previously described (Schardt *et al.*, 1982) and stored at −70°C. Since thyroid microsomal fractions may be contaminated with small amounts of Tg (Goodburn, Williams & Marks, 1982), sera were preincubated at 4°C overnight with Tg to a final concentration of 100 μg/ml before analysis for Mic antibody (Schardt *et al.*, 1982). Mic and Tg antibodies of IgG class were measured in duplicate aliquots of sera diluted 1:100 to 1:1000 by ELISA techniques (Schardt *et al.*, 1982; McLachlan *et al.*, 1982) and the results have been expressed as an ELISA index defined as follows:

$$\text{ELISA index} = \frac{\text{Optical density of test sample}}{\text{Optical density of standard Hashimoto serum sample}}$$

In Mic antibody assays the standard was a Hashimoto serum (negative for Tg antibody) with a haemagglutination titre of 1:204,800 (diluted 4,000 times). For Tg antibody assays the reference serum had a haemaglutination titre of 1:5,120 (diluted 400 times).

The distribution of Mic antibodies among the IgG subclasses was determined by a previously described ELISA technique (Parkes *et al.*, 1984). Briefly quadruplicate ELISA plates coated with thyroid microsomes were interacted with test serum diluted 1:100 and, in some cases, 1:300 and 1:1,000. Each plate was then exposed to one of four murine monoclonal antibodies specific for the human IgG subclass 1, 2, 3 or 4 (NL16, GOM2, ZG4 & RJ4 respectively, Unipath Ltd, Bedford, UK) Subsequently, the plates were treated with horse-radish peroxidase conjugated anti-mouse IgG (Sigma Chemical Company, London, UK) and substrate (*o*-phenylene diamine). The optical density (OD) at 492 nm was measured using a Titertek Multiskan (Flow Laboratories Limited). A similar technique was used to determine the distribution of Tg antibodies among the IgG subclass using ELISA plates coated with Tg (4 μg/ml). Serum from a normal donor (diluted 1:100) gave background OD values ranging from 0·00 to 0·03.

Mic antibody subclass assays were standardized as follows: serum from a Hashimoto patient

was diluted, on the basis of values obtained in pilot studies, to give an OD between 0·5 and 1·0 in the IgG-1 subclass assay. Similar standards were established for the assays for IgG-2, 3 and 4. The values obtained in an assay performed at the beginning of the study were regarded as arbitrary reference values for the four subclasses. Multiple aliquots of each subclass standard were prepared as quality controls and stored at −70°C. In all subsequent assays, duplicate aliquots of these quality controls were included and used to provide a correction factor as follows:

$$\frac{\text{Optical density of initial IgG subclass quality control (Reference value)}}{\text{Optical density of quality control in a particular assay}}$$

The OD values obtained for test samples in individual assays were then multiplied by the relevant factor for each subclass. Further, the factors were used to estimate inter-assay variation; for each subclass assay the mean correction factor was close to 1·0 and the coefficient of variation ranged between 18–27%, values which are comparable with those obtained for the measurement of total Mic or Tg antibody (Schardt *et al.*, 1982; McLachlan *et al.*, 1982).

RESULTS

In serum from 15 women from whom an early pregnancy sample was available, the IgG class Mic antibody levels determined by ELISA were lower 2 months postpartum, increased twofold 5 months postpartum and had returned 10–12 months postpartum to the early pregnancy level (Fig. 1). The pattern of change observed at 2, 5 and 10–12 months postpartum for this subgroup of women was essentially similar to that seen in serum samples from the whole group of 34 individuals considered in this study (Fig. 1).

The distribution of Mic antibody among the IgG subclasses is illustrated in Fig. 2 for sera from the 15 women from whom an early pregnancy sample was available. The percentage contribution made by each IgG subclass to Mic antibody was essentially similar in early pregnancy and 2, 5 and 10–12 months postpartum (Fig. 2) despite the changes in total IgG class Mic antibody (Fig. 1). Further, Mic antibodies were principally of the IgG-1 or IgG-4 subclasses with only minor contributions from IgG-2 and IgG-3. In the remaining 19 women, the distribution of Mic antibody among the IgG subclasses was similar at each time interval studied (data not shown).

Thyroid function was normal (serum TSH below 4 mU/l in all 34 women 6–8 weeks postpartum. Subsequently, during the year following delivery, transient hypothyroidism with a maximal TSH greater than 20 mU/l developed in 13 individuals; 11 other women had a TSH value of between 4–10 mU/l and in 10 women the serum TSH remained below 4 mU/l. However, in this latter group, five

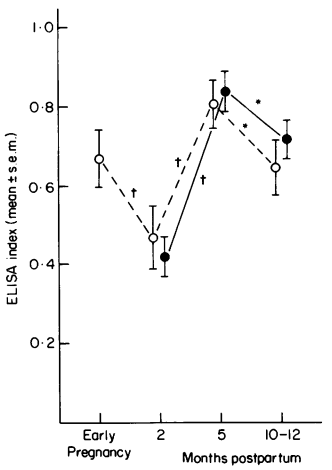

Fig. 1. Total IgG class Mic antibody levels in the postpartum period in all women (●, $n = 34$) and in a subgroup in which an additional serum sample from the first trimester of pregnancy was included (○, $n = 15$). Significance of differences between the time points by t-test for paired data; (*) $P < 0{\cdot}01$; (†) $P < 0{\cdot}001$.

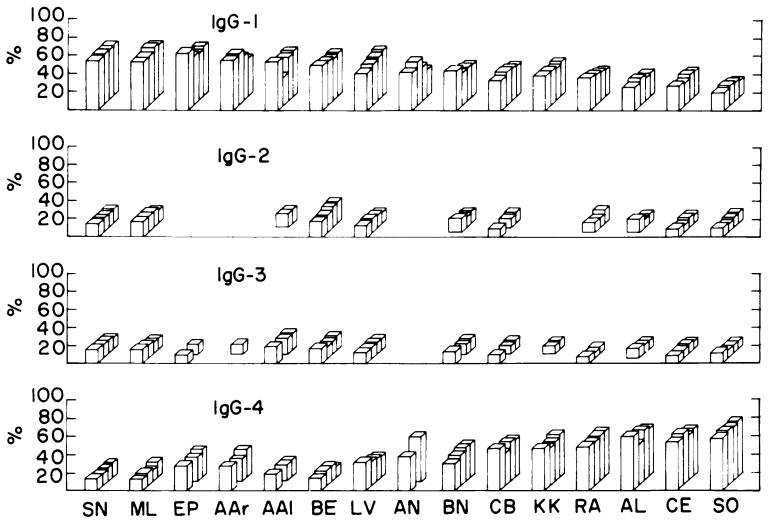

Fig. 2. Percentage contribution made by each IgG subclass to Mic antibody in sera obtained from 15 women early in pregnancy (front column) or 2 months (second column), 5 months (third column) and 10–12 months (back column) postpartum. Mic antibodies were principally IgG-1 or IgG-4 with only minor contribution from IgG-2 and IgG-3. The absence of a column indicates optical density values for a particular IgG subclass which were below background.

women developed Graves' thyrotoxicosis [on the basis of high radioiodine uptake and/or high levels of TSH-receptor stimulating antibodies (Jansson *et al.*, 1985)]. These individuals were excluded from further consideration of the role of Mic antibodies because of the complex interactions between Mic antibodies and the stimulating TSH receptor antibodies. A comparison was therefore carried out between the 13 women with moderate or severe (mostly symptomatic) hypothyroidism (TSH >20 mU/l) and the 16 women with minor or no hypothyroidism (TSH <10 mU/l). The total Mic antibody level (IgG class) was higher in the former than in the latter (Fig. 3) and the difference was significant 5 and 10–12 months postpartum ($P<0{\cdot}001$ & $P<0{\cdot}05$ respectively by *t*-test between groups). The level of Mic antibody of subclass IgG-1 (in terms of OD) was significantly higher throughout the postpartum period in the group which developed hypothyroidism ($P<0{\cdot}05$, $P<0{\cdot}001$ & $P<0{\cdot}01$ for 2, 5 & 10–12 months postpartum, respectively) (Fig. 3). In contrast, the levels of Mic antibody of subclass IgG4 were similar in both groups of women (Fig. 3).

The possible influence of the HLA-DR4 phenotype on Mic antibody subclass distribution was investigated at the time of hypothyroidism, that is 5 months postpartum. No difference was observed between the two groups to suggest an association between DR4 and Mic antibody subclass restriction (data not given).

Tg antibodies were present only in small amounts, the maximal mean ELISA index $\pm$ s.d. postpartum being $0{\cdot}38 \pm 0{\cdot}35$ in 14 women for whom Tg antibodies were analysed. Tg antibodies were almost exclusively of subclass IgG-1 but the low levels precluded the possibility of providing reliable estimates of the proportions made by other IgG subclasses.

DISCUSSION

In this study of postpartum autoimmune thyroiditis the changes observed in serum levels of IgG class Mic antibody measured by ELISA from early pregnancy and at 2, 5 and 10–12 months postpartum were essentially similar to those obtained by haemagglutination (Jansson *et al.*, 1984a). Despite the change in total amount of Mic antibody, the percentage contribution made by each IgG subclass was virtually unchanged from early pregnancy to 12 months postpartum, the major

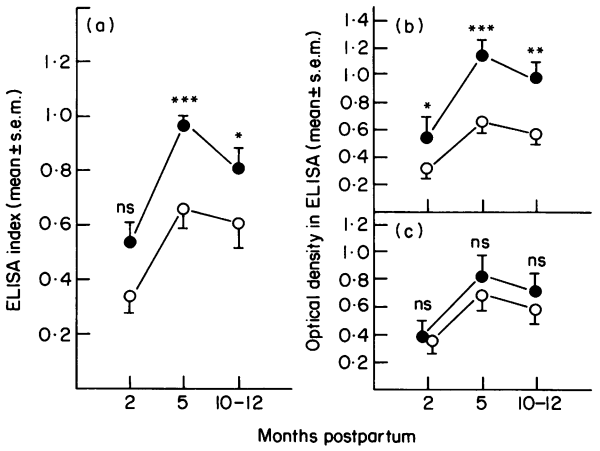

Fig. 3. Total IgG Mic antibodies (a) and subclass IgG-1 (b) and IgG-4 (c) Mic antibodies in two subgroups of women with a maximal TSH >20 mU/l (●, $n=13$) or <10 mU/l (○, $n=16$) in the postpartum period. Significance of differences by t-test between these groups is indicated: ns = not significant, (*) $P<0.05$; (**) $P<0.01$; (***) $P<0.001$.

contributions being derived from IgG-1 and IgG-4. The development of hypothyroidism, however, was associated with increased amounts of Mic antibody of subclass IgG-1.

Postpartum hypothyroidism is preceded by a thyrotoxic state presumably due to release of thyroid hormones, which may involve K cell-mediated Mic antibody cytotoxicity (Amino & Miyai, 1983; Jansson *et al.*, 1984c). If Mic antibody is responsible for destruction of thyroid epithelial cells it is likely that the subclass IgG-1 would be potentially more destructive because of its ability to fix complement, a property which appears to be absent from antibodies of subclass IgG-4 (Spiegelberg, 1974; Shakib & Stanworth, 1980). However, it is also possible that antibodies of IgG-1 merely reflect the activation of a particular cytotoxic T cell subset which destroys thyroid cells without the need for autoantibodies (Creemers, Rose & Kong, 1983). Furthermore it should be observed that the reversible hypothyroid phase of postpartum thyroiditis, which is accompanied by goiter development, is not likely to be due to destructive mechanisms alone. Differences in thyroid cellular regrowth capacity and interference with the metabolic function of the thyrocytes may be of additional importance.

In normal human serum the distribution of IgG subclasses is 60–70% IgG-1, 25–30% IgG-2, 4–7% IgG-3 and 2–4% IgG-4 (Schur, 1972; French & Harrison, 1984). Although IgG-1 is the major subclass, antibodies directed at specific antigens may be principally of another subclass; for example antibodies against *Haemophilus influenza* are predominantly IgG-2 (Andersson *et al.*, 1981). Restriction to a particular subclass has also been observed for autoantibodies in several autoimmune diseases (Zouali, Jefferis & Eyquem, 1984; Lefvert & Bergström, 1977; Fulpius, Miskin & Reich, 1980; Dean, Bottazzo & Cudworth, 1983; Riggione, Stokes & Thompson, 1983). In Hashimoto patients with high titres of thyroid autoantibodies, Tg antibodies were frequently IgG-4 (Thompson *et al.*, 1983), whereas Mic antibodies were usually IgG-1 and IgG-4 (Parkes *et al.*, 1984). The results reported in this paper for Mic antibodies in the group of women with postpartum autoimmune thyroid disease were therefore essentially similar to those obtained for patients with chronic Hashimoto's thyroiditis. Tg antibody levels as measured by ELISA were low, in accordance with the observations made by haemagglutination tests (Jansson *et al.*, 1984). Consequently, it was only possible to record presence or absence of Tg antibody and it is of interest to note that when present they were almost entirely of subclass IgG-1.

It is not yet clear what parameters are responsible for the IgG subclass distribution of antibodies although on the basis of experiments in mice it is likely that the nature of the antigen, its manner of presentation and the type of T cell help may all play a role (Mongini, Stein & Paul, 1981; Teale, 1983; Sarvas *et al.*, 1983). T cells recognize antigens in conjunction with HLA-DR molecules on

antigen-presenting cells and possibly on target organ cells as well, as suggested by the demonstration of HLA-DR positive thyroid epithelial cells in thyroid tissue from patients with autoimmune thyroid disease but not in normal controls (Hanafusa *et al.*, 1983; Jansson, Karlsson & Forsum, 1984b; Warford *et al.*, 1984). Recently, we observed that HLA-DR4 is prevalent among women with Mic antibodies and this association is even stronger in women who develop transient postpartum hypothyroidism (Jansson *et al.*, 1985). However, the results presented in this report suggest that the IgG subclass distribution of Mic antibodies is not likely to be under control by genes in the HLA-DR region.

Although the precise role of Mic antibodies in thyroid destruction is not known, the association between postpartum hypothyroidism and Mic antibodies of IgG-1 (a subclass capable of fixing complement) suggests that they are potentially important in the development of autoimmune hypothyroidism. If this is the case, the IgG subclass distribution of Mic antibodies could be used for the prediction of hypothyroidism not only in autoimmune postpartum thyroiditis but also in other variants of autoimmune thyroid disease.

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