Correlation of the antibody titers with serum prolactin levels and their clinical course in patients with anti-prolactin autoantibody

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Patients with anti-prolactin (PRL) autoantibody were surveyed among 208 patients with hyperprolactinemia (PRL ≥ 30 µg/l) and 228 subjects with normal PRL levels, and the relationship of the antibody titers with serum PRL levels and their clinical course were studied. Diagnosis of possessing the anti-PRL autoantibody was based on the polyethylene glycol method, displacement of the binding of [125I]PRL with the serum by unlabeled PRL and the binding of PRL to protein G, the affinity gel for immunoglobulin G. Prolactin was measured by an immunoradiometric assay that we found was not affected by the anti-PRL autoantibody. A significantly high frequency of anti-PRL autoantibody in patients with idiopathic hyperprolactinemia (16%) and a positive correlation between titers of the autoantibody and serum PRL levels (r = 0.74, p < 0.01) may indicate that the anti-PRL autoantibody itself is another cause of hyperprolactinemia, probably owing to the delayed clearance of PRL. Most patients with anti-PRL autoantibody lacked the clinical symptoms of hyperprolactinemia, such as amenorrhea and galactorrhea, and spontaneous pregnancy occurred despite the marked hyperprolactinemic state, indicating that the biological activity of PRL was attenuated by the autoantibody. In addition, PRL levels and the titers of anti-PRL autoantibody were not changed significantly during the observation period of up to 5 years without any medical intervention. These results suggest that the anti-PRL autoantibody itself is one of the causes of hyperprolactinemia and that medical intervention is unnecessary for this type of hyperprolactinemia.

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Hyperprolactinemia has many causes: pregnancy; prolactinoma; non-functioning pituitary macroadenoma and parasellar tumors, such as meningioma and craniopharyngioma, which compress the stalk or hypothalamus; PRL stimulatory drugs, such as dopaminergic antagonists and catecholamine re-uptake inhibitors; hypothyroidism; chest wall diseases; hepatorenal disorders; idiopathic hyperprolactinemia (1–6).

Recently, anti-PRL autoantibody has been detected in sera from some patients with idiopathic hyperprolactinemia (7, 8). The autoantibody was found belonging to immunoglobulin G with low affinity and high capacity. The patients with anti-PRL autoantibody lacked the clinical symptoms of hyperprolactinemia, such as amenorrhea and galactorrhea, which is similar to that of macroadenoma (9–11). There are still several points to be addressed:

(i) What is the frequency of anti-PRL autoantibody in a larger population?
(ii) Is the autoantibody itself a cause of hyperprolactinemia?
(iii) Does the autoantibody persist for a long period or disappear in a short time?
(iv) What is the mechanism of production of the autoantibody?
(v) How does the diagnosis of autoantibody-associated hyperprolactinemia differ from that of macroadenoma or prolactinoma?
(vi) Is it necessary to treat hyperprolactinemia in patients with anti-PRL autoantibody?

To elucidate these points, we surveyed anti-PRL autoantibody among 208 hyperprolactinemic patients and 228 normoprolactinemic subjects and performed clinical and endocrinological evaluations.

Subjects and methods

Subjects

Among the serum samples ordered for the measurement of PRL values in our laboratory between January 1990 and January 1993, those with a PRL value of greater than 30 µg/l were collected randomly. This survey yielded 208 patients with hyperprolactinemia (47 males and 161 females, aged 15–72 years). The initial PRL value was measured for various reasons according to the medical records, i.e. work-up for the
pituitary adenoma, menstrual irregularities, galactorrhea, infertility, check for the side-effect of psychiatric medication, and general endocrine evaluations.

The diagnoses of hyperprolactinemia, made on the basis of the patient’s medical records, including clinical, laboratory and radiological data, were: prolactinoma in 37 patients; non-functioning pituitary macroadenoma in 11; parasellar tumors such as meningioma, germinoma and craniopharyngioma in seven; drug-induced hyperprolactinemia in 63; pregnancy in six; hypothyroidism in six; chest-wall diseases in three; and idiopathic hyperprolactinemia in 75. The diagnoses of prolactinoma, non-functioning pituitary macroadenoma and parasellar tumors were made on the basis of pathological, endocrinological and radiographical examinations. The diagnosis of drug-induced hyperprolactinemia is difficult to make because the PRL value usually is not measured before the medication and it is impossible to stop the medication to confirm the normal PRL level from an ethical point of view. Therefore, we diagnosed the patients as having drug-induced hyperprolactinemia when they were taking drugs that apparently were considered to raise the PRL level, such as dopamine antagonists (phenothiazine, butyrophenones, sulpiride) and catecholamine re-uptake inhibitors (tricyclic antidepressants). Fifty-five of the 63 drug-induced hyperprolactinemic patients were taking sulpiride. All patients with drug-induced hyperprolactinemia were those who were followed regularly at the departments of internal medicine and psychiatry in our hospital. The serum PRL level ranged from 51 to 6000 µg/l in patients with prolactinoma, from 30 to 400 µg/l in patients with drug-induced hyperprolactinemia, from 30 to 935 µg/l in patients with idiopathic hyperprolactinemia and from 30 to 288 µg/l in patients with other causes. The PRL level was measured also in the serum samples collected for the determination of hepatitis B antigen among the medical staff in our hospital and 228 subjects (66 males and 162 females, aged 20–69 years) with a PRL level of less than 30 µg/l were studied as normoprolactinemic subjects. Seven subjects among the medical staff were found to have elevated PRL levels and were excluded from further study. None of the patients had systemic autoimmune disorders, hepatorenal disease or was taking methimazole.

Endocrinological work-up was performed on six patients with anti-PRL autoantibody after obtaining their informed consent: dopamine (2 µg/kg min⁻¹, div), bromocriptine (2.5 mg, po), sulpiride (100 mg, im) and TRH (500 µg, iv) loading tests. Blood was collected early in the morning after an overnight fast and stored until the assay was carried out. The study was approved by the Regional Ethical Committee of Kobe City General Hospital.

Evaluations for anti-PRL autoantibody

Survey for the anti-PRL autoantibody in the serum was conducted by the polyethylene glycol (PEG) method as described previously (7, 8). Two hundred and fifty grams of PEG (PEG 6000, Wako Chemical, Osaka, Japan) was dissolved in distilled water, making the volume up to 11. Human PRL (NIDDK-hPRL-I-7) was iodinated by the chloramine T method. Serum samples (100 µl) and [125I]PRL (10000–15000 cpm/50 µl phosphate buffer) were incubated at 37°C for 1 h and 150 µl of the 25% PEG was added. The incubation mixture then was mixed vigorously and centrifuged at 3000 rpm for 30 min. The sediments were washed once with 12.5% PEG and their radioactivity was measured with a γ-counter. The intra- and interassay coefficients of variation of the PEG method were 5.2% and 6.3%, respectively. Serum samples were judged as containing anti-PRL autoantibody when they had the following two conditions:

(i) The ratio of radioactivity of the sediment exceeded 8.9%; the mean + 2 so in 20 healthy volunteers aged 20–29 years who had normal results of routine hematological and biochemical tests.

(ii) The binding of [125I]PRL with the serum was dose dependently displaced by unlabeled PRL (NIDDK-hPRL-I-7: 1600–50,000 µg/l serum).

In addition, to confirm that the binding component in the serum was immunoglobulin, a protein G column (Mab Trap G, Pharmacia LKB, Uppsala, Sweden) was used. Protein G binds only immunoglobulin G (IgG) and its subclasses, separating out IgA, IgM, IgD and albumin, which may bind to other affinity gels such as protein A (12). When the anti-PRL autoantibody belonging to IgG is present, substantial amounts of the autoantibody-bound PRL may bind to the column. After equilibration of the column with 0.2 mol/l sodium phosphate buffer (pH 7.0), serum samples were applied onto the column and the unbound proteins were washed away with the same buffer. Then, the bound PRL-IgG complex was eluted with 1.0 mol/l glycine HCl buffer (pH 2.7) and neutralized immediately with 1.0 mol/l TRIS.HCl buffer (pH 9.0). When the PRL standard or the serum from patients with prolactinoma was applied onto the column, less than 1% of the PRL was bound to protein G.

**Assay for PRL**

The concentrations of PRL were measured in duplicate by immunoradiometric assay (IRMA, Dinabot, Tokyo, Japan). Serum samples (25 µl) were incubated with [125I]-labeled antibody (150 000 cpm/200 µl) and antibody-bound beads for 3 h at room temperature with continuous shaking. The beads were washed twice with 1 ml of saline and the radioactivity bound to the beads was measured with a γ-counter. The PRL standards of IRMA kit were based on NIH VLS 4. The limit of sensitivity of the assay was 1.25 µg/l. The intra- and interassay coefficients of variation determined with the use of pooled patients’ sera (PRL: 12.8 µg/l) were...
1.8% and 5.3%, respectively. The normal PRL level in our laboratory was below 30 µg/l. Free and total PRL were extracted according to the method used for insulin extraction from the antibody-containing serum but with slight modification (13). To measure free PRL, 200 µl of 25% PEG solution was added to 200 µl of serum and mixed vigorously, followed by centrifugation at 3000 rpm for 30 min. The supernatant in which autoantibody-bound PRL was removed was assayed for free PRL. To measure the total PRL, 30 µl of 1 mol/l HCl was added to 250 µl of serum, making the pH of the serum 2.6 to dissociate PRL from the autoantibody. After a 30 min incubation at room temperature, 340 µl of 25% PEG solution was added and the mixture was shaken vigorously. Then, 60 µl of 2 mol/l TRIS, HCl buffer (pH 8.25) was added for neutralization and the mixture was centrifuged at 3000 rpm for 30 min. The supernatant in which only the autoantibody was removed was assayed for total PRL. Bound PRL (%) was determined by (Total PRL – Free PRL)/Total PRL × 100. Direct measurement of PRL by IRMA resulted in almost equal values to the total values, despite the presence of anti-PRL autoantibody, i.e. no affect on IRMA by the autoantibody was observed. The ratio of the PRL level determined by direct measurement (with autoantibody) to the total level (without autoantibody) in 10 patients with anti-PRL autoantibody was 112 ± 14%.

Data were expressed at means ± sd and were analyzed using Student’s t-test. Correlation coefficients were determined by linear regression analysis. The chi-squared test was used to compare the frequency of possession of the anti-PRL autoantibody.

Results

Figure 1 shows the percentage of radioactivity of the precipitate by the PEG method (titers of anti-PRL autoantibody, if present). The dotted area represents the mean ± 2 sd of the 20 normoprolactinemic healthy volunteers with normal routine laboratory tests. Closed circles indicate the samples in which binding of [125I]-PRL with the serum was dose dependently displaced by unlabeled PRL, i.e. those with anti-PRL autoantibody. The anti-PRL autoantibody was detected in 12 of the 75 patients with idiopathic hyperprolactinemia (16%), three of the 63 with drug-induced hyperprolactinemia (4.8%), one of the 37 with prolactinoma (2.7%), one of the 33 with hyperprolactinemia from other causes (3.0%), and three of the 228 with normoprolactinemia (1.3%). The frequency of possession of the anti-PRL autoantibody in idiopathic hyperprolactinemia was significantly higher than that in normoprolactinemia (p < 0.001) and those in cause-proven hyperprolactinemia (p < 0.05). There was no significant difference in the presence or absence of the anti-PRL autoantibody among cause-proven hyperprolactinemia and normoprolactinemia. The titers of the autoantibody observed in normoprolactinemia were all just above the mean ± 2 sd of the control. The survey for the anti-PRL autoantibody by the PEG method yielded false-positive results, i.e., above the mean ± 2 sd of the control by the PEG method but not displaced by unlabeled PRL in 1.1% of the subjects, probably owing to hypergammaglobulinemia or other serum factors increasing the non-specific binding. The frequency of false-positive
results was not significantly different among the groups.

Table 1 shows the clinical features, the levels of PRL and the characteristics of the autoantibody in hyperprolactinemic patients with anti-PRL autoantibody. None of the patients with anti-PRL autoantibody had generalized autoimmune diseases or evidence of lymphocytic hypophysitis. Patient 17 had a transsphenoidal adenectomy 13 years earlier. Clinical symptoms such as amenorrhea and galactorrhea, endocrinological abnormalities such as hyperprolactinemia, impaired PRL responses to TRH and sulpiride and radiological evaluations supported the diagnosis of prolactinoma, and it was confirmed surgically. However, the serum PRL level began to rise 2 years after the operation, without radiological evidence of the recurrence of prolactinoma. She started to take bromocriptine. A head computed tomography (CT) scan and/or magnetic resonance imaging (MRI) revealed no evidence of pituitary adenoma in those patients with anti-PRL autoantibody.

The clinical characteristics of hyperprolactinemia, such as amenorrhea and galactorrhea, were not observed in most patients with anti-PRL autoantibody. Minimal galactorrhea was observed in patients 2 and 13. The PRL levels when bromocriptine was not administered ranged from 39 to 935 μg/l (279 ± 265 μg/l). The proportion of the autoantibody-bound PRL, determined by (Total PRL – Free PRL)/Total PRL × 100, ranged from 69.5 to 95.9% (83.4 ± 7.2%). Prolactin bound to protein G with a significantly high proportion (47.9 ± 14.4% vs less than 1% in controls) and the autoantibody was found belonging to IgG class. A smaller proportion of protein G-bound PRL (IgG-bound PRL) than that calculated from free and total PRL may be attributed to the dissociation of some PRL from the autoantibody during the washing step of the protein G column. Scatchard analysis revealed a low-affinity, high-capacity autoantibody: the association constant ($K_a$) was $3.2 ± 4.2 \times 10^6$ l/mol and the maximal

\[ \text{Fig. 2. Relationship between the titers of anti-PRL autoantibody and the serum PRL levels. Serum samples containing anti-PRL autoantibody are those in patients with idiopathic hyperprolactinemia and in normal subjects. Asterisk indicates patient 2. A significant positive correlation (r = 0.74, p < 0.01) was present between them.} \]
and incomplete in all six patients. Administration of sulpiride (100 mg, im) elicited a significantly higher PRL secretion (except for patient 2) as compared with prolactinoma.

Figure 4 shows the changes of serum PRL levels and anti-PRL autoantibody titers in four patients with anti-PRL autoantibody whom we could follow for several years. Hyperprolactinemia persisted during these periods, and patients 3 and 4 became pregnant spontaneously despite the marked hyperprolactinemic state (PRL: 200–300 µg/l). Serum PRL concentrations began to increase during the first trimester and peaked at term with extremely high levels (PRL more than 1000 µg/l); normal lactation started after delivery in both patients. The anti-PRL autoantibody titers were not changed significantly in any of the four patients during these periods, although they were decreased slightly in accordance with the increase in PRL levels during pregnancy in patient 3.

Figure 5 shows the clinical course and the results of PRL suppression and stimulation tests of patient 17. The anti-PRL autoantibody titers in the last 3 years showed a tendency to increase. The PRL response to sulpiride or TRH was totally abolished before surgery, while that to sulpiride became normalized despite persisting hyperprolactinemia 13 years after the operation.

**Discussion**

Anti-PRL autoantibody was detected in hyperprolactinemic patients at a frequency of 16% in idiopathic hyperprolactinemia, 4.8% in drug-induced hyperprolactinemia, 2.7% in prolactinoma and 3.0% in hyperprolactinemia of other causes. The result that the frequency of possession of the anti-PRL autoantibody was significantly higher in idiopathic hyperprolactinemia than in the other cause-proven hyperprolactinemia suggests that the anti-PRL autoantibody is “another” cause of hyperprolactinemia. This is supported further by the observation that a significant positive correlation existed between the titers of the autoantibody and the serum PRL levels. It is likely that the anti-PRL autoantibody stores endogenous PRL according to the titers. Because the clearance of the autoantibody-bound PRL in the kidney is probably decreased owing to its larger molecular size than free PRL, hyperprolactinemia may occur. In the present study, we confirmed the previous finding (7) that the suppression of PRL levels by dopamine and bromocriptine was delayed and incomplete in six patients with anti-PRL autoantibody, to support this hypothesis. We observed the anti-PRL autoantibody even in normoprolactinemic subjects, although the frequency and the titers were low. Long-term follow-up is necessary to see if these subjects develop hyperprolactinemia in the future.

The physiological significance of the anti-PRL autoantibody remains to be clarified.
autoantibody other than to raise the serum PRL levels is to attenuate the biological activity of PRL. Lack of clinical symptoms of hyperprolactinemia in most patients with the autoantibody, and normal pregnancy in the presence of marked hyperprolactinemia support this assumption. It is likely that the anti-PRL autoantibody inhibits PRL action in the target cells by interfering with the receptor binding. The time-course of the anti-PRL autoantibody titers and PRL levels showed that they did not change significantly during the observation period up to 5 years. Slight suppression of the titers during pregnancy may indicate that the binding of $^{[125]}$I-PRL with the autoantibody was slightly dissociated owing to the elevated endogenous PRL level. Although it is not clear when the autoantibody was produced, the present findings suggest that the generation of the anti-PRL autoantibody is not a transient phenomenon.

Little is known about the underlying mechanism of the production of the anti-PRL autoantibody. In patients with insulin autoimmune syndrome (14–16), methimazole is postulated to be related to the production of the autoantibody. Methimazole contains a thiol group that may act on the disulfide bond in the insulin molecule, resulting in structural and immunological alterations of endogenous insulin, which triggers the initiation of the immune response. However, no patients with anti-PRL autoantibody had a history of taking methimazole. One of the patients with anti-PRL autoantibody (patient 1) had a history of taking domperidone, an anti-dopaminergic agent, for gastritis and had hyperprolactinemia with transient galactorrhea (7). Although the patient stopped taking the medicine, marked hyperprolactinemia persisted for more than 2 years without clinical symptoms of hyperprolactinemia. However, it is unlikely that PRL stimulative agents themselves are related to the production of the anti-PRL autoantibody because the frequency of the presence of the autoantibody in patients with drug-induced hyperprolactinemia was not significantly high (4.8%). Further studies are necessary to elucidate the mechanism of the production of the anti-PRL autoantibody.

Macroprolactinemia has many similarities to the anti-PRL autoantibody-related hyperprolactinemia: large molecular weight PRL and the lack of clinical
symptoms of hyperprolactinemia (9–11). Macroprolactinemia is considered to be a genetic disorder because the fetal cord blood of the patients also contains macroprolactin. However, because IgG and PRL readily pass through the placenta, there is a possibility that some of the macroprolactinemia cases are caused by the anti-PRL autoantibody.

Hyperprolactinemia with a PRL level higher than 200 μg/l is thought to be pathognomonic of prolactinoma (3). Because many patients with anti-PRL autoantibody have an elevated PRL level to such a degree, differential diagnosis is needed. Negative results by CT or MRI of the pituitary gland and the lack of clinical symptoms of hyperprolactinemia, such as amenorrhea and galactorrhea, favor the diagnosis of autoantibody-associated hyperprolactinemia. The responsiveness of PRL secretion to sulpiride also seems to be useful for the differential diagnosis. The PRL response to sulpiride was almost always reduced or absent in patients with prolactinoma, while it was normal or augmented in autoantibody-associated hyperprolactinemia in five of the six patients. The explanation for the impaired PRL response in prolactinoma is that continuous hyperprolactinemia by the adenomas leads to chronically increased hypothalamic–hypophysial portal dopamine concentrations, resulting in a down-regulation of lactotroph dopamine receptors and in dopamine resistance (17, 18). On the other hand, in autoantibody-associated hyperprolactinemia, the autoantibody-bound PRL may not so much elevate the central dopaminergic tone as free PRL, and the down-regulation of lactotroph dopamine receptors may not occur. Patient 17 is of interest in this respect. She had a transsphenoidal adenectomy for prolactinoma 13 years previously. Her serum PRL level began to rise 2 years after the operation, without radiological evidence of the recurrence of tumor. The observation that the PRL response to sulpiride was normal and that to the dopamine or bromocriptine was blunted suggests that hyperprolactinemia in the present state was due to the anti-PRL autoantibody, although whether the autoantibody co-existed with prolactinoma 13 years ago or it was produced afterwards was not clear. Patient 2 showed features different from the other patients with anti-PRL autoantibody; she had clinical symptoms of hyperprolactinemia, her PRL level was high for the autoantibody titer and no PRL response to sulpiride was observed. Because the binding potential of the autoantibody in patient 2 was not significantly different from that in the other patients, it is possible that patient 2 has not only the anti-PRL autoantibody but also microadenoma or other factors to elevate the PRL levels.

 Clinically, whether the autoantibody-associated hyperprolactinemia requires medical therapy or not is important. The present findings that clinical symptoms of hyperprolactinemia seldom occurred and normal pregnancy was possible despite marked hyperprolactinemia suggest that chronic medical therapy is not indicated for autoantibody-associated hyperprolactinemia.

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