Autoantibodies to gastrin in patients with pernicious anaemia—a novel antibody

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Summary

Autoantibodies arise when there is a breakdown in immunological tolerance. Autoantibodies to parietal cells and intrinsic factor are found in autoimmune atrophic gastritis (AAG) and are associated with elevated plasma gastrin. Endogenous gastrin autoantibodies have not been described to date. The aim of this study was to investigate the occurrence of autoantibodies to gastrin. Plasma from 50000 patients, including more than 2000 with AAG, was tested. Gastrin was measured by radioimmunoassay (RIA) in whole plasma and the presence of autoantibody determined by using a control which omitted assay antibody. The quantity and affinity of gastrin autoantibodies was assessed. Three patients had autoantibodies to gastrin. All three had AAG and pernicious anaemia (PA). The antibodies were of low titre and relatively high affinity. Free circulating plasma gastrin levels were within the normal range, but total gastrin levels were elevated. This is the first description of autoantibodies to endogenous gastrin. Plasma from 50000 patients, including more than 2000 with AAG, was tested. Gastrin was measured by radioimmunoassay (RIA) in whole plasma and the presence of autoantibody determined by using a control which omitted assay antibody. The quantity and affinity of gastrin autoantibodies was assessed. Three patients had autoantibodies to gastrin. All three had AAG and pernicious anaemia (PA). The antibodies were of low titre and relatively high affinity. Free circulating plasma gastrin levels were within the normal range, but total gastrin levels were elevated. This is the first description of autoantibodies to endogenous gastrin. The incidence of antibodies to gastrin is low, they are found in association with PA, and they may lead to falsely low measurements of plasma gastrin.

Introduction

Mechanisms exist in the body to prevent the mounting of an immune response against the body’s own constituents. Spontaneous or exogenously-induced flaws in immunological tolerance may however develop, and autoantibodies then occur. Such antibodies are well-characterized, and include antibodies directed against cells, cellular components and peptides. Autoantibodies to cells include the antral gastrin (G) cell, which in some cases of antral gastritis would suggest an autoimmune aetiology.1,2 Other cellular antibodies include those to the pancreatic islet cell, which were discovered in 1974 and provide a marker for anti-islet autoimmunity in normoglycaemic patients.3,4 Antibodies to cellular components include antimitochondrial antibodies, antinuclear antibodies and antinucleolar antibodies. Antibodies to exogenous peptides such as insulin are described. These may be induced in response to any form of injected insulin, and evidence suggests that they may be involved in insulin resistance. Autoantibodies to endogenous peptides are rare but are illustrated in the case of insulin again, with the development of anti-insulin antibodies, in patients who have never received exogenous insulin. These insulin autoantibodies are associated with autoimmune insulin syndrome which results in spontaneous or reactive hypoglycaemia with high circulating concentrations of total immuno-reactive insulin.5

Pernicious anaemia (PA) is associated with autoantibodies to intrinsic factor6 and the parietal cell.7 In PA, gastrin levels are normally, but not invariably, elevated as a consequence of achlorhydria due to the absence of the negative feedback mechanism.8,9 We have investigated the existence and prevalence of autoantibodies to gastrin in plasma samples analysed in our laboratory, with particular interest in patients with hypergastrinaemia and other autoimmune disease.
Methods

Patients

In this laboratory we provide a routine diagnostic service for all of Northern Ireland, which has a population of 1.6 million. We have processed in excess of 50,000 plasma samples for routine gastrin analysis over a 25-year period. These samples have included more than 2000 specimens from PA patients. Patients are diagnosed as having PA if they have autoantibodies to intrinsic factor or parietal cells, megaloblastic anaemia and are successfully treated by vitamin B12 replacement therapy. Patients whose blood specimens were sent to this laboratory for routine analysis of gastrin were included in this study.

Measurements

Routinely, circulating gastrin concentration is estimated in whole plasma. An antibody is used which was raised to human gastrin 17 conjugated to chicken egg albumen. It detects amidated gastrins only, and measures G34 and G17 in equimolar quantities. Human gastrin 17 is used as standard in concentrations between 1.56 and 100 pmol/l. Radiolabelled gastrin tracer is used in the assay at 11 pmol/l. Radioimmunoassay (RIA) is used where antibody-bound is separated from free antigen using dextran-coated charcoal. Lack of an alcohol extraction step (where large-sized proteins are removed from the system), along with the estimation of non-specific interference by the inclusion of blank tubes for every plasma sample (where assay antibody is omitted), results in an estimation of endogenous antibody to gastrin which may be present in the plasma.

Where autoantibodies to gastrin were detected, the following procedures were followed. (i) Quantification of endogenous antibody in plasma was made by assessment of plasma binding to ^125^I-labelled gastrin under RIA conditions using serial dilutions of the plasma. (ii) Affinity of endogenous antibody was assessed by the ability of the plasma to bind ^125^I-labelled gastrin tracer and assessment of the displacement of the labelled gastrin with low concentrations of standard gastrin. (iii) Estimation of circulating free gastrin was made after plasma extraction with alcohol. (iv) Evaluation of total gastrin and total antibody was made after acid treatment of plasma, which results in dissociation of antibody-bound antigen. The pH of the plasma was decreased to <2.0, and the products were purified by Sep Pac and assayed for gastrin and antigastrin antibody. (v) Endogenous antibody and gastrin was purified using gel chromatography. A column (G100, 1.0 x 100 cm) was prepared and equilibrated with phosphate buffer (pH 7.4, 0.05M) containing bovine albumen (RIA grade, 0.2%). The column was calibrated using rabbit gamma globulin and ^125^I gastrin-17. Elution was with phosphate buffer at a flow rate of 4 ml/h at 4 °C. Patient plasma (2 ml) was applied to the column and the eluted fractions were assayed in the absence of assay antibody (assessment of endogenous antibody) and in the presence of assay antibody (assessment of endogenous gastrin). Total gastrin fraction from acid treatment and Sep Pac purification of patient plasma was also applied to the column, eluted and assayed under the same conditions. (vi) Acid-purified antibody from patient plasma was used to construct calibration curves where ^125^I gastrin was used as tracer and human G17 as standard.

Results

The plasma from three patients in the total group studied (more than 50,000 subjects) showed the ability to bind ^125^I gastrin in the absence of assay antibody. All three patients with autoantibodies to gastrin had pernicious anaemia (more than 2000 patients, in the total group, had pernicious anaemia). Clinical details from these three patients with positive antigastrin autoantibodies, are shown below.

Patient 1

A 42-year-old male with insulin-dependent diabetes mellitus who was diagnosed at the age of 4 years. He suffered frequent hypoglycaemic episodes in childhood and adolescence. PA was diagnosed at the age of 36 years, and he was commenced on vitamin B12. His mother had pernicious anaemia and diabetes mellitus. In addition to antigastrin antibodies, he was also positive for antithyroid antibodies and antibodies to intrinsic factor. He was not dyspeptic.

Patient 2

A 35-year-old female who had rheumatoid arthritis diagnosed at the age of 27, when she presented with pain and swelling of her hands and feet during pregnancy. She also suffered from allergic rhinitis. PA was diagnosed at the age of 34 and she was commenced on vitamin B12. Her brother suffered from rheumatoid arthritis. In addition to antigastrin antibodies, she was also positive for antiparietal cell antibodies. She was not dyspeptic.

Patient 3

A 78-year-old female who had a past history of mild hypertension. Vitamin B12 was low, and a diagnosis
Autoantibodies to gastrin were detected in three patients. Treatment was commenced. She tested positive for cell, antinuclear and thyroid microsomal antibodies, in addition to antigastrin antibodies, antiparietal cell antibodies and intrinsic factor antibody. She was not dyspeptic.

### All three patients

Free gastrin levels in extracted plasma were within the normal range for this laboratory (0–40 pmol/l), while gastrin levels in whole plasma were increased in two of the three patients (50 and 69 pmol/l). Total, antibody-bound and free gastrin levels, however, were elevated in all three patients at 129, 233 and 317 pmol/l. Total gastrin was 10 to 14.0 times higher than free plasma gastrin as measured in extracted plasma and five times higher than free gastrin, measured directly in plasma (Table 1).

Autoantibodies to gastrin found in all three patients were of low titre and relatively high affinity. At a dilution of 1:10, the plasmas bound between 45 and 58% of $^{125}$I-labelled gastrin (Figure 1).

With gel chromatography, two peaks were eluted, one corresponding to globulins in molecular size and capable of binding to labelled gastrin, and the second of small molecular size, corresponding to gastrin. When plasma was acid-treated and purified with Sep Pac, and the gastrin fraction applied to gel filtration, the globulin peak was absent and the quantity of gastrin increased. The results of chromatography for patient 3 are illustrated in Figure 2.

Calibration curves constructed from patient plasma using $^{125}$I-labelled gastrin as tracer and G17 as standard gave ID50 values of 170, 215 and 290 pmol/l. Calibration curves were constructed from data for all three patients (Figure 3).

### Discussion

In this laboratory, autoantibodies to gastrin were readily detectable because of the methodology routinely used for the assay of gastrin in plasma. The rarity of antigastrin autoantibody is emphasized by

### Table 1 Gastrin levels in patients positive for antigastrin antibody

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracted plasma gastrin level (free) (pmol/l)</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Whole plasma gastrin level (free) (pmol/l)</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>Total gastrin level (bound and free) (pmol/l)</td>
<td>129</td>
<td>233</td>
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Figure 1. Antibody dilution curves under radioimmunoassay conditions using plasma from the three patients with antibody to gastrin. No control patient showed binding to labelled gastrin >3% at any dilution.

Figure 2. The elution profile for gel purification (G100) of plasma from patient 3. The column was calibrated with rabbit gamma globulin and $^{125}$I gastrin tracer. Fractions were assayed in the presence (to estimate gastrin) and absence (to illustrate antibody) of assay antibody. The continuous line shows results of untreated plasma and the broken line shows results of the gastrin fraction after treatment with acid and purification by Sep Pac.

Figure 3. Calibration curves constructed under radioimmunoassay conditions using untreated plasma from patients 1, 2 and 3, showing decrease in binding to $^{125}$I gastrin tracer with the addition of increasing amounts of gastrin standard.
the extremely low incidence. It is significant that all three patients suffered from PA in addition to other autoimmune disorders. None of the patients showed dyspeptic symptoms, and there was no particular syndrome directly associated with the gastrin autoimmunity. The ability of patient plasma to bind gastrin tracer has clearly been shown to be the result of endogenous antibodies, rather than the result of non-specific binding, by the production of calibration curves for gastrin and the partial purification of the antibody by chromatography.

In PA, gastrin levels become grossly elevated due to the absence of the negative feedback mechanism. Gastrin circulates in several different molecular forms, and in patients with PA, the processing of gastrin is incomplete. C-terminally extended gastrins in particular, rise considerably in patients with atrophic gastritis and PA. These larger gastrins which are incompletely processed at the C terminus may be more immunogenic than G-17 and G-34, and in particular where a patient is already showing autoimmunity.

The gastrin autoantibodies found in this study were of low titre and unexpectedly high affinity. In a comparative study with insulin antibodies, Valasalo compared the binding characteristics of insulin antibodies (IAA) with those of antibodies to exogenous insulin (IBA) and found that antibodies induced by treatment have a greatly increased binding capacity compared to endogenously-induced autoantibodies to insulin, leading to a significantly higher specific insulin binding in the case of intragenic antibodies.

The development of autoimmune diseases is often associated with the emergence of particular antibodies. Certainly, it is difficult to say whether gastrin autoantibodies appeared at the onset of the disease process in these three patients described. As the antibodies were detected with the first gastrin measurement which was used as a diagnostic tool, it is likely that they appeared at least at an early stage in the disease process. Circulating gastrin levels are usually, but not invariably elevated in autoimmune atrophic gastritis and PA. It is possible that endogenous gastrin antibodies may obscure the elevation of gastrin in some patients.

In conclusion, gastrin autoantibodies are very rare and may result in a falsely low circulating gastrin concentration. In the patients presented here, gastrin autoantibodies were associated with pernicious anaemia and the presence of other autoimmune disorders. Gastrin autoimmunity was not associated with peptic ulcer disease, and there was no evidence to suggest any deleterious effect of the anti-gastrin antibodies.

References