Subclass distribution of mucosal IgG-producing cells in gastritis

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SUMMARY  IgG-mediated immune reactions are probably involved in the maintenance of gastritis and glandular atrophy; the mucosal IgG-subclass pattern may therefore influence the effect of local hypersensitivity mechanisms. In this study the proportions of IgG1-, IgG2-, IgG3-, and IgG4-producing immunocytes were determined by paired immunofluorescence staining in specimens from simple gastritis, gastritis after Billroth II (BII) resection, and gastritis associated with dermatitis herpetiformis (DH). The results were related to histopathological degree of inflammation and atrophy. Generally, IgG1 immunocytes predominated (48–60%) in all types of gastritis. With increasing severity of inflammation, the IgG2-cell proportion was significantly increased from 4–6% to 26–34% in simple and BII gastritis, whereas the ratio of IgG1 immunocytes was correspondingly decreased from 58–69% to 38–43%. In the same types of gastritis the proportion of IgG3 cells was increased in association with severe (35–38%) compared with mild (15–23%) atrophy, whereas the proportion of IgG1 cells was correspondingly decreased. In severe gastritis associated with DH, the proportion of IgG1 cells was relatively high (60%) and that of IgG2 cells relatively low (13%), and severe atrophy did not seem to influence significantly the subclass proportions.

Previous immunohistochemical studies of gastric mucosa1–3 have shown that, with increasing severity of inflammatory changes, the number of IgG-producing cells is raised – in simple gastritis (SG), in gastritis after Billroth II (BII) resections and in gastritis associated with dermatitis herpetiformis (DH). This implies that the normal mucosal immunological homeostasis, with a predominantly IgA-producing cell population, is altered in favour of a non-secretory local IgG response in severe gastritis. As discussed elsewhere in relation to inflammatory mucosal disease, such an altered local homeostasis may contribute to aggravation and maintenance of inflammatory mechanisms.4

No information exists concerning the mucosal distribution of IgG subclass producing immunocytes in normal and diseased gastric mucosa. As very few mucosal IgG cells occur in the absence of inflammation, immunohistochemical quantification of their subclasses distribution in the histologically normal mucosa is hampered by great methodological difficulties. In the present study the IgG-subclass distribution of mucosal immunocytes was determined in various types of gastritis and was related to the degree of inflammation and atrophy. Such information is of interest because the biological properties vary considerably among the IgG subclasses.5

Methods

PATIENTS AND TISSUE PREPARATION
The gastric body specimens were of the following three categories. (1) Simple gastritis (SG) group: small mucosal pieces of surgically resected stomachs from patients operated for duodenal or gastric ulcer or from kidney donors. (2) Dermatitis herpetiformis (DH) group: obtained by a multiple biopsy capsule

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Figure  Paired immunofluorescence staining for IgG (left, rhodamine) and IgGl subclass (right, fluorescein) in the same field from section of gastric body mucosa. Examples of identical cells are indicated by arrows. Bar=40 um.

from patients with DH. (3) Billroth II (BII) group: obtained endoscopically from duodenal ulcer patients subjected to BII resections 25–30 years previously. All tissue specimens were extracted in cold phosphate buffered isotonic saline for 48 hours prior to cold ethanol fixation and paraffin embedding.6 Serial sections were cut at 6 μm and either stained by a haematoxylin-azophloxine-saffron (HAS) method7 or by two-colour immunohistochemistry. The HAS-stained sections were graded by conventional histopathological evaluation for gastritis and atrophy.8 In addition, selected antral specimens (n= 13) from the SG group were examined by the same procedures.

IMMUNOHISTOCHEMICAL REAGENTS AND STAINING PROCEDURE
Dewaxed serial sections were incubated with murine monoclonal antibodies (ascitic fluid) to IgG1 (clone 2C7), IgG2 (clone GOM2), IgG3 (clone CBI-AH7), or IgG4 (clone RJ4); these reagents were selected on the basis of an international evaluation study9 and immunohistochemical performance experiments in our laboratory.10 Ascitic fluid (1:800) was applied for 20 hours at room temperature; the second incubation included a mix of fluorescein isothiocyanate labelled rabbit antimouse IgG and rhodamine B sulphonyl chloride-labelled anti-human IgG.11 After the final wash, the sections were mounted in buffered polyvinyl alcohol.11

MICROSCOPY, CELL COUNTING, AND EVALUATION OF RESULTS
The sections were evaluated in a Leitz Orthoplan microscope equipped with a Ploem-type vertical illuminator with interference filters for selective observation of fluorescein (green) or rhodamine (red) emission. Counting of positive immunocytes was performed with an ×25 oil immersion objective and an ×10 ocular lens. IgG-producing cells with a distinct cytoplasmic fluorescence were recorded (Figure). In each tissue specimen usually several hundred (median, 840; range, 168–1755) IgG immunocytes (red) were counted and examined for
concomitant subclass staining (green). The proportion of each subclasses was calculated in relation to the total number of IgG-producing cells evaluated in the same section. The sum of the four subclass proportions in the four serial sections from the same tissue specimen was close to 100% (median, 100-3; range, 85-119%). This result attested to the reliability of the method. Variation from 100% for individual specimens did not only reflect methodological problems but could also include biological variations in the subclass distribution between serial sections.

Differences between median values of IgG subclass producing cells were determined by Wilcoxon's two-tailed test for unpaired samples. Results were given as median and range.

**Results**

**IgG-subclass proportions in different specimen categories**

The median sum of the four IgG-subclass percentages in serial sections from individual specimens was in the SG group 101%, in the BII group 98%, in the DH group 92% (Table 1), and in the antral specimens 104% (range, 89-119%).

IgG1 immunocytes predominated in all specimen categories (Table 1). There were more IgG2 than IgG3 cells in SG, whereas the latter predominated significantly over the former subclass in the BII and DH groups. IgG4 cells accounted for a small proportion in all groups.

When statistical comparisons were performed between specimen groups, the proportion of IgG3 was significantly higher in the BII group than in the SG group, whereas the contrary was found for IgG4 (Table 1).

In the 13 antral specimens the proportions of IgG1, IgG2, IgG3, and IgG4 cells were 42%, 34%, 20-8%, and 0-7%, respectively; this was not significantly different from the result in the body specimens of the whole SG group.

**IgG-subclass proportions related to grade of gastritis**

There was an increased proportion of IgG2 cells in specimens with moderate to severe gastritis (grades 2 and 3) of the SG and BII groups (Table 2). The IgG1 proportion was correspondingly decreased, significantly so for the SG group. Similar although not significant inflammation related changes were found in the antral SG specimens (Table 3).

Within each grade of gastritis no differences were found between specimen groups, except for a higher proportion of IgG4 and IgG2 in mild and severe SG, respectively – the first compared with the BII group and the second with the DH group (Table 2).

**IgG-subclass proportions related to grade of atrophy**

In body specimens with increased severity of atrophy, the proportion of IgG3 cells tended to be raised and IgG1 cells decreased, particularly in the SG and the BII groups (Table 4). A significantly increased proportion of IgG3 cells (24%) was likewise found in antral SG specimens (n=9) with atrophy of grades 1 and 2 as compared with the percentage (10%) in specimens with grade 0 (n=4). Conversely, severe atrophy did not seem to influence the IgG-subclass proportions in the DH specimens with severe gastritis (Table 4). Because of the wide scatter of the data, however, no differences were revealed between the specimen groups within each grade of atrophy.

**Discussion**

IgG1 was the predominating subclass produced locally in SG and in gastritis associated with BII resection or DH. The IgG1 subclass likewise predominates among IgG immunocytes in the intestinal lesions of inflammatory bowel disease and coeliac disease (submitted data). The present study revealed two interesting alterations of the gastric subclass pattern. First, with increasing degree of SG and...
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Table 2  Median and range of IgG subclass proportions (%) in gastric body specimens with simple gastritis (SG), gastritis after Billroth II resection (BII), and gastritis associated with dermatitis herpetiformis (DH), related to grade of gastritis

<table>
<thead>
<tr>
<th>Grade of gastritis</th>
<th>Mild (grade 1)</th>
<th>Moderate/severe (grades 2 and 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG IgG1</td>
<td>69.2% (34.0-93.7)</td>
<td>43.4 (26.3-67.1)</td>
</tr>
<tr>
<td>IgG2</td>
<td>3.9 (1.3-51.3)</td>
<td>23.9* (13.3-30.4)</td>
</tr>
<tr>
<td>IgG3</td>
<td>1.4 (0-2.6)</td>
<td>1.4 (0-2.7)</td>
</tr>
<tr>
<td>n=8</td>
<td>n=14</td>
<td></td>
</tr>
<tr>
<td>BII IgG1</td>
<td>57.8 (28.6-81.8)</td>
<td>38.1 (30.3-69.0)</td>
</tr>
<tr>
<td>IgG2</td>
<td>6.4 (1.8-32.7)</td>
<td>25.9* (9.8-42.8)</td>
</tr>
<tr>
<td>IgG3</td>
<td>24.8 (5.0-66.3)</td>
<td>28.0 (8.4-57.6)</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.5 (0-1.8)</td>
<td>0.6 (0-4-8)</td>
</tr>
<tr>
<td>n=12</td>
<td>n=13</td>
<td></td>
</tr>
<tr>
<td>DH IgG1</td>
<td>ND</td>
<td>60.3 (28.6-73.1)</td>
</tr>
<tr>
<td>IgG2</td>
<td>ND</td>
<td>12.6 (1.3-36.9)</td>
</tr>
<tr>
<td>IgG3</td>
<td>ND</td>
<td>23.4 (4.1-26.2)</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.7 (0-3.9)</td>
<td></td>
</tr>
<tr>
<td>n=11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n=number of specimens; *=significantly raised subclass proportion compared with other grade of gastritis; ‡=significantly raised subclass proportion compared with one of the other specimen groups with the same grade of gastritis; ND=not determined because of small number of specimens.

gastritis after BII resection, the proportion of IgG2 cells increased whereas that of IgG1 cells decreased correspondingly. In the DH group, however, the proportion of IgG1 cells was not decreased in moderate to severe gastritis compared with mild gastritis of the two other categories. With this exception, a shift towards IgG2 production seemed to be linked to intensified mucosal immune responses. A similar raised proportion of jejunal IgG2 immunocytes is seen in untreated compared with treated coeliac disease or food allergy (submitted data). There is likewise a significantly increased proportion of IgG2 cells in the lesion of Crohn colitis in contrast with active ulcerative colitis, which, however, shows more pronounced inflammation (and more involvement of systemic-type immunity) than Crohn's disease, coeliac disease and chronic gastritis.

Table 3  Median and range of IgG subclass proportions (%) in antral specimens with simple gastritis

<table>
<thead>
<tr>
<th>Grade of gastritis</th>
<th>Moderate (grade 2) n=6</th>
<th>Severe (grade 3) n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>50.8a (31.0-67.2)</td>
<td>36.0a (32.2-49.8)</td>
</tr>
<tr>
<td>IgG2</td>
<td>17.1a (7.9-57.7)</td>
<td>49.7a (30.4-61.8)</td>
</tr>
<tr>
<td>IgG3</td>
<td>18.5a (6.1-35.1)</td>
<td>22.5a (7.9-32.3)</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.8 (0-1.8)</td>
<td>0.7 (0-4-1.7)</td>
</tr>
</tbody>
</table>

n=number of specimens; a=significantly raised subclass proportion within same grade of gastritis compared with one (a1), two (a2), or three (a3) of the other subclasses.

The second significant finding was a relatively large proportion of IgG3 cells associated with severe atrophy in the gastric mucosa, although this could not be shown in DH-associated atrophic gastritis. In the latter category the proportion of IgG1 cells remained fairly unaltered even in severe atrophy. We have previously reported that IgG immunocytes are relatively more increased in the basal zone of the body mucosa in association with atrophy in SG. The present study showed a preferential association between atrophy in SG and BII gastritis and increase of the IgG3 subclass. As this subclass shows particularly strong complement activating properties and capacity for binding to Fc receptors on mononuclear cells, our findings might reflect an immunopathological link.

The subclass distribution of extracellular mucosal IgG could not be evaluated in the present study because the tissue specimens were subjected to prewashing. Because extracellular IgG is serum derived and locally produced, the subclass distribution in serum would be of importance in the mucosa. Normally this distribution is known to be about 60-70% IgG1, 20-30% IgG2, 5-8% IgG3, and 0-7-5% IgG4. Possible alterations in this distribution in relation to gastritis are, however, unknown.

The mechanisms regulating the IgG-subclass responses are obscure; both immunological and genetic variables may be involved. The nature of the antigen definitely plays a part in isotype expression. IgG1 and IgG3 antibodies are triggered mainly by protein antigens - for example, virus infections, as...
opposed to IgG2, which is stimulated preferentially by bacterial carbohydrates. 17

Such stimulatory differences may explain the disparity between the intestinal mucosa and the nasal mucosa, which tends to show a preference of IgG3 over IgG2 cells. 18 Whether the tendency for IgG3 to dominate over IgG2 in the gastric mucosa (except in severe SG) reflects increased penetration of food proteins, such as in reflux gastritis after BI1 resection, or viral infection, is unknown. The specificities of the local IgG cells have to be mapped to obtain information to this end.

Genetic factors may additionally influence the IgG-subclass response. If such variables were at play in our material, this would most likely be the case in the DH group which was best defined. It is unknown, however, whether certain IgG heavy-chain markers are associated with DH.

Finally, local immunoregulatory events may in theory influence the IgG-subclass expression. It has recently been suggested from studies of human myeloma cells that IgA1 immunocytes mainly differentiate from IgG1-expressing precursors. 19 Frequent vectorial switching from IgG1 to IgA1 expression in the gastric mucosa may thus explain the relatively reduced proportion of IgG1-producing cells seen with increasing inflammation. IgA1-producing cells are indeed by far the most predominant immunocyte class in the gastric mucosa. 20

The main conclusions of the present study are that IgG1 is the major IgG subclass produced in human gastric mucosa. With increasing degree of simple gastritis, the proportion of IgG2 cells increases; in severe gastritis after BI1 resections, both IgG2 and IgG3 cells tend to increase; and in severe DH-associated gastritis, there tends to be a preference of IgG3 over IgG2 cells. In severe atrophy of the gastric mucosa, IgG3 cells increase, except in DH-associated gastritis. These alterations may reflect local immune responses linked to aetiological or immunopathological events in the development of chronic gastritis.

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References