Hyposplenism and Gluten-Sensitive Enteropathy
Natural History, Incidence, and Relationship to Diet and Small Bowel Morphology

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Splenic function was quantitatively assessed using "pitted" erythrocyte counts in 177 patients with gluten-sensitive enteropathy. Hyposplenism was found to be a common, but not inevitable, complication of gluten-sensitive enteropathy that fluctuated with disease activity. Splenic function improved after withdrawal of gluten from the diet and a close relationship was demonstrated between hyposplenism and the morphology of the small intestine. The severity of the hyposplenism increased with advancing age and prolongation of exposure to dietary gluten. Splenic function did not vary with the HLA-A, -B, and -DR antigens, but, due to the presence of HLA-B8 and -DR3 in the vast majority of patients, a role for this haplotype in the causation of the hyposplenism cannot be excluded.

In 1888, Gee, in his celebrated paper "On the Coeliac Affection," stated "that the spleen (is) sometimes enlarged" (1). Since then, it has become clear that hyposplenism and splenic atrophy, rather than splenomegaly, are commonly associated with gluten-sensitive enteropathy (GSE) or celiac disease (2-6). The significance of this complication of GSE is unclear, partly as a result of our incomplete understanding of its natural history. The reported incidences of hyposplenism in GSE vary widely, ranging from 16% (2) to 77% (3). One study suggested that the incidence of hyposplenism in patients with GSE was related to the duration of untreated disease (6), but another found no correlation between splenic function and the length of the initial exposure to dietary gluten (7). There is also conflicting evidence regarding the effect on hyposplenism of gluten withdrawal from the diet. Recent reports suggest that the hyposplenism of GSE is improved by a gluten-free diet (8,9), in contrast to another assertion that the hyposplenism is irreversible (7). It is further considered that splenic function in GSE is not related to the state of the small intestinal mucosa (7), but to date there is no conclusive evidence in this regard.

Much of the uncertainty about the role of hyposplenism in GSE stems from the relatively small numbers of patients studied. The technique most commonly used to assess splenic function was the clearance of radioactively labeled, damaged erythrocytes from the circulation. This method is difficult to standardize (10), time-consuming, and unsuitable for repeated studies and use in younger patients. "Pitted" erythrocyte counts overcome many of these problems and have been used to quantitatively assess splenic function, not only in GSE (6,8,11) but also in dermatitis herpetiformis (6), neonates (12,13), sickle-cell anemia (14), splenic irradiation (15), and splenosis (16-19). In patients with GSE, pitted erythrocyte counts have been found to correlate with the clearance of radioactively labeled, heat-damaged erythrocytes.
erythrocytes from the circulation and with splenic size (6). In this study, we used pitted erythrocyte counts in a large group of patients with GSE to determine the incidence and natural history of this complication of GSE and to determine its relationship to dietary status and small bowel morphology.

**Materials and Methods**

**Patients**

One hundred seventy-seven patients with GSE were studied. All underwent small bowel biopsy at the time of diagnosis, and in each case there was a histologic or clinical response, or both, to removal of gluten from the diet. The male/female ratio was 2:3 and the age range was 2.5-80 yr (mean 42.2 yr). Two control groups consisting of 118 healthy volunteers and 77 splenectomized subjects were also studied. The ages of the normal controls ranged from 14 to 80 yr (mean 37.4 yr) and there was an equal sex distribution. The ages of the splenectomized controls ranged from 7 to 79 yr (mean 39.5 yr) and the male/female ratio was 2:1. All subjects underwent splenectomy between 1 and 10 yr before the time of study.

**Small Bowel Biopsy Specimens**

Small bowel biopsy specimens were obtained with a Watson capsule or, in a small number of cases, through the fiberoptic endoscope. All biopsies were performed during the appropriate investigation and follow-up of patients with proven or suspected GSE. The biopsy specimen was graded histologically on a scale of 0-111 as previously described in detail (20). Briefly, grade 0/1 represents normal appearances or minor abnormalities, grade 11 represents partial villus atrophy, and grade 111 represents subtotal or total villus atrophy.

**Diet**

All patients with GSE who underwent small bowel biopsy were interviewed by one experienced clinician (F.S.) to determine the quality of the gluten-free diet.

**Pitted Erythrocyte Counts**

Pitted erythrocyte counts were performed at least once on all patients with GSE and once only on the normal and splenectomized controls using the method of Pearson et al. (16). Three drops of fresh venous blood were mixed with 1 ml of 3% glutaraldehyde solution at pH 7.4. In each case, 1000 consecutive erythrocytes, in a wet preparation, were examined using a Nikon interference phase microscope (Nikon Inc., Garden City, N.Y.) (×1000). The result was expressed as the percentage of erythrocytes having one or more pit. All counts were performed by one observer (J.O.G.) without knowledge of the clinical status of the subject.

**Tissue Typing**

HLA-A and -B typing was performed by the standard microlymphocytotoxicity test; HLA-DR typing was performed by the long B-cell enriched microlymphocytotoxicity test (21).

**Statistical Methods**

Analysis was performed by computer, using the Mann–Whitney U-test and the paired Student’s t-test.

**Consent**

Informed written consent was obtained from the individual, parent, or guardian in all cases.

**Results**

**Age at Time of Study**

The first pitted erythrocyte count obtained for each of the 177 patients with GSE was used (Figure 1). Pitted erythrocyte counts were lower in those <20 yr old than in all groups ≥30 yr old (p < 0.05). The counts showed little variation in the three decades covering the 20–50-yr age span. The highest counts were seen in the 50–60-yr-old group, and the differences between this and all younger groups were significant (p < 0.05). The mean pitted erythrocyte counts fell after the age of 60 yr. Pitted erythrocyte counts in the normal controls were higher in those >60 yr old (p < 0.05), corresponding to the decrease in mean splenic volume found in this age group. Age did not influence the pitted erythrocyte counts in the splenectomized subjects.

![Figure 1](image-url)
Age at Time of Diagnosis of Gluten-Sensitive Enteropathy

Pitted erythrocyte counts increased with patient age at the time of diagnosis of GSE (Figure 2). The counts in patients >50 yr old were higher than those in patients <20 yr old (p < 0.05). The peak seen in the 50–60 yr old group in Figure 1 is not reproduced.

Relationship Between Splenic Function and Small Bowel Morphology

Patients with GSE were divided into three age groups on the basis of the pattern of results described above, i.e., <20 yr old, 20–50 yr old, and >50 yr old. Each age group was subdivided on the basis of the morphologic grade of the small bowel mucosa. Data are represented for 188 biopsy specimens from 149 patients with GSE (Figure 3), as more than one set of data was accepted from the same subject if at separate studies they fell into different morphologic categories. The number of subjects in the <20-yr-old group is relatively small but no differences were found between the morphologic groupings. In the two older age groups, pitted erythrocyte counts were lower in patients with grade 0/I biopsy specimens than in those with either grade II or grade III specimens (p < 0.02). No significant differences were found between counts in patients with grade II and grade III biopsy specimens.

Gluten-Free Diet and Splenic Function

Eighteen patients with elevated pitted erythrocyte counts on normal diets were studied at intervals of 1, 2, 4, and ≥6 mo after withdrawal of gluten from the diet. All the patients were not available at every interval, and the longest period of study was 18 mo. The pitted erythrocyte counts were highest while the patients were on normal diets (Figure 4) and fell significantly at all time intervals after gluten withdrawal (p < 0.05). The counts were lower after 4 and ≥6 mo on a gluten-free diet than after only 1 mo of treatment (p < 0.02). A significant fall also occurred in pitted erythrocyte counts between the 2- and 4-mo intervals (p < 0.04), but no further decrease was found after 4 mo. All these comparisons refer to paired counts from individuals studied at both times in question. At the time of completion of the study, splenic function had returned to normal in 14 of the 18 patients (77.7%). Three of the 4 patients whose pitted erythrocyte counts remained elevated underwent small bowel biopsy at a later date that showed grade III morphologic changes.

Incidence of Hyposplenism in Gluten-Sensitive Enteropathy

Hyposplenism was defined as present if the pitted erythrocyte count was greater than the upper limit of the range found in the age-matched control group. Age, dietary status, and small bowel morphology influence splenic function, and meaningful estimations of the incidence of hyposplenism in GSE must take these factors into account. The figures listed in Table 1 refer only to adults with GSE. Hyposplenism was found in 76.2% of 42 patients on normal diets. Sixty patients apparently on gluten-free diets were found to have abnormal small bowel

Figure 2. Pitted erythrocyte counts are shown as mean and SEM for each group or subgroup (n, figure under the respective horizontal lines). The pitted erythrocyte counts increase with prolongation of the exposure to dietary gluten in gluten-sensitive enteropathy patients. The pitted erythrocyte counts in the control groups are indicated on the basis of age at the time of study.

Figure 3. Pitted erythrocyte counts in gluten-sensitive enteropathy patients with grade 0/I (●), grade II (■), and grade III (○) small bowel biopsy specimens in three age groups as indicated. In the 20–50-yr-old and >50-yr-old groups the counts were higher in patients with either grade II or III biopsy specimens than in those with grade 0/I biopsy specimens (p < 0.02).
Table 1. Incidence of Hyposplenism in Gluten-Sensitive Enteropathy

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal function</th>
<th>Hyposplenism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 111</td>
<td>23.8% (10)</td>
<td>76.2% (32)</td>
</tr>
<tr>
<td>Grade 11</td>
<td>20.6% (7)</td>
<td>79.4% (27)</td>
</tr>
<tr>
<td>Grade 0/1</td>
<td>91.4% (42)</td>
<td>8.6% (4)</td>
</tr>
<tr>
<td>No biopsy</td>
<td>64.3% (18)</td>
<td>36.7% (10)</td>
</tr>
</tbody>
</table>

The incidence of normal splenic function and hyposplenism in subgroups of adults with gluten-sensitive enteropathy divided on the basis of diet and small bowel morphology. Number of subjects is given in parentheses.

Discussion

This study represents the largest investigation to date of splenic function in patients with GSE. It demonstrates hyposplenism to be a common and dynamic complication of this disease. Hyposplenism was present in 76.2% of untreated adult GSE patients and improved over a 4-mo period after withdrawal of gluten from the diet. Hyposplenism occurred in 69%-79% of patients with damaged small intestinal mucosas in comparison with only 8.6% of patients with normal or near normal mucosas. It is interesting that no difference in splenic function was found between patients with moderate

Table 2. HLA-A and -B Antigens and Pitted Erythrocyte Counts

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Frequency</th>
<th>Count</th>
<th>Frequency</th>
<th>Count</th>
<th>Frequency</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>69.3</td>
<td>10.5</td>
<td>3.0 (0.28)</td>
<td>14.9</td>
<td>13.6 (1.3)</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>40.5</td>
<td>9.3</td>
<td>3.0 (0.33)</td>
<td>42.2</td>
<td>12.3 (1.4)</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>21.6</td>
<td>9.8</td>
<td>3.0 (0.31)</td>
<td>16.9</td>
<td>16.0 (1.9)</td>
<td></td>
</tr>
<tr>
<td>A9</td>
<td>9.1</td>
<td>11.7</td>
<td>3.4 (0.33)</td>
<td>16.9</td>
<td>15.8 (1.8)</td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>4.6</td>
<td>8.5</td>
<td>3.6 (0.33)</td>
<td>6.0</td>
<td>14.6 (1.5)</td>
<td></td>
</tr>
<tr>
<td>B7</td>
<td>19.6</td>
<td>10.6</td>
<td>3.1 (0.29)</td>
<td>25.4</td>
<td>12.5 (1.3)</td>
<td></td>
</tr>
<tr>
<td>B8</td>
<td>85.0</td>
<td>10.5</td>
<td>3.4 (0.32)</td>
<td>34.5</td>
<td>13.9 (1.9)</td>
<td></td>
</tr>
<tr>
<td>B12</td>
<td>20.9</td>
<td>11.6</td>
<td>2.8 (0.27)</td>
<td>35.9</td>
<td>12.2 (1.7)</td>
<td></td>
</tr>
<tr>
<td>B14</td>
<td>17.0</td>
<td>10.0</td>
<td>3.2 (0.30)</td>
<td>4.8</td>
<td>13.3 (1.6)</td>
<td></td>
</tr>
</tbody>
</table>

GSE, gluten-sensitive enteropathy. The frequency of the principal HLA-A and -B antigens in the GSE patients, normal controls, and splenectomized controls are indicated as percentages. The counts are indicated as mean (SEM).
between the presence and severity of hyposplenism and the level of circulating immune complexes. A close correlation has been found.

Blockade of the reticuloendothelial system by immune complexes is a result of splenic atrophy. Hyposplenism is not reversible, although in some patients there is evidence of an irreversible loss of function, presumably due to an absence of a variation in splenic function with age. The pathogenesis of the functional hyposplenism is unique to GSE among bowel disorders, but is well recognized in association with ulcerative colitis and Crohn's disease (22). As in GSE, the severity of the hyposplenism fluctuates with the activity of the underlying disease. The pathogenesis of the hyposplenism of GSE and inflammatory bowel disease is unknown. A possible cause is transient saturation or blockade of the reticuloendothelial system by immune complexes. A close correlation has been found between the presence and severity of hyposplenism and the level of circulating immune complexes in rheumatoid arthritis (23) and systemic lupus erythematosus (24,25). In one study, the hyposplenism was reversed by plasma exchange (24). Circulating immune complexes have been reported, although in widely varying percentages, in both GSE and inflammatory bowel disease (26–31), and immune complexes occur more commonly in untreated GSE patients than in those on gluten-free diets (26). Although studies correlating circulating immune complexes and splenic function in GSE are needed, there is some evidence to suggest that immune complexes alone are not the complete explanation of the pathogenesis of the functional hyposplenism. In dermatitis herpetiformis, a condition closely allied to GSE, no correlation was found between the defect in reticuloendothelial function and the presence of circulating immune complexes (32). This study, however, demonstrated a defect in Fc-receptor function associated with the HLA-B8/DRw3 haplotype, not only in dermatitis herpetiformis but also in 50% of normal controls. In this present study, the HLA-B8 and -DR3 antigens were present in 85% and 91.4% of GSE patients, respectively. It is plausible that the defect in Fc-receptor function associated with these HLA antigens, coupled with immune complexes or another as yet unidentified "blocking factor," is responsible for the functional hyposplenism of GSE.

**Table 3. HLA-DR Antigens and Pitted Erythrocyte Counts in Gluten-Sensitive Enteropathy**

<table>
<thead>
<tr>
<th>Antigen(s)</th>
<th>Frequency</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR3/1</td>
<td>9.5</td>
<td>9.0 (1.3)</td>
</tr>
<tr>
<td>DR3/2</td>
<td>10.5</td>
<td>10.9 (2.6)</td>
</tr>
<tr>
<td>DR3/5</td>
<td>10.5</td>
<td>7.4 (0.8)</td>
</tr>
<tr>
<td>DR3/6</td>
<td>8.6</td>
<td>7.2 (1.4)</td>
</tr>
<tr>
<td>DR3/7</td>
<td>24.8</td>
<td>8.5 (0.8)</td>
</tr>
<tr>
<td>DR3/9</td>
<td>6.7</td>
<td>10.0 (2.2)</td>
</tr>
<tr>
<td>DR3 absent</td>
<td>8.6</td>
<td>8.7 (1.7)</td>
</tr>
</tbody>
</table>

The principal HLA-DR antigens found in 105 patients with gluten-sensitive enteropathy are shown as percentages with their associated pitted erythrocyte counts given as mean (SEM). The counts did not vary significantly with the DR antigens.

(grade 11) and severe (grade 111) mucosal damage, suggesting that almost complete regeneration of the small intestinal mucosa must occur before the hyposplenism can be maximally reversed. Hyposplenism was found in 36.7% of patients who were taking gluten-free diets but who did not undergo small bowel biopsy at the time of assessment. This figure is very similar to the incidence of hyposplenism reported by Corazza et al. (6) who accepted dietary status alone as evidence of adequate treatment. The severity of hyposplenism in GSE clearly increased both with age and with the duration of exposure to dietary gluten. Hyposplenism is not an inevitable complication of GSE, however, as shown by a subgroup in this study who had normal splenic function while on gluten-containing diets and who had severely damaged small intestinal mucosas. These patients account for between 20% and 25% of the adults with GSE and report a cross section of the study group in terms of age and sex distribution. This subgroup of patients may lack genetic determinants predisposing to the development of hyposplenism, as suggested by Trewby et al. (7), although the absence of a variation in splenic function with HLA antigens does not lend support to this postulation.

The hyposplenism of GSE is largely functional or reversible, although in some patients there is evidence of an irreversible loss of function, presumably as a result of splenic atrophy. Hyposplenism is not unique to GSE among bowel disorders, but is well recognized in association with ulcerative colitis and Crohn's disease (22). As in GSE, the severity of the hyposplenism fluctuates with the activity of the underlying disease. The pathogenesis of the hyposplenism of GSE and inflammatory bowel disease is unknown. A possible cause is transient saturation or blockade of the reticuloendothelial system by immune complexes. A close correlation has been found between the presence and severity of hyposplenism and the level of circulating immune complexes in glutensensitive enteropathy (23) and systemic lupus erythematosus (24,25). In one study, the hyposplenism was reversed by plasma exchange (24). Circulating immune complexes have been reported, although in widely varying percentages, in both GSE and inflammatory bowel disease (26–31), and immune complexes occur more commonly in untreated GSE patients than in those on gluten-free diets (26). Although studies correlating circulating immune complexes and splenic function in GSE are needed, there is some evidence to suggest that immune complexes alone are not the complete explanation of the pathogenesis of the functional hyposplenism. In dermatitis herpetiformis, a condition closely allied to GSE, no correlation was found between the defect in reticuloendothelial function and the presence of circulating immune complexes (32). This study, however, demonstrated a defect in Fc-receptor function associated with the HLA-B8/DRw3 haplotype, not only in dermatitis herpetiformis but also in 50% of normal controls. In this present study, the HLA-B8 and -DR3 antigens were present in 85% and 91.4% of GSE patients, respectively. It is plausible that the defect in Fc-receptor function associated with these HLA antigens, coupled with immune complexes or another as yet unidentified "blocking factor," is responsible for the functional hyposplenism of GSE.

**References**