Abstract. Peripheral blood lymphocytes from patients with dermatitis herpetiformis and healthy controls were investigated in order to determine the percentages of B-lymphocytes, T-lymphocytes and cells with receptors for the Fc part of human IgG (EA-RFC). Furthermore, the serum concentrations of IgG, IgA, IgM, IgD and IgE and the complement factors C3 and C4 were also measured. An increase in the mean percentages of lymphocytes with membrane-bound IgA and IgD, as well as an increase in the mean percentage of cells with receptors for the Fc part of human IgG (EA-RFC) were found in the patient group as compared with the control group. No differences between patient group and control group were found concerning the mean percentages of B-lymphocytes or T-lymphocytes. The mean serum concentrations of the various immunoglobulins and the complement factors investigated did not differ significantly between the two groups.

Key words: Dermatitis herpetiformis; Lymphocyte subpopulations; Immunoglobulins; Complement factors

Dermatitis herpetiformis is a polymorphic skin eruption which often appears symmetrically on forearms, legs, loins and buttocks. Severe itching may occur, but there are usually no general complaints. Blisters are often formed on an erythematous base (15). The clinical picture is not absolutely conclusive for diagnosis and consequently additional confirmation by histological examination is needed. This usually reveals neutrophilic intrapapillary microabscesses and subepidermal blisters (13, 15) and, on immunohistological examination, IgA deposits at the dermo-epidermal junction of normal and perilesional skin can be demonstrated (22). An increased number of IgA-containing plasma cells have been demonstrated in the jejunal mucosa (2). Furthermore an increased concentration of IgA has been found in the jejunal fluid (16, 20) as well as in saliva (20) of patients with dermatitis herpetiformis. Some patients also have an elevated serum concentration of IgA (21).

The aim of this study was to investigate some immunological parameters in peripheral blood of patients with dermatitis herpetiformis, such as proportions of different lymphocyte populations and concentrations of immunoglobulins and complement factors. The study was concentrated particularly on the detection of the IgA-positive peripheral lymphocytes, because of the reported increased activity of the IgA system in this disease.

MATERIALS AND METHODS

Patients. 11 patients, 5 females and 6 males with a mean age of 44 years, range 12-72 years; and 11 controls, 8 females and 3 males with a mean age of 31 years, range 22-55 years, were investigated. The mean duration of the disease was 14.7 years, with a range from 2 to 30 years. All patients had skin lesions. 9 patients adhered to a gluten-free diet and aldesulphone sodium or dapsone. One patient took dapsone only, and another patient used gluten-free diet but no tablets. All patients agreed upon request to refrain from both diet and sulphones the last week before the blood was drawn. They were all investigated utilizing direct immunofluorescence technique on skin tissue specimens of involved and uninvolved skin. All but one patient had IgA deposits at the dermo-epidermal junction. The latter patient was treated with dapsone and gluten-free diet with good therapeutic effect and presented a positive histology with neutrophilic intrapapillary microabscesses and subepidermal blisters in the involved skin. A biopsy of the jejunal mucosa revealed villous atrophy.

Separation of cells. Cells were separated by the Isopaque-Ficoll technique (5).

Detection of T-lymphocytes. T-lymphocytes were detected by a specific rabbit anti-human T-lymphocyte antisera and by a rosette technique utilizing sheep erythrocytes (3, 9, 11).

The anti-T-lymphocyte antisera was raised against thymocytes, extracted from pieces of thymus obtained from children undergoing cardiac surgery. The thymocyte suspension was injected into rabbits, and the antisera obtained from the rabbits was rendered T-lymphocyte specific by extensive absorptions with human AB and O erythrocytes, pooled human IgG, and B-lymphocytes.
from patients with chronic lymphocytic leukemia. The specificity of this antiserum has been determined in another study (paper in preparation). The anti-T antiserum was then used in an indirect immunofluorescence technique with fluoresceinated goat anti-rabbit antiserum as the second layer.

B-lymphocytes. Antisera against different Ig-classes and IgG-F(ab')2-fragments were raised in rabbits, and used in the direct immunofluorescence technique to demonstrate membrane-bound immunoglobulins on human B-lymphocytes (12).

EA-RFC. Cells forming rosettes with IgG sensitized erythrocytes (EA-RFC) were detected after reaction of the lymphocytes with human IgG (anti-C+D) coated human O,R,R2 erythrocytes (11).

Serum immunoglobulins and complement factors. IgG, IgA, IgM, IgD, C3 and C4 concentrations were measured by the single radial immunodiffusion method (18). Serum IgE concentrations were measured by means of radioimmunoassay (Pharmacia Diagnostics Phadebas IgE test).

Immunofluorescence staining and microscopy. Immunofluorescence staining was performed with the direct method for all antisera with the exception of the anti-T-lymphocyte antiserum. 4 x 10^5 lymphocytes in Medium 199 (Grand Island Biological Company, Grand Island, New York) were incubated with 0.1 ml of a dilution of 1/4-1/16 of FITC-conjugated antiserum. F/P ratios 1.6-3.0, for 20 min at +4°C. Thereafter the cells were washed once in Hanks' BSS (GIBCo., Grand Island, New York), and once in McCoy's 5A medium (GIBCo, Bio-cult, Glasgow, Scotland). The staining of the T-lymphocytes was performed utilizing the indirect immunofluorescence technique by first permitting the unconjugated anti-T antiserum to react with the lymphocyte suspension for 20 min at +4°C. Then the cells were washed twice in Medium 199, before FITC-conjugated goat anti-rabbit immunoglobulin antiserum (Behringwerke AG, Marburg-Lahn, W. Germany) was added and permitted to react for 20 min at +4°C. The cells were then washed as described for the direct immunofluorescence procedure (3, 12).

A Leitz Orthoplan Microscope with an Osram HBO-200 high pressure Mercury Vapor-burner light source and a Leitz vertical illuminator was used as previously described (10). Lymphocytes were examined with oil immersion objective (×54) and a minimum of 200 cells per preparation were counted. Granulocytes and monocytes were excluded, as judged by the size, intracellular staining, and the shape of the nucleus.

**RESULTS**

**Determination of B- and T-lymphocytes**

Immunofluorescence staining was performed by permitting FITC-conjugated anti-Ig class and anti-F(ab')2 antiserum to react with cells isolated by the Isopaque-Ficoll gradient centrifugation method from 11 patients with dermatitis herpetiformis and 10 healthy controls. The percentages of lymphocytes positive with anti-IgA antiserum had a mean of 5.2 in the patient group and a mean of 2.3 in the control group. The difference between the two groups is significant (p<0.002) (Fig. 1). Lymphocytes positive with anti-IgD antiserum varied from a mean percentage of 4.5 in the control group to a
Dermatitis herpetiformis

Table I. Comparison of the percentages of lymphocytes positive with various anti-class and anti-F(ab')2 antisera in 11 patients with dermatitis herpetiformis and 10 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Dermatitis herpetiformis</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Ly-a-F(ab')2</td>
<td>13.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Ly-IgG</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Ly-IgM</td>
<td>6.9</td>
<td>1.8</td>
</tr>
<tr>
<td>Ly-IgE</td>
<td>&lt;1*</td>
<td></td>
</tr>
</tbody>
</table>

* Median.

mean percentage of 7.3 in the patient group (p<0.02) (Fig. 2). No statistical differences between the two groups concerning the mean percentages of lymphocytes positive with anti-F(ab')2 (Ly-a-F(ab')2), anti-IgG (Ly-IgG), anti-IgM (Ly-IgM) or anti-IgE (Ly-IgE) antisera were found (Table I).

Lymphocytes that formed rosettes with sheep erythrocytes showed no statistically significant difference between the two groups (Fig. 3). Table II shows a comparison of the percentages of lymphocytes staining with the anti-T lymphocyte antiserum and lymphocytes forming rosettes with sheep erythrocytes in 11 patients. The anti-T antiserum stained a mean of 63% of the lymphocytes while a mean of 53% of the lymphocytes formed rosettes with sheep erythrocytes. This difference is significant (p<0.01).

Incubation effects on the membrane-bound immunoglobulins

To investigate whether the immunoglobulin molecules on the lymphocyte membranes were passively adsorbed or actively produced by the cells, some incubation experiments were performed. Lymphocytes isolated from 5 patients with dermatitis herpetiformis were stained before and after incubation at 37°C for 40 min in McCoy's 5A medium with 20% foetal calf serum (Grand Island Biological Company, Grand Island, New York) with FITC-conjugated anti-F(ab')2 and anti-IgA antisera.

The percentage of lymphocytes positive with the anti-F(ab')2 antiserum decreased from a mean of 11.0 before the incubation to a mean of 8.52 after the incubation, while the mean percentage of cells positive with the anti-IgA antiserum was essentially the same before as after the incubation, viz. 5.88 and 5.52 respectively (Table IV).

To further investigate a possible passive adsorption of IgA-molecules on to the lymphocyte membranes, suspensions of lymphocytes from 4 patients with dermatitis herpetiformis were incubated in McCoy's medium containing IgA myeloma protein in a concentration of 10 mg/ml, which is approximately 4-5 times the normal IgA serum concentration. Staining before and after the incubation with FITC-conjugated anti-F(ab')2 and anti-IgA antisera gave essentially the same percentage of cells positive with anti-IgA, while a small decrease in cells positive with the anti-F(ab')2 antiserum was observed after the incubation (Table V).

Double staining experiments

Lymphocyte suspensions from 8 patients were stained with a mixture of FITC-conjugated anti-IgD and rhodamine-conjugated anti-IgA, and with amix-

Table II. Comparison of the percentages of lymphocytes staining with the anti-T lymphocyte antiserum and lymphocytes forming rosettes with sheep erythrocytes in 11 patients with dermatitis herpetiformis

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Anti-T</th>
<th>E-rosettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. J.</td>
<td>63</td>
<td>49</td>
</tr>
<tr>
<td>A. K. M.</td>
<td>53</td>
<td>38</td>
</tr>
<tr>
<td>E. M.</td>
<td>71</td>
<td>60</td>
</tr>
<tr>
<td>L. J.</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>K. G.</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>K. S.</td>
<td>77</td>
<td>64</td>
</tr>
<tr>
<td>M. P. Ø.</td>
<td>56</td>
<td>43</td>
</tr>
<tr>
<td>M. Ø.</td>
<td>68</td>
<td>63</td>
</tr>
<tr>
<td>R. A.</td>
<td>65</td>
<td>59</td>
</tr>
<tr>
<td>R. M.</td>
<td>67</td>
<td>52</td>
</tr>
<tr>
<td>T. B.</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td>Mean</td>
<td>63.00%</td>
<td>53.27%</td>
</tr>
</tbody>
</table>

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Table III. Serum concentrations (in g/l) of IgG, IgA, IgM and the complement factors C3 and C4 and of IgE in U/ml in 11 patients with dermatitis herpetiformis and 11 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Dermatitis herpetiformis</th>
<th>Healthy controls</th>
<th>Test for difference dermatitis herpetiformis versus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>IgG</td>
<td>13.5</td>
<td>3.3</td>
<td>12.2</td>
</tr>
<tr>
<td>IgA</td>
<td>2.4</td>
<td>1.0</td>
<td>1.8</td>
</tr>
<tr>
<td>IgM</td>
<td>1.2</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>IgE</td>
<td>50*</td>
<td>-</td>
<td>80*</td>
</tr>
<tr>
<td>C3</td>
<td>0.71</td>
<td>0.19</td>
<td>0.70</td>
</tr>
<tr>
<td>C4</td>
<td>0.266</td>
<td>0.086</td>
<td>0.210*</td>
</tr>
</tbody>
</table>

* Median.

Lymphocytes with receptors for the Fc-part of human IgG (EA-RFC)

Cells forming rosettes with IgG (anti-C + D) coated human OR, R₂ erythrocytes were significantly increased in the patient group, with a mean percentage of 29.2 as compared with the control group, with a mean percentage of 19.8 (p<0.02) (Fig. 4).

Concentrations of serum immunoglobulins and complement factors

The concentrations of the immunoglobulin classes IgG, IgA, IgD, IgM and the complement factors C3 and C4 were measured by means of single radial immunodiffusion, while serum IgE was measured by radioimmunoassay. We observed a small but not significant increase in the mean serum concentrations of IgA, and a small but not significant decrease in the mean serum concentration of IgM in the patient group as compared with the control group (Table III). The mean serum concentrations of IgG and IgE, and the mean serum concentrations of the complement factors C3 and C4 showed no statistically significant differences between the patient group and the control group (Table III). IgD was measured in 8 patients, and all had normal serum concentrations.

DISCUSSION

In the present study an augmentation of IgA-positive lymphocytes was demonstrated in a group of patients with dermatitis herpetiformis as compared with a group of healthy controls. The observed augmentation of IgA-positive lymphocytes may well reflect the increased number of IgA-positive plasma cells reported (2), since...
Table V. Lymphocytes from 4 patients with dermatitis herpetiformis

Percentages of the lymphocytes staining with FITC-anti-F(ab')<sub>2</sub> and FITC-anti-lgA antisera before and after incubation in McCoy’s 5A Medium containing 20% foetal calf serum and a concentration of 10 mg/ml of lgA myeloma protein; for 40 min at 37°C

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before incubation</th>
<th>After incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-F(ab')&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Anti-lgA</td>
</tr>
<tr>
<td>I. J.</td>
<td>9.4</td>
<td>6.0</td>
</tr>
<tr>
<td>K. G.</td>
<td>12.5</td>
<td>5.3</td>
</tr>
<tr>
<td>R. L.</td>
<td>19.5</td>
<td>7.4</td>
</tr>
<tr>
<td>P. S.</td>
<td>12.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Mean</td>
<td>13.48</td>
<td>5.23</td>
</tr>
</tbody>
</table>

Lymphocytes are generally assumed to be the precursors of mature lg-producing cells. The increased number of lgA-positive lymphocytes found is also in agreement with other reports of an increased activity of the lgA-immunoglobulin system in dermatitis herpetiformis (8, 20, 21, 22).

The results of the incubation experiments performed in this study indicated that no lgA molecules were passively adsorbed to the lymphocyte membranes. This suggests that the lymphocytes positive with the FITC-conjugated anti-lgA antiserum represented cells that actively synthesized lgA molecules. Similar observations have also been reported by others (17). There is also a possibility that the membrane-bound lg may possess anti-immunoglobulin activity. This is most likely to occur when the conjugate source is goat, sheep or guinea pig, because heterophil antibodies to gammaglobulin from these species are often produced in man (4). We tried to avoid this problem by utilizing conjugated rabbit antibodies, towards which heterophil antibodies are seldom directed in man (3).

Furthermore, the present study revealed an increase in the mean percentage of lgD-positive lymphocytes in patients with dermatitis herpetiformis. A similar observation has been made in patients with allergic contact dermatitis (6, 7), but the significance of an increased number of lgD-positive lymphocytes in these diseases is not yet fully understood. It is accepted that most lymphocytes with surface lgD also have surface lgM. A recent study, however, presents evidence for a shift from lgD to lgA synthesis in the maturation of B-lymphocytes, suggesting that at least some of the developing cells destined to synthesize lgA for secretion pass through a stage in which immunoglobulins D and A are present together on the cell membrane (14).

An increase in both lgA- and lgD-positive lymphocytes as found in this study could be explained in this way. We therefore did some double-staining experiments. Lymphocyte suspensions from 8 patients were stained with a mixture of FITC-conjugated anti-lgD and rhodamine-conjugated anti-lgA and with a mixture of FITC-conjugated anti-lgM and rhodamine-conjugated anti-lgA. We were not able to detect lymphocytes with lgA and lgD together, or lgM and lgA together on the same lymphocyte membrane.

Our investigation also demonstrated an increase in the mean percentage of EA-RFC in the patient group. In vitro it has been shown that a part of these cells mediate cellular antibody-dependent cytotoxicity (24). The EA-RFC may therefore participate in the pathogenesis of dermatitis herpetiformis. This problem, however, needs further elucidation and studies are under way along these lines.

The mean percentage of lymphocytes staining with the anti-T antiserum was found to be higher than the mean percentage of lymphocytes forming rosettes with sheep erythrocytes.

E-rosette formation is dependent on experimental conditions. One important factor is the force with which the pellet is resuspended after the incubation at +4°C. Formed E-rosettes are easily disrupted. The expression of the sheep red blood cell receptor may reflect a more advanced stage of maturation of T-lymphocytes, while the anti-T antiserum may react with the total T-lymphocyte population (1). This could explain our findings, which are in agreement with other reports (1).

Two of the patients in this study had serum lgA levels above normal values. Similar findings have been reported by others (21), but the mean serum lgA concentration in the patient group, as compared with the control group, demonstrated a small increase that was not statistically significant.

A characteristic finding in dermatitis herpetiformis is the deposits of lgA at the dermo-epidermal junction. The antibody specificity of
these IgA molecules is unknown but may partly be
directed against antigenic determinants on gluten
molecules, since sensitivity to gluten is linked to
dermatitis herpetiformis. Cross reactivity between
and a recent report suggests that immune com­
glutens and is carried to the skin and it is
proposed that complexed gluten activates C3
and initiates an inflammatory reaction (19), thus
explaining the skin symptoms in dermatitis her­
ketiformis.

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