Lack of gamma A-immunoglobulin in serum of patients with steatorrhoea

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EDITORIAL SYNOPSIS The possibility is put forward that the deficiency of gamma A-immunoglobulin in the patients described might be a primary defect and not secondary to impaired protein synthesis due to reduced intestinal absorption.

The human intestinal mucosa is known to contain a large population of plasma cells. In the course of an immunohistochemical study on intestinal biopsies from normal subjects, we have recently shown that the majority of these mucosal plasma cells contain the \( \gamma \)A-type of immunoglobulin (Crabbé, Carbonara, and Heremans, 1965). It seemed to us of interest to extend this kind of analysis to patients suffering from various intestinal diseases, especially idiopathic steatorrhoea, where the intestinal mucosa is heavily infiltrated with plasma cells. The results obtained in such patients prompted us to study in more detail the immunoglobulins present in their serum.

The present report deals with three patients who have in common the presence of steatorrhoea and the absence or severe decrease of \( \gamma \)A-immunoglobulin in the serum. Two of these patients have a normal level of serum '\( \gamma \) globulin', as judged by paper electrophoresis, while the third is hypogamma-globulinaemic.

CASE REPORTS

CASE 1 A 59-year-old unmarried woman was admitted to the hospital on 5 February 1965 for a study of intestinal absorption function. The family history was not remarkable and the patient denied any symptoms of coeliac disease during childhood. She had been very well, working as an employee, until 1961, when she noted the progressive onset of weight loss, anorexia, fatigue, and swelling of the ankles. She began to complain of bone pain which was at first restricted to the left groin, but later spread to other bones. She was treated, without success, for 'neuritis' and 'rheumatism'. In 1963, an X-ray examination disclosed a diffuse decrease in density of the bones and a non-consolidated fracture of the left femoral neck. Hypocalcaemia and increased activity of serum alkaline phosphatase were present. Despite the absence of diarrhoea, malabsorption syndrome was suspected and the presence of steatorrhoea was confirmed by the microscopic examination of a stool specimen. The patient was given injections of calcium gluconate and supplements of vitamin preparations with some benefit, but the administration of large doses of pancreatic extracts failed to correct the steatorrhoea.

On admission the patient appeared as a pale, active, middle-aged woman in no apparent distress. Her weight was 51 kg and her height was 150 cm. The blood pressure was 115/7 cm. of Hg and her pulse was 76 per minute and regular. A soft, apical systolic murmur was present. The abdomen was slightly protuberant; bowel sounds were not hyperactive. Mild bilateral pitting ankle oedema was present. Digital pressure on several bones and vertebrae was painful. The neurological examination was within normal limits.

The relevant laboratory data are presented in Tables I and II. The absorption of D-xylose was evaluated from the cumulative amount excreted at five hours after an oral dose of 25 g. None of the three patients described here showed any sign of renal insufficiency. Quantitative determination of faecal fat was performed by the method of van de Kamer, ten Bokkel Huinink, and Weijers (1949) on a four-day stool collection, the patient receiving a diet containing 80 g of fat.

The radiological examination of the small bowel showed very slight diffuse dilatation of the loops and some flocculation of the contrast medium. No localized lesion was recognized. These changes were interpreted as being minimal and non-specific. A jejunal biopsy was performed by means of the Crosby-Kugler capsule. The specimen was obtained a few centimetres beyond the ligament of Treitz. Under the dissecting microscope the mucosa appeared to be completely flat. The histological examination showed so-called 'subtotal villous atrophy' with increased infiltration with lymphocytes and plasma cells in the lamina propria. Adequately stained sections revealed the absence of P.A.S.-positive material in the chorion. The changes were considered compatible with the diagnosis of idiopathic steatorrhoea.

The patient was placed on a strict gluten-free diet and

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was given supplements of vitamins, iron, and folic acid. After three months of this regimen, a slight subjective improvement was noted and the abdominal distension had definitely decreased. Total serum proteins had increased to 7.29 g./100 ml., R.B.C.s to 4,290,000 per c.mm., and haemoglobin to 12.3 g./100 ml.

CASE 2 A 57-year-old widow was admitted on 8 March 1965, in order better to define the origin of her steatorrhoea. No history of coeliac disease in childhood could be elicited and the family study was not contributory. The patient admitted to having frequent upper respiratory infections for many years. Any skin wounds were easily infected. These problems, however, were never severe enough to be incapacitating. In 1948 she noted the onset of intermittent, crampy abdominal pain which was followed by the passage of frequent, liquid, pale stools. In 1951, a diagnosis of steatorrhoea and anaemia was proposed. Except for an episode of acute intestinal infection of unknown aetiology in 1962, she has continued passing two to three loose, offensive stools daily. Anaemia, general weakness, and back pain became progressively more severe.

The patient appeared as a middle-aged, slender, pale woman in no acute distress. Her weight was 46 kg. and her height was 160 cm. Clubbing of the finger and toenails, abdominal distension, and a painless, regular liver edge palpable 2 cm. below the right costal margin were the only abnormal findings on physical examination. The blood pressure was 13:5/9 cm. of Hg.

The results of the laboratory examinations are given in Tables I and II. The 131I-PVP test indicated 1.6% faecal excretion in four days, a result which is slightly abnormal and of the order of magnitude obtained in intestinal malabsorption of any aetiology. Bacteriological examination of a specimen of stool failed to show any pathological microorganism. The jejunal biopsy displayed a completely flat mucosa under the dissecting microscope. Subtotal villous atrophy was present. A single lymphoid follicle was found in the chorion of the histological preparation. The cellular population of the lamina propria of the biopsy specimen consisted mostly of small lymphocytes, histiocytes, and eosinophils. Very few typical plasma cells could be recognized. A biopsy of the rectal mucosa showed the presence of many plasma cells under the surface epithelium and between the glandular crypts. A radiological examination of the small bowel showed diffuse enlargement of the intestinal loops, with some flocculation of the barium paste. The picture was that of a deficiency pattern. A radiograph of the chest failed to reveal the presence of any bronchial dilatation.

The patient was placed on a strict gluten-free diet supplemented by vitamins, and received a course of oral terramycin therapy.

CASE 3 This patient is a 61-year-old man who for many years has been suffering from diarrhoea, marked general weakness, and intermittent spontaneous tetany. He had been critically ill in 1963 when an episode of bronchopneumonia complicated the underlying disease. A definite improvement in his condition was obtained by treatment consisting of antibiotics and steroids given orally. He recently suffered a relapse, while still taking these drugs.

Physical examination was almost within normal limits, except for the presence of clubbing of the finger nails, a positive Trouseau’s sign, and a low systolic blood pressure at 10 cm. of Hg.

The few laboratory data available are listed in Tables I and II. The presence of steatorrhoea was evident as judged by the macroscopic appearance of the stools and has been confirmed by microscopic examination. Radiographs showed a pronounced and diffuse increase in the radiolucency of the bones. The loops of the small intestine were much dilated and a prominent deficiency pattern was evident on the films. A proximal jejunal biopsy showed a flat mucosa which, on microscopic examination, assumed the appearance of ‘partial villous atrophy’ with heavy infiltrates of plasma cells and tissue eosinophils.

**METHODS**

**ANALYSIS OF THE SERUM PROTEINS** Electrophoresis of the serum proteins was carried out in agar-gel according to the technique of Wieme (1959). Immunoelectrophoresis was performed according to Scheidegger (1955), using a multivalent horse antiserum directed against total human serum (Pasteur Institute, Paris), as well as specific rabbit antisera directed against each of the three human immunoglobulins. For quantitative determination of the
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immunoglobulins present in the sera, the method of single radial immuno-diffusion in agar-gel, recently described by Mancini, Vaerman, Carbonara, and Heremans (1964), was used.

IMMUNOHISTOCHEMICAL TECHNIQUES The methods used for immunohistochemical studies of the intestinal mucosal specimens have been extensively described in a previous article (Crabbé et al., 1965). The specific antisera directed against each of the three types of immunoglobulins were labelled with fluorescein isothiocyanate. The number of cells containing each type of immunoglobulin was evaluated from planimetric measurements on enlarged photographs of microscopic sections, by means of the mathematical formula presented in our previous publication (Crabbé et al., 1965).

RESULTS

IMMUNOELECTROPHORESIS OF THE SERUM PROTEINS The common finding in the three sera was the absence of the precipitin line corresponding to yA-immunoglobulin (Fig. 1). The line of the yG-immunoglobulin was well developed in the sera of cases 1 and 3, whereas it was very weak in case 2. A weak line corresponding to yM-immunoglobulin could be recognized in cases 1 and 3, but was apparently absent in case 2.

QUANTITATIVE DETERMINATION OF THE THREE IMMUNOGLOBULINS The concentrations of the three immunoglobulins in the sera of our patients are given in Table III. The normal values indicated for comparison are unpublished data obtained by Mancini. No yA-immunoglobulin was detected in sera 1 and 3, and only trace amounts (10 mg./100 ml.) in serum 2, whereas yM-immunoglobulin was very low in serum 2 (7 mg./100 ml.) and normal in sera 1 and 3.

TABLE III

| Immunoglobulin Values (mg./100 ml.) |
|---|---|---|---|
| yA | yM | yG |
| **Normals** | | | |
| Males Mean | 230-8 | 130-4 | 1,203-9 |
| S.D. | 78.0 | 63-1 | 160-7 |
| Females Mean | 277-3 | 168-5 | 1,228-3 |
| S.D. | 111-5 | 58-6 | 287-9 |
| **Cases** | | | |
| 1 | 0 | 120 | 1,260 |
| 2 | 10 | 7 | 260 |
| 3 | 0 | 180 | 2,192 |

The mean values found in normal subjects are given for comparison.

The serum of each of the three patients was used to absorb a multivalent horse antiserum, in a ratio of 3:7 (vol./vol.). In each case, this treatment failed to remove the anti-yA activity of the antiserum.

IMMUNOFLOUORESCENT STUDIES OF THE INTESTINAL MUCOSA The results obtained for the patients 2 and 3 with each antiserum are shown in Figures 5 to 11. Figures 2 to 4 represent, for comparison, the aspect of a normal specimen treated with the same antisera. In Table IV are given the values for the population.

TABLE IV

| POPULATION DENSITIES (NUMBER OF CELLS PER C.MM.) OF THE THREE TYPES OF IMMUNOGLOBULIN-CONTAINING PLASMA CELLS PRESENT IN THE INTESTINAL MUCOSA |
|---|---|---|---|
| **Normal** | | | |
| Mean | 352,290 | 51,490 | 15,450 |
| S.D. | 122,348 | 22,271 | 14,680 |
| **Case 1** | | | |
| Jejunum | 57,594 | 431,628 | 17,249 |
| Rectum | 3,423 | 2,057 | 17,611 |
| **Case 2** | | | |
| Jejunum | 3,200 | 430,025 | 28,723 |
| Rectum | 26,094 | 581,535 | 12,264 |

1 Normal values are average obtained on 60 fields from 10 normal subjects.
2 Each figure represents the mean value from six high-power field determinations.
FIG. 2. Section of a normal human jejunal mucosal biopsy after incubation with specific anti-γA-immunoglobulin fluorescent antiserum. Note the large number of fluorescent cells under the epithelium of the villi and between the crypts of Lieberkuhn.

FIG. 3. Same biopsy as in Fig. 2; section incubated with specific anti-γM-immunoglobulin antiserum. Fluorescent cells are relatively few.

FIG. 4. Same biopsy as in Figs. 2 and 3, after incubation with anti-γG-immunoglobulin antiserum. Fluorescent cells are very few. The narrow fluorescent lining extending along the basal part of the epithelium corresponds to the capillary network.
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FIG. 5. Biopsy specimen obtained from case 3. This section has been incubated with specific anti-\(\gamma\)A antiserum and shows a strikingly small number of specifically fluorescent cells in the chorion. Many small brilliantly fluorescent spots on the photograph correspond to tissue eosinophils.

FIG. 6. Biopsy specimen of the same patient, after incubation with anti-\(\gamma\)M-antiserum. Note the large number of specifically fluorescent cells in the densely cellular chorion under the surface epithelium.

FIG. 7. Same biopsy as in Figures 5 and 6. This section has been incubated with anti-\(\gamma\)G-immunoglobulin antiserum. The photograph shows a diffuse fluorescence in the interstitial spaces, a feature which complicates the definition of fluorescent cells at this magnification. The capillary network under the epithelium is well outlined (low magnification).
FIG. 8. Jejunal biopsy obtained from case 2. This section has been incubated with anti-γA-antiserum. The photograph shows the chorion (located under the surface epithelium) to be devoid of any specifically fluorescent cells. The two brilliantly fluorescent spots correspond to tissue eosinophils (high magnification).

FIG. 9. Section of intestinal mucosa incubated with anti-γM-immunoglobulin antiserum (case 2). One very weakly fluorescent cell is located near the centre of the photograph. The other fluorescent spots are tissue eosinophils (high magnification).

FIG. 10. Same biopsy as in Figs. 8 and 9, incubated with anti-γG-antiserum. Two specifically fluorescent cells may be seen in the chorion under the epithelium. Note the faintly fluorescent capillary network (high magnification).
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The patients often have infections before the malabsorption syndrome becomes apparent, this was not the case in our patient who was incapacitated much more by the intestinal condition than by any bronchopulmonary problem. The same finding has also been reported in other patients (Cohen, Paley, and Janowitz, 1961).

The aetiology of the malabsorption syndrome in hypogammaglobulinaemic patients is not clear. Pathogenic microorganisms were not found in the faeces in our case nor in other cases reported in the literature (Firkin and Blackburn, 1958). Yet, it has been assumed that a decreased resistance to infection with possible invasion of the small intestine by microorganisms could play a role in the pathogenesis of the steatorrhoea (Kabler, 1960). As far as we know, no direct evidence for such a concept has been brought forward. These patients are often resistant to a gluten-free diet and it is not unusual for antibiotics, associated or not with injections of gamma globulins, to fail in correcting the steatorrhoea (Allen and Hadden, 1964; Kabler, 1960).

The small intestinal mucosa in such cases has shown a variable picture. Shortening, clubbing, or even fusion of the villi is not uncommonly found (Forssman and Herner, 1964; Cohen et al., 1961) and, in some patients, the lamina propria has been reported to be heavily infiltrated with lymphoid cells (Case record of the Massachusetts General Hospital, 1963). The presence in the mucosa of an active lymphoid follicle, as seen in our case, was also reported by Allen and Hadden (1964) in one of their patients. The most striking change in the mucosa of our patient was the almost complete absence of plasma cells in the lamina propria. Most of the interstitial cells assumed the appearance of small lymphocytes and histiocytes. Many tissue eosinophils were also present. Obviously, these changes did not affect the entire intestinal tract, since many plasma cells could be recognized under the epithelium of the rectal mucosa.

Using immunohistochemical methods, we have previously shown that most of the plasma cells present in the normal intestinal mucosa contain γA-immunoglobulin (Crabbé et al., 1966). The same observation has been made by Rubin, Fauci, Sleisenger, and Jeffries (1965). The mean population densities for cells containing γA-, γM-, and γG-immunoglobulins have been found to be respectively 352,290 per c.mm., 51,490 per c.mm., and 15,450 per c.mm. (Crabbé and Heremans, 1966). In contrast, it is interesting to note that the specimen of jejunal mucosa obtained from case 2 was very poor in cells containing immunoglobulins. It may be inferred from the data shown in Table IV that the very low plasma cell count was essentially due to the scarcity of γA- and γM-immunoglobulin-containing cells.

FIG. 11. Rectal mucosal biopsy obtained from case 2. After incubation with anti-γA-antiserum, the section shows many fluorescent cells located between the glandular crypts.

Densities of the cells containing each type of immunoglobulin, in each of the three patients, as well as in normal controls.

DISCUSSION

The three patients described here are suffering from a malabsorption syndrome which seems to be secondary to a diffuse involvement of the small intestine, as evidenced by the radiological pattern, low D-xylene absorption, and histological appearance of the jejunal mucosa.

Case 2 is an example of the association of hypogammaglobulinaemia and steatorrhoea. The simultaneous occurrence of these two conditions in the same patient has been described in the literature. In acquired hypogammaglobulinaemia, Gitlin, Gross, and Janeway (1959) estimate the incidence of diarrhoea or a 'sprue-like' syndrome to be approximately 20%. The presence of steatorrhoea in the congenital type of hypogammaglobulinaemia has also been described (Allen and Hadden, 1964). While the patients often have a long history of respiratory
Again, this was localized in parts of, or in the whole length of, the small intestine, since in the rectal mucosa the respective proportions of $\gamma A$-, $\gamma M$-, and $\gamma G$-immunoglobulin-containing plasma cells were normal (Table IV).

The deficiency in the serum of case 2 of $\gamma A$- and $\gamma M$-immunoglobulins is not surprising since these proteins are often lacking in the serum of cases of hypogammaglobulinaemia (West, Hong, and Holland, 1962). It was more surprising to find a complete and isolated absence of $\gamma A$-immunoglobulin in the sera of cases 1 and 3. On clinical, biological, and histological grounds, these two patients would be considered as having idiopathic steatorrhoea. That most patients suffering from this disease are specifically sensitive to the proteins of gluten has often been reported. As yet we have no evidence that our patients are indeed responding to the elimination of these proteins from their diet, and no history of coeliac disease during childhood could be obtained from any of them. Yet the return to normal of serum proteins as well as the progressive correction of the anaemia as seen in case 1, three months after the institution of a gluten-free diet, suggest that this patient might be sensitive to the proteins in wheat flour.

The histological appearance of the jejunal mucosa in cases 1 and 3 was very similar and consisted in an important flattening and widening of the villi, associated with a heavy infiltration, mostly with plasma cells, in the lamina propria. The similarity between these two cases extended to the immuno-histochemical pattern of the lamina propria, where the densities for $\gamma A$- cells were inferior to those obtained for $\gamma M$- cells, and only slightly higher than those found for $\gamma G$-immunoglobulin-containing cells. In our experience, this is the only situation where this reversal of the $\gamma A$: $\gamma M$ cell ratio has been encountered in the human jejunal mucosa. The present results are different from those obtained by Rubin et al. (1965) in patients with adult coeliac disease. In their cases the $\gamma A$-immunoglobulin-containing cells were stated to predominate, as they do in normal subjects.

Gilbert and Hong (1964) have described a patient with a malabsorption syndrome whose serum contained almost no $\gamma A$- immunoglobulin, whereas the $\gamma M$- and $\gamma G$-immunoglobulins were moderately reduced. In children, West et al. (1962) found a malabsorption syndrome in two patients among 13 having low serum levels of $\gamma A$-immunoglobulin with near-normal values for the other two immunoglobulins. On the other hand, a congenital disorder characterized by ataxia, telangiectasia, and the frequent absence of $\gamma A$-immunoglobulin has been described (Peterson, Kelly, and Good, 1964; Fireman, Boesman, and Gitlin, 1964), but, as far as we know, no intestinal absorption studies have been reported on such patients. The isolated deficiency of $\gamma A$-immunoglobulin has also been described in female relatives of patients with congenital agammaglobulinaemia (Heremans, 1960; Burtin, Buffe, and Grabar, 1964), in a very small number of apparently healthy adults (Rockey, Hanson, Heremans, and Kunkel, 1964), and in one patient with systemic lupus erythematosus (Bachmann, Laurell, and Svenonius, 1965). In a study on an unselected population comprising 6,995 adults, Bachmann (1965) found the incidence of a $\gamma A$-globulinaemia to be of 1:700. We do not know of any report of the association in the adult of a malabsorption state with a $\gamma A$-globulinaemia as the only detectable defect in the immune system. It should be noted, however, that the lack of this protein is entirely missed on paper electrophoresis and that such cases might be more frequent, were immuno-electrophoretic analyses of serum proteins carried out on every patient suffering from idiopathic steatorrhoea.

In 1961, Huizenga, Wollaeger, Green, and McKenzie noted that the hypogammaglobulinaemia seen in certain cases of non-tropical sprue could be secondary to impaired synthesis of proteins due to the poor intestinal absorption of certain nutrients. That the absence of $\gamma A$-immunoglobulin cannot be explained by such a mechanism in our patients is suggested by the fact that case 1 still completely lacked $\gamma A$-immunoglobulin at a time when her serum proteins had returned to a normal concentration after a period on a gluten-free diet. It is indeed difficult to imagine an isolated defect in the synthesis of a single protein that would be due to a state of malnutrition. For all we know, the deficiency of $\gamma A$-immunoglobulin in the patients described here may be a primary defect.

The possible relationship between the disease of our patients and idiopathic steatorrhoea, adult coeliac disease, or gluten-induced enteropathy remains to be elucidated. The results of this study suggest that it may be profitable to examine the immunoglobulins of every patient suffering from this poorly understood syndrome.

**SUMMARY**

Three adult patients suffering from chronic steatorrhoea were found to have a serum deficient in $\gamma A$-immunoglobulin. Two of them had normal levels of 'y-globulins', as seen on paper electrophoresis, the third one being hypogammaglobulinaemic. Immuno-
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h histochemical studies performed on the jejunal mucosal biopsy of these patients showed that the hypogammaglobulinaemic subject had almost no plasma cells containing γA- and γM-immunoglobulins in the lamina propria. The other two patients showed a reversal in the ratio of γA-cells: γM-cells. The number of cells containing γM-immunoglobulin was greatly increased, while the density of γA-containing cells was reduced to very low levels. It is suggested that every patient suffering from idiopathic steatorrhoea be studied for the presence of γA-immunoglobulin in the serum.

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ADDENDUM

Since our manuscript was submitted for publication, we have been able to re-evaluate the condition of case 1, six months after the institution of a gluten-free diet. The following results have been obtained. The weight is unchanged at 51 kg. The R.B.C.s are now at 4,080,000 per c.m.m. and haemoglobin is 12·4 g. per 100 ml.; the serum iron level has risen to 90 μg. per 100 ml. (no parenteral therapy has been given). The total serum proteins are at 7-41 g. per 100 ml.; the paper electrophoretic pattern is normal but no precipitation line corresponding to γA-immunoglobulin is seen on immunoelectrophoretic analysis. The urinary excretion of D-xylene is 4·32 g. after a 25 g. oral load. The daily faecal fat excretion (calculated from a four-day collection) is 2·3 g. Finally, the upper jejunal biopsy shows a coarsely convoluted aspect under the dissecting microscope; histologically, several villi are present, still very short and broad. Immunohistochemical examination shows that most of the plasma cells present in the jejunal biopsy and in a rectal mucosal specimen contain γM-immunoglobulin.

REFERENCES


