Immunological disturbances in the pathogenesis of malabsorption

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Before considering the pathogenesis of malabsorption, I will briefly review the known immune mechanisms of the normal gut. The *post hoc* effects that malabsorption can have upon immunity must also be mentioned, so that at the time a patient presents and is investigated these will not be confused with true *propter hoc* effects immunological disturbances can have on malabsorption.

The Normal Immune Defence of the Gut

Normal immune mechanisms involve humoral and cellular components and for the gut more is known of the former.

In 1922, Davies showed that antibodies against *Shigella dysenteriae* were produced locally in the gut before they could be detected in the serum. After Heremans discovered IgA-globulin in human serum, Gabl and Wachter (1961) identified IgA as the major immunoglobulin in human saliva. Hanson (1961) established that IgA in the secretions contained additional carbohydrate antigen, now known as secretory piece, and Tomasi and Zigelbaum (1962) showed that secretory IgA was an 11s molecule of larger size than 7s serum IgA. Crabbé, Carbonaro, and Heremans (1965) found the lamina propria of the gut contained large numbers of plasma cells, 80% of which were synthesizing IgA, 12% IgM, and 8% IgG. We now know there are also very small numbers of cells synthesizing IgD or IgE.

The IgA is released from these cells as a 7s monomer into a subepithelial pool of IgA from which some 7s IgA can travel back through the lymphatics and portal veins. Thus a raised serum level of IgA is a common nonspecific response to diseases of the small gut, especially those involving the wall, such as Crohn's and Whipple's diseases (Hobbs, 1969a). However it seems that normally much of the lamina propria pool of IgA is transported across the gut epithelium, becoming dimerised in the process, and to the dimer is added secretory piece which is synthesised by the epithelial cells. The molecules of 11s secretory IgA which gain the gut lumen, and especially its lining mucus, differ from 7s IgA in two important ways.

First, while still susceptible to acid and pepsin in the gastric juice, the secretory IgA added thereafter is largely resistant to normal intestinal pH and enzymes, so that it can persist through to the faeces. This accounts for polio virus coproantibody (Kono, Ikawa, Yaoi, Hamada, Ashihara, and Kawamaki, 1966), conclusively shown to be IgA by Berger, Ainbender, Hodes, Zepp, and Hevizi (1967). Secondly, secretory IgA can fix complement (Adinolfi, Glynn, Lindsay, and Milne, 1966) and can thereby cause holes to form in the lipid membranes of microorganisms. In the case of Gram-negative bacteria, which have an underlying polysaccharide backbone, lysozyme (also present in the secretions) can enter through such holes in the lipid membrane and complete the destruction of the bacteria. Thus the combination of 11s secretory IgA, complement, and lysozyme provides a powerful antibacterial mechanism.

Secretory IgA is also powerful against many viruses, and this form of immunity is regionally initiated and has a long memory. Thus Ogra and Karzon (1970) found that after instilling live polio vaccine into the distal colon (in patients with 'double barrel' colostomies) that the secretory IgA response was essentially limited to the distal colon. No antibody appeared in the nasopharynx and only a little in the proximal colon. This regional concept has important implications both for modes of immunization and in disease. Regional memory seems also much better than systemic in that children previously given oral Sabin vaccine were found to be still capable of eliminating further oral doses 10 years later, whereas those given parenteral Salk vaccine would often fail to do this within a year. Secretory IgA with its cofactors thus offers the main line of humoral defence of the gut, but other classes of antibody can be detected. Of these the second line seems to be IgM from two areas of evidence. Hazenberg (1968) has shown that IgM like IgA is cleared across the gut at a rate some 30 times greater than albumin, and therefore IgM must also enjoy some kind of active transport or local secretion. Local increase of IgM plasma cells is also the major compensation seen in subjects with congenital

absence of IgA. It is possible local IgE formation may protect against helminth infections, but I am unaware of any evidence that local IgG or IgD can have a protective role and know of six patients with isolated IgG deficiency who suffered no gut symptoms whatsoever.

With regard to the cellular contribution to gut immune defence much less is known. Since the work of Yoffe, evidence has been mounting that enormous numbers of lymphocytes enter the gut epithelium each day, probably to be lost through it. What they do is unknown. In patients with isolated defects of cellular immunity gut symptoms are not prominent, but they are the rule in combined defects of cellular and humoral immunity, and much more so than in patients with isolated defects of humoral immunity. The cellular components must therefore have some protective role in the gut.

The Effects of Malabsorption upon Immunity

Malabsorption states can through the loss of proteins and lymphocytes result in immune defects, and it also is possible that lack of absorption of vitamins, etc, could aggravate such a situation.

When a great deal of protein is lost (over 10 g/day) a hypercatabolic state is induced in the body, probably largely by the liver which in a frantic attempt to synthesize new proteins breaks down many proteins. While albumin synthesis may be increased by 100% (and rarely more) this is often inadequate, so that the serum albumin level falls and offers some indication of the severity of hypercatabolism. With albumin levels under 2 g/100 ml caused by a protein-losing enteropathy it is usual to find subnormal serum levels of IgG globulin (< 500 mg/100 ml). This is because the catabolism of IgG can be increased some nine-fold (Andersen, 1963), reducing the normal long T $\frac{1}{2}$ of 28 days down to some four days. While the aminoacids from such catabolism become available to the liver, etc, they do not become so readily available to the plasma cells in the bone marrow, where some 60% of IgG is synthesized. This is the main mechanism for the fall in IgG level. It is not due to losses of IgG into the gut, which are usually trivial in terms of the daily turnover. In jejunal juice (preserved *in situ* by trasyolol and EACA) from patients with protein-losing enteropathy the highest level of IgG I have found has been 38 mg/100 ml. Even at 8 l daily this would only come to some 3 g, whereas the daily IgG catabolism in such a patient could be 27 g. Increases in IgA and IgM catabolism were also shown by Strober, Wochner, Carbone, and Waldmann (1967), but in their paper these authors only plotted bars at 1 SD below the mean normal serum levels.

In actual fact because IgA and IgM already have short half-lives (five-six days), increased catabolism, while lowering the serum level, does not usually reduce it below 2 SD. Thus in hypercatabolic malabsorption states it is typical to have a serum pattern of subnormal albumin and IgG levels, but with IgA and IgM within the normal ranges albeit the lower half. Also because the quality of these immunoglobulins is usually good, and because IgG alone is subnormal, this secondary antibody-deficient state is more apparent than truly symptomatic, and infection is unusual. In one form of protein-losing enteropathy, intestinal lymphangiectasia, there can result in addition severe lymphopenia, probably from an excessive loss of lymphocytes into the gut. Such patients can suffer superadded infection due to their secondary immune deficiencies.

It could be that severe deficiencies of folate, B₁₂, and other substances could impair immune responses but since today we normally institute replacement therapy, these will not be considered further.

Finally, it is known that coeliac disease can induce a subnormal serum IgM (Hobbs and Hepner, 1966) due to overall impaired IgM synthesis (Brown, Cooper, and Hepner, 1969) and that, after many years, atrophy of spleen and lymph nodes can ensue (McCarthy, Fraser, Evans, and Read, 1966). A resultant loss of immunosurveillance could account for the increased incidence of regional carcinomata and lymphoma recorded by Harris, Cooke, Thompson, and Waterhouse (1967) and Austad, Cornes, Gough, McCarthy, and Read (1967). Impaired lymphocyte transformation is associated with several small gut diseases, and certainly is a result of the disease in some patients, for transformation becomes normal after treatment.

Immune Deficiency Predisposing to Malabsorption

Combined cellular and humoral deficiency as a rule results in intractable diarrhoea with malabsorption, and infants affected congenitally fail to thrive and usually die within six months of birth.

Of the mainly humoral deficiencies it is those with virtual absence (< 1 mg/100 ml) of IgA and/or IgM that may lead to gut symptoms. Thus up to 40% of children with Bruton's disease (severe deficiency of IgG, IgA, and IgM) suffer malabsorption. Gut symptoms are only present in some 20% of adults with acquired antibody deficiency syndromes, whether genetic or secondary to other diseases (eg, chronic lymphatic leukaemia) and this seems to be because it is unusual for adults to acquire complete absence of IgA, and even in some personal patients with serum levels of 2 to 20 mg/100 ml, secretory IgA persisted within our normal ranges (parotid saliva

3-21; jejunal juice 5-38 mg 11s IgA/100 ml). That the malabsorption can indeed result from severe deficiency of IgA and IgM (whether genetic or secondary) is shown by the success of treatment with whole plasma after treatment with γ -globulin (IgG mainly) had failed (Binder and Reynolds, 1967). Furthermore in dysgammaglobulinaemia type I (Giedion and Scheidegger, 1957), in which only isolated deficiency of both IgA and IgM is present, gut symptoms are usual (see Hobbs, 1969b).

Isolated absence of IgA occurs in about 1-500 of the population and of such subjects only some 10% had gut symptoms (Hobbs, 1968a). A major factor determining whether or not symptoms will ensue seems to be the quality of the compensation possible in the IgM. Thus our healthy subjects with IgA deficiency show jejunal lamina propria with 90% of the plasma cells producing IgM, and with high levels of IgM in the secretions. Some of our symptomatic patients lack this, or have poor quality IgM as judged by poor levels of isohaemagglutinins. Among patients with malabsorption, about 3% have absence of IgA, about 15 times the expected incidence. Their diseases include the coeliac syndrome, tropical sprue, typical Crohn's, nodular lymphoid hyperplasia, and even nonspecific malabsorption, responding to antibiotics only, so that IgA deficiency can act as a general predisposition to small gut disease.

Isolated IgM deficiency is most commonly secondary (Hobbs, 1968b) but primary IgM deficiency has in my experience been associated with malabsorption in three patients, two of whom had Whipple's disease.

NODULAR LYMPHOID HYPERPLASIA

Since this lesion in the small gut, usually accompanied with *Giardia lamblia* infestation, was described by Hermans, Huizenga, Hoffman, Brown, and Markowitz (1966) it has been associated with longstanding (1) IgA + IgM deficiency, (2) IgG + IgA + IgM deficiency, (3) isolated IgA deficiency, and (4) normal levels of IgA and IgM. In 3 and 4, IgM has been of poor quality with poor isohaemagglutinin levels. It would seem that if IgA cannot protect the gut and IgM is inadequate, then the cellular components proliferate in an attempt to compensate and can result in lymphoid nodular hyperplasia, and in half the subjects splenomegaly also occurs. Fresh frozen whole plasma can ameliorate symptoms (Gryboski, Self, Clemett, and Herskovic, 1968) although anaphylactic reactions from antibodies to IgA must be avoided. Lymphoid nodular hyperplasia in the large gut can be an extension of the above, but is much more often of little clinical significance (Franken, 1970).

ALLERGY TO COW'S MILK

Using rigid criteria, Freier and Kletter (1970) estimate true allergy to cow's milk to occur in about 0.5% of children. While onset usually occurs at 1 month or on weaning thereafter it may occur much later. Vomiting, diarrhoea, and secondary malabsorption often result and in some cases anaemia due to occult bleeding and protein-losing enteropathy can occur, as well as symptoms in the chest and skin. High levels of serum and faecal antibodies to cow's milk are suggestive and strongly positive intradermal tests to cow's milk point to true allergy, though the real proof of the diagnosis is improvement on milk withdrawal with relapse after rechallenge (beware of 'shock'). In this way milk can be proven to increase ^{131}I -albumin catabolism in some cases of protein-losing enteropathy, cured by milk withdrawal, and this seems analogous to pollen nephrosis. Allergy to other foods such as egg, bananas, and even orange juice can be similarly established.

COELIAC DISEASE

Summarizing current immunological knowledge of coeliac disease, Hobbs, Hepner, Douglas, Crabbé, and Johansson (1969) concluded that the only regular difference between coeliac and other gut diseases was an excess of IgM produced locally in the jejunum. Precipitating antibodies to gluten fraction III could be found in jejunal juice, and the work of Mietens (1967) suggests these belong to the IgM class. In coeliac subjects where gluten withdrawal has achieved restoration to normal of the jejunal mucosa, infusion of a pure fraction of α -gliadin can regenerate the typical lesions. Nothing is seen before four hours, but mucosal damage often occurs by 18 hours (Hekkens, Haex, and Willighagen, 1971). This really excludes immediate type hypersensitivity and is too soon for delayed type hypersensitivity. The timing suggests to me a local Arthus type reaction, which could be initiated by a local excess of IgM precipitating and complement-fixing antibodies to gluten. Coeliac subjects do produce these, and most probably locally in the jejunum. In contrast other subjects seem to produce antibodies in IgA and 7s IgA within the mucosa which cannot fix complement or cause an Arthus reaction. Together with Drs A. J. Beale, A. P. Douglas, and W. E. Parish (to be published) I have been looking at the antibody production of coeliac subjects. They produce normal responses with IgG antibodies to tetanus toxoid, but 13 of 30 IgA responses to oral polio viruses were subnormal and a further six were poor. This suggests the coeliac subject may have a poor IgA response to gluten and thus overuses the second gut line of IgM. Therein

may be their undoing, and there may even be a regional failure of IgA in the small gut only.

ALPHA-CHAIN DISEASE

Described by Seligmann, Danon, Hurez Mihaesco, and Preud'homme (1968), this disease represents a true regional disorder of the small gut IgA system, IgA in the parotid and rectum being normal. It occurs mainly in young Arabs, and although we have found it in two native born Pakistanis, this could be due to the incursions of Alexander the Great. In the teens and young adulthood, patients present with intractable malabsorption, which becomes steadily worse. A stove-pipe thickening of the whole small gut is found, and the whole lamina propria is stuffed with primitive plasma cells. These cells produce only the α -chain of IgA-globulin, and have a lower content than do normal IgA cells as judged by fluorescence. Later the tumour can disseminate and we have seen infiltration of the tonsils and the bone marrow. There is suppression of normal IgG and IgM serum levels, and the α -chain can be seen as a diffuse band in the α_2 - β region, with a characteristic precipitin line into the α_2 region on immunoelectrophoresis against anti- α . The α -chains belong to subclass I of IgA. There is also a late characteristic elevation of serum alkaline phosphatase, most of which is of the intestinal isoenzyme. Arabian lymphoma of the small gut had been known for many years, but its unique α -chain component was first recognized by Seligmann. Two of our patients soon died before being fully recognized but the third has done very well for one year on high intermittent dosage of melphelan, his malabsorption being corrected. Dr R. Ballieux (personal communication) has recently identified an α -chain disorder confined to the lung of a Dutch child, so the regional concept of secretory IgA is gaining strength.

OTHER SMALL GUT DISEASES

While much research in Crohn's disease has revealed disorders of cellular immunity, I am not yet convinced that these are *a priori* predisposing to the disease process. Similar defects are found as a result of coeliac disease and an increasing number of other diseases. Impaired lymphocyte function in Whipple's disease has also not yet been shown to precede the infection, and in our patients has improved with treatment. The causes of intestinal lymphangiectasia and other protein-losing enteropathies are unknown to me, but may have an immunological basis.

Summary

In the normal immune defence of the gut, the major

humoral role is played by secretory IgA (which shows regional localization and memory) with a secondary role for IgM and possibly for IgE. Cellular immunity undoubtedly also has a protective role, but how in the gut is not yet clear.

Malabsorption states can result in (1) IgG deficiency, (2) lymphopenia, and (3) IgM deficiency. Longstanding coeliac disease can lead to lymphoid atrophy.

Malabsorption results as a rule from combined immune deficiency or inadequate IgA and IgM (which can later result in lymphoid nodular hyperplasia). True allergy to cow's milk and other foods can also cause malabsorption and protein-losing enteropathy. One regional disorder of IgA is α -chain disease of the small gut, and another may be a local poor response of IgA to gluten, resulting in a local IgM-mediated reaction to cause coeliac disease.

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