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Annotation

Immune Mechanisms and the Gut

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The lymphoid tissue of the gastrointestinal tract is a central component of the body's immune system. It is the major site of exposure to foreign antigenic materials such as those present in micro-organisms or food, and the patterns of lymphocyte sensitisation which result have widespread implications for both health and disease. The capacity to develop protective immunity carries with it the potential for coincidental tissue damage to occur. The onset of such hypersensitivity reactions in some patients implies the presence of various predisposing factors. These include the mode of presentation of the antigen, the competence of the immune system encountered, and the genetic constitution of the individual concerned. The importance of these factors can only be appreciated in the context of the normal physiology of the immune system in the gut. The object of this paper is to discuss this and its clinical relevance to paediatric practice, particularly in relation to gastrointestinal infections and food allergy.

The Gut as an Immunological Organ

The immune response requires an afferent limb to present antigen to lymphocytes capable of recognition and proliferation, and an efferent limb to disseminate immuno-competent daughter cells able to produce specific antibody, or lymphokine at the site of antigen exposure. It is now clear that the gut is uniquely structured to promote antigen-lymphocyte interaction. A large proportion of the body's lymphocytes reside in the gastrointestinal tract. They are scattered throughout its length in the lamina propria and epithelium, with aggregations occurring in the Peyer's patches, appendix and tonsils. The cells present include two distinct populations of lymphocytes, one which has passed through the thymus during maturation and participates in cell-mediated reactions (T cells) and one which was first described in the bone marrow and is able to produce antibodies (B cells). There are no afferent lymphatics. Early concepts of antigen access to the mucosa assumed that molecules could pass between or through the epithelial cells at all points. It is now apparent that the Peyer's patches are covered by a specialised epithelium which preferentially allows antigen entry [31]. Beneath this epithelium lies the dome area which contains a large proportion of T lymphocytes. This overlies an area of cell traffic

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containing post-capillary venules, macrophages and B lymphocytes [32]. Sensitised cells pass in efferent lymphatics to the mesenteric lymph nodes and thence to the thoracic duct and the circulation. T and B cell proliferation occurs outside the patches themselves, characteristically taking place in the draining lymph nodes and spleen [34]. The efferent limb of the immune response is mediated by lymphocytes which return to populate the mucosa. Several complicated patterns of recirculation have been identified in animals [13]. Clearly, a system which allows widespread distribution of antigen-reactive cells throughout the mucosa in this way is both elegant and efficient.

Immune Responses Associated with the Gut

The immunologically mediated changes in the gut which have been recognised for many years include luminal antibody production, cellular infiltration of the mucosa and the development of immunoglobulin producing cells in the lamina propria. Other changes which reflect gut immune responses include the presence of circulating antibodies to food proteins, anaphylactic reactions, evidence of complement activation following oral challenge, and the development of mucosal enteropathies related to ingested antigen and infections. The correlation of these observations with the classical types I-IV immune reactions described by Gell and Coombs [5] has proved difficult.

Humoral Immunity

The ability of the gut to produce protective antibodies against bacillary dysentry regardless of serum antibody levels was shown by Besredka [3] and by Davies [9] in the early 1920s. It was some 40 years later that IgA was recognised as the predominant immunoglobulin in external secretions [42] and in the lamina propria [6]. It is synthesised as a dimer in the mucosa and becomes associated with secretory component produced in the epithelial cells. The capacity of the newborn to produce IgA is impaired [43] and the establishment of a normal population of immunoglobulin-bearing cells in the mucosa appears to be closely related to the intestinal contents [7]. IgA antibodies are active against bacteria, viruses and toxins. They also prevent bacterial adherence to the mucosa [45], and in rats have been shown to prevent albumin absorption following intra-gastric immunisation [17]. Such modulation of antigen access has profound implications.

Cells producing other immunoglobulin classes are also found in lower numbers in the mucosa and IgG, IgM and IgE can be demonstrated in intestinal secretions. IgM may be associated with secretory component, IgG and IgE are not, and are thought to be derived from plasma. In the context of the distribution of IgE producing lymphocytes in the body, it is noteable that they are particularly found in the mucosae [41].

Cellular Immunity

To date it has been difficult to study cellular immune reactions in the gut. The essential histological features of human small bowel enteropathies have been mimiced by allograft rejection studies in mice [25] and in graft-versus-host disease [33]. A common factor in both situations is a local cell-mediated immune reaction. Direct evidence of functional T lymphocytes in the small intestine has come from studies of lymphokine production. Ferguson et al. [12] showed that migration inhibition factor was released from jejunal biopsy specimens from patients with coeliac disease when they were incubated with alpha-gliadin.

An important model for the study of cell-mediated immunity in the gut is parasitic worm infection in rodents. Inbred rats subjected to neonatal thymectomy had significantly greater infection than did control animals [22, 30]. In this system it is unlikely that the mechanism concerned is confined to the classical cell-mediated reaction. The parasite N. brasiliensis is known to stimulate remarkable IgE production, a response which is particularly dependent on T-B cell interaction. The partial villous atrophy which occurs in this nematode infection does not develop in thymus-deprived rats [11].

Cellular infiltration of the columnar epithelium and the lamina propria is well documented in jejunal enteropathies [21, 28]. The function of intra-epithelial lymphocytes remains obscure, it is currently thought that they are effector T cells [10]. Their sentinel position at the mucosal surface suggests an important role. The infrastructure of cellular cooperation which underlies all immune reactions has yet to be identified in the gut. Sub-populations of suppressor and helper T lymphocytes will eventually be recognised and in vivo evidence of cell-mediated immunity and antibody dependent cellular cytotoxicity will doubtless emerge.

Tolerance

Specific immunological unresponsiveness is known as tolerance. The induction of tolerance to parenteral antigen by previous oral immunisation was originally shown by Chase in 1946 [4]. The mechanisms are still not understood, it has been suggested that IgA binding

of antigen molecules either reduces their uptake or renders them tolerogenic. It has been shown that feeding ovalbumin to mice reduces its subsequent absorption, while simultaneously inducing a state of systemic tolerance [40]. Introduction of minute amounts of hapten into the portal system similarly produces tolerance [2]. The mechanism of antigen handling may thus be crucial to the development or non-development of immunity.

Hypersensitivity Reactions

What then is the evidence that hypersensitivity reactions [5] occur in the gut in man, and what is their relevance to disease states?

Type I (immediate hypersensitivity) reactions involve IgE antibodies which on combination with antigen release histamine and other vaso-active substances. Their role in gastrointestinal disorders in man is uncertain. Although symptoms such as diarrhoea, vomiting, urticaria and asthma may follow the ingestion of specific proteins, there is rarely an obvious correlation with circulating IgE antibodies [8]. Further studies on IgE antibody activity in the mucosa are required. In animals with worm infestations Type I reactions appear to be more concerned with expulsion of the parasite than associated with specific anti-nematode IgE antibodies.

Type II (cytotoxic) hypersensitivity reactions involve complement fixing antibodies and antigens which form an integral part of, or become attached to, the surface of an individual's own cells. These reactions have usually been described as affecting red cells, white cells or platelets in the circulation. The demonstration of "autoantibodies" is not conclusive evidence that they are of primary or secondary significance. There is no confirmation that Type II mechanisms operate in gastrointestinal disease. However, locally secreted antibodies which only reacted with epithelial cell-membrane constituents would be very difficult to demonstrate unequivocally.

Type III (Arthus type) hypersensitivity reactions develop when antigen-antibody-complement complexes occur. Tissue damage then results from the generation of chemotactic factors and local changes in capillary permeability. The antibody classes capable of complement activation are IgM and IgG. IgA is unable to fix complement by the classical pathway although aggregated secretory IgA can activate complement by the alternate pathway, as evidenced by bacteriolysis [18]. Very low concentrations of complement are found in intestinal secretions and complement components are readily inactivated by proteolytic enzymes. Nevertheless, complement activation has been documented in the serum following cows' milk protein challenge in sensitive individuals [27] and following gluten challenge in coeliac disease [26]. It is most likely that these changes reflect a type III reaction in the lamina propria, antigen having gained access across a damaged epithelium.

Type IV (delayed hypersensitivity) reactions occur between antigen and antigen-sensitive lymphocytes, exemplified by the Mantoux test. Although it seems likely, as discussed above, that cellular immune reactions occur in the mucosa, in vivo evidence is inconclusive. Peripheral lymphocyte stimulation in the presence of a sub-fraction of gluten has been reported in patients with coeliac disease [16]. However the interpretation of this is as controversial as the significance of circulating antibodies to dietary antigens in individual patients.

Gastrointestinal Infections

The normal bacterial flora of the gut is determined by interactions between the host and the micro-organisms. Non-immune factors are important. They include lactoferrin, lysozymes, proteolytic enzymes, and the integrity of the glycocalyx. The contribution of immunological mechanisms can be examined in immunodeficiency disorders. The commonest of these is selective IgA deficiency. Interestingly, most of these patients are symptom-free and do not have an increased susceptibility to gastrointestinal infection, although Giardia lamblia infestation and enteropathies are well recognised to occur in some of them [37]. Giardiasis is more common in hypogammaglobulinaemia [36], however it is the children with cellular immune defects such as the Di George syndrome or severe combined immunodeficiency who suffer the greatest incidence of bacterial, fungal or viral infections [14, 19].

The pathogenicity of strains of *E. coli* is determined by their ability to adhere to the mucosa, and their capacity to produce enterotoxin. In animals adherence has been linked to the presence of genetically determined mucosal receptors [38]. The mucosa is capable of producing specific IgA antibodies against *E. coli* [24] but their protective role is not clear. It has been shown that in neonatal animals fed on colostrum, passively transferred immunoglobulins can be protective [23]. In man, gastroenteritis and necrotising enterocolitis are less common in breast-fed infants [35, 1]. The development of a vaccine is complicated by the changing serotypes of the organisms. Immunisation against the enterotoxin might be more practical. Cholera-toxin binding to microvilli can be blocked by antibody [44], but in vivo application is awaited.

The recognition of rotavirus, coronavirus and similar particles in faeces has done much to explain the high proportion of diarrhoeal diseases in man and animals in which no bacteriological cause had been found. Studies in piglets have shown that maternal colostrum can be protective [38] and that the preparation of specific vaccine is a practical proposition [29]. Aspects of the mucosal immunology of the poliovirus have been well studied in the development of the Sabin vaccine. The findings indicated that an oral live attenuated vaccine-provoked protective immunity is principally localised in secretory IgA. Despite this finding, other mechanisms are implicated by the work of Mebus et al. [29] who showed that immunity to calf gastroenteritis virus preceded the appearance of specific antibody activity.

The association between malnutrition and increased susceptibility to infection [15] is an important cause of mortality. Malnutrition may compromise mucosal immunity, while nutritional status is further impaired by the consequences of infection such as anorexia, dehydration, and malabsorption.

Food Allergy

It is clear from the above discussion that abnormalities in antigen presentation or lymphocyte responsiveness could predispose to the development of hypersensitivity reactions in the bowel mucosa. Currently, there is no diagnostic investigation which unequivocally establishes that this has occurred. The many screening tests suggested reflect changes remote from the gut itself. The interpretation of positive skin tests or circulating antibodies to food proteins is notoriously unreliable. The clinician must still rely on meticulous observation, and will find that many children's symptoms may not be reproducible, and that in some patients, non-immunological mechanisms may be involved—such as in lactose malabsorption. Nevertheless, food allergy does exist and is most commonly found in association with cow's milk or egg protein in those children who present with gastrointestinal symptoms. Some patients react immediately, in others the symptoms are delayed and it is evident, both clinically and experimentally, that several different types of reactions are occurring. Until we have a clearer understanding of the fundamental basis of these differences, the distinction between primary and secondary phenomena is blurred. Current views suggest that there is a group of infants who become sensitised to cow's milk protein during an episode of gastroenteritis [20]. Such hypersensitivity is relatively short-lived and even in this group the presence of a jejunal enteropathy is not always associated with continuing disturbances. It has been suggested that infants who are symptomatic on exposure to cow's milk after breast feeding may have been sensitised in the neonatal period at a time when their mucosal IgA production was immature [39].

It will be interesting to see if the current upsurge in breast feeding and in the use of hypoallergenic milks will have any discernible effect on gastrointestinal disorders in infancy or later life.

The balance required in the gastrointestinal tract between the selective absorption of nutrients and mucosal defence has thus resulted in the development of a complex immune system. The inaccessibility of the epithelium and its vascular drainage to both examination and experimentation has heightened its obscurity. Application of the modern techniques of endoscopy, tissue culture, cell separation and lymphocyte identification offer exciting prospects for the future. Only then will the relevance of aberrations of the gut immune system to disease become truly apparent.

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