Progress report

The secretory immune system of the intestine

The intestinal epithelium comprises a single sheet of cells which separates the ‘milieu interieur’ from a wide variety of potential antigens in the intestinal lumen. These include microorganisms, dietary antigens, drugs, and food contaminants and it is not surprising, therefore, that the intestinal tract is remarkably well endowed with immunocytes. These are present as collections of lymphoid cells, as for example in Peyer’s patches and the appendix, and as a diffuse population of lymphocytes and plasma cells in the lamina propria and in the epithelium of the intestinal tract.

There has been evidence for many years to suggest that the intestine is capable of mounting a local immune response independent of systemic immunity. In 1919 Besredka demonstrated that oral immunization of rabbits with killed Shiga bacillus provided solid protection against fatal dysenteric infection, irrespective of the serum agglutination titre. He therefore suggested that there was a local intestinal immune system which was protective. Further support for this concept was provided in 1922 by Davies, who showed that in patients suffering from bacillary dysentery antibodies appear in the stools several days before serum antibodies are detectable. Since then, evidence has accumulated to strengthen the concept that the intestinal tract, in common with other mucosal surfaces, is capable of mounting a local immune response which provides protection at its mucosal surface.

This review describes the immunocytes which are involved in the intestinal secretory immune system and the nature and properties of the immunoglobulins secreted by these cells. The functional role of this system is then discussed.

Cells Mediating the Humoral Response

One of the striking features of the normal intestinal mucosa is its rich endowment with plasma cells in the lamina propria. Immunofluorescent studies using specific fluorescein-labelled antisera reveal a marked predominance of IgA-containing cells which greatly outnumber those producing IgM and IgG, the ratios of IgA:IgM:IgG cells being approximately 20:3:1. IgE-producing plasma cells are also present in significant numbers but IgD cells while present, are scarce.

The heavy population of immunocytes found in the intestinal mucosa appears to be the result of exposure of the neonatal intestine to antigen stimulation. In germ-free animals the intestinal submucosa is practically devoid of plasma cells but if such animals are exposed to a normal environment, the intestinal plasma cells increase to levels similar to those found in conventional animals within three to four weeks.

Although little is known of the lifespan or fate of the intestinal plasma
cell population, some evidence is now available concerning the origin and function of these cells. In the first place, it is established that plasma cells are derived from the large lymphocytes. When large lymphocytes, collected from rat thoracic duct and labelled in vivo with tritiated thymidine, are transfused intravenously into siblings of an inbred strain, they migrate principally to the wall of the intestine, where they lodge in the lamina propria. In this situation they resemble immature plasma cells in appearance. The output of large lymphocytes from the thoracic duct is maintained by a continuous production of new cells from the intestinal lymphoid tissue. Their output is relatively unaffected by prolonged drainage from a thoracic duct fistula and over 90% of these cells in the thoracic duct are labelled in vivo when tritiated thymidine is given over a 24-hour period, indicating synthesis within that period. These observations led Gowans and Knight to suggest that before large lymphocytes are released from intestinal lymphoid tissue, they are sensitized by antigenic material originating in the gut. The work of Griscelli et al provides further evidence for this concept of 'preferential localization' of sensitized large lymphocytes. These workers have confirmed that large dividing lymphocytes from the thoracic duct 'home' to the intestinal mucosa and intestinal lymphoid tissue (Peyer's patches and mesenteric nodes). In addition, they reported that large lymphocytes obtained from mesenteric nodes and infused into syngeneic recipients also localize preferentially to gut-associated lymphoid tissue. The mechanism of 'preferential localization' of large lymphocytes is probably dependent on an antigen recognition system. It is postulated that the surface of large lymphocytes is coated with antibody and that these cells, sensitized by exposure to gut antigens, are trapped as they travel through the intestinal mucosa and its lymphoid tissue where they become the plasma cells involved in the secretory immune response.

The role which other lymphocytes play in the intestinal mucosa is not well understood. Intraepithelial lymphocytes are conspicuous in the mucosal epithelium, but although it is known that they move across the basement membrane, little is known of their origin, fate, or function.

The bursa of Fabricius is clearly defined as the central lymphoid tissue controlling humoral immunity in the chicken. The question whether a 'bursa equivalent' exists in mammals, however, remains unanswered. While there is little evidence to support the concept that intestinal epithelial lymphocytes have a bursal function, Peyer's patches and the appendix may fulfil this role in mammals. This view is supported by the observation that there is depression of humoral responses in rabbits following extirpation of Peyer's patches and the appendix, whole-body irradiation, and subsequent reconstitution of the immune system with transplanted cells. Furthermore, Bienenstock et al showed that the immunocytes in Peyer's patches show no response to oral or parenteral immunization, despite the presence of antigen in the intestinal lamina propria and despite active specific antibody production by lamina propria plasma cells. This suggests that the Peyer's patches are not directly involved in local antibody production. More recently, the bone marrow has been proposed as the site of maturation of lymphocytes destined to become antibody-producing cells.

**Intestinal Fluid Immunoglobulins**

Accurate quantitation of immunoglobulin concentrations in intestinal fluid...
is complicated by several factors. These include contamination with saliva, gastric juice, and bile, and by transudation into the lumen of immunoglobulins derived from the plasma. In addition, some intestinal fluid immunoglobulins are broken down by proteolytic enzymes, IgG and IgM being the most vulnerable immunoglobulin classes. Despite these difficulties, the relative concentrations of IgA, IgM, and IgG are in substantial agreement with the distribution of these different classes of plasma cells in the intestinal mucosa, IgA being the predominant immunoglobulin component of the intestinal secretion. Although IgD- and IgE-containing plasma cells are present in intestinal mucosa, neither immunoglobulin has yet been detected in normal intestinal secretions using available techniques. IgE has been found, however, in the exocrine secretions of some allergic subjects.

A specific form of IgA, known as secretory IgA, is characteristic of the secretory immune system. Unlike serum IgA, which is predominantly a 7S monomer (MW 170 000), secretory IgA is an 11S dimer (MW 390 000) consisting of two monomers of 7S IgA attached to an additional non-immunoglobulin component, the secretory piece (SP). This name is to be preferred to the alternative name of 'transport' or 'T piece' which implies a function for which at present there is no evidence. An important property of secretory piece is that it renders IgA less susceptible to proteolysis.

Secretory piece can be isolated from 11S secretory IgA as a 4.2S glycopeptide (MW 58 000). It is found free in neonatal intestinal secretions and in the secretions of patients with IgA deficiency. Secretory piece is linked by both covalent and non-covalent bonds to the IgA dimer and three separate lines of evidence suggest that secretory piece binds to the alpha chain of IgA. First secretory piece is specific for IgA and fails to bind 19S IgM or 7S IgG, although intestinal fluid IgM may be linked to secretory piece in patients with secretory IgA deficiency. Secondly, following partial dissociation of secretory IgA by guanidine, a component has been characterized which appears to consist of two alpha chains joined by secretory piece. Thirdly, in the secretions of patients with alpha chain disease, in which abnormal plasma cells in the gut secrete free alpha chain protein, this has been shown to contain bound secretory piece. As alpha chain protein consists mainly of the Fc fragment, this finding suggests that secretory piece binds to the Fc portion of the alpha chain. In addition to secretory piece, a new component, named 'J' (for joining) has recently been reported in the dimer form of human IgA. This single study describes the finding of a component (MW approximately 23 000) which is independent of secretory piece. It is linked by disulphide bridges to the alpha chain and may be responsible for the dimer structure characteristic of secretory IgA.

Detailed investigation of the IgM and IgG found in the intestinal secretions has been hampered by their low concentrations which make isolation procedures difficult. 19S IgM is found in low concentrations in the intestinal fluid of normal subjects but is increased in patients with IgA deficiency. IgG is present as 7S immunoglobulin but antigenically recognizable fragments of IgG, presumably breakdown products due to proteolysis, can also be detected.

Synthesis, Assembly, and Secretory Pathway of Intestinal Immunoglobulins

There is increasing evidence that most of the immunoglobulin present in
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Secretory piece is synthesized independently of IgA, probably in the epithelial cell as demonstrated by fluorescent antibody techniques which localize specific secretory piece staining to epithelial cells, intercellular spaces, and surface mucus. The concept of independent secretory piece synthesis is supported by the finding of free secretory piece in the exocrine secretions of patients with IgA deficiency and in neonates where the concentrations of free secretory piece diminish as development progresses and IgA synthesis increases.

Little is known of the site and method of assembly of secretory IgA. Secretory IgA isoagglutinins isolated from human colostrum contain both type K and type L subunits, suggesting that 7S IgA monomers are synthesized by the subepithelial plasma cell and that following secretion from the cell random dimerization and linkage to secretory piece occurs. Conflicting evidence, however, has been reported following studies of colostral IgA collected from rabbits which were heterozygous for light chain allotypic specificities. Quantitative precipitin techniques showed that the secretory IgA consisted of one allelic type or the other, but not both, indicating that rabbit colostral IgA is synthesized as a dimer in single cells. This conflict may represent species differences but further work is required to elucidate this point.

The secretory pathway of IgA from subepithelial plasma cells to the intestinal lumen remains a mystery. Fluorescent antibody studies of the intestinal mucosa demonstrate IgA staining in subepithelial plasma cells, along the basement membrane, between epithelial cells, and in the apical portions of epithelial cells. It has been suggested that IgA synthesized in lamina propria plasma cells passes through the basement membrane into the intercellular channels, where its further passage is blocked by the tight epithelial cell junction, the zona occludens. IgA passes from the intercellular channel into the apex of the epithelial cell and is subsequently secreted into the intestinal lumen. The site of complexing with secretory piece is unknown.

Little is known about the secretory pathway of locally synthesized IgM and IgG. Studies of local IgM synthesis and secretion in the parotid gland demonstrate that secretory piece is not normally linked to IgM or IgG in parotid saliva, even in patients with IgA deficiency who synthesize and secrete free secretory piece into the saliva. Recently, however, Thompson has shown that in such patients some IgM may be linked to secretory piece in serum and in intestinal juice. Furthermore, in patients with selective IgA deficiency whose intestinal plasma cells and intestinal fluid immunoglobulins are predominantly IgM instead of IgA, IgM can be demonstrated in the intercellular channels and apices of epithelial cells. This may reflect a similar secretory route for locally secreted IgM as for IgA.

The Functional Role of the Secretory Immune System

The biological implications of secretory antibody are becoming increasingly clear. Such antibodies, termed 'coproantibodies' in intestinal secretions, are thought to play an important role in the resistance of the intestinal mucosa.
to invasion by pathogens. The intestinal mucosa has the capacity to display local immunological responses which are both distinct, and, when locally stimulated, independent of the serum response. Susceptibility to infection and resistance from infection following immunization are not related directly to the serum antibody titre but are related to the presence and titre of coproantibody. Studies on experimental cholera in guinea pigs lend support to this view. Following intragastric inoculation of live or killed cholera antigen, the appearance, peak titre, and decline of titre of coproantibodies preceded that for the corresponding serum agglutinins. Parenteral inoculation, however, elicited a synchronous serum agglutinin and ‘coproantibody’ response, the latter apparently being due to the leakage of serum antibody into the intestinal tract. Only the presence of genuine coproantibody stimulated by intragastric inoculation seems to protect against subsequent infection with *Vibrio cholerae*. Further studies have suggested that coproantibody against *V. cholerae* is produced locally in the intestinal mucosa. Recently, more detailed studies have shown that following oral immunization of infants with extracts of pathogenic *E. coli* there was a marked increase in specific antibody titres in the duodenal fluid, while no significant antibody response was detectable in the serum. Absorption experiments revealed that the antibody activity in duodenal fluid obtained eight to 12 days after immunization was confined to the IgM class and that in the late collection (20–44 days) most of the antibody activity was IgA class.

This suggested that a typical primary immune response to the administered antigens had occurred within the gastrointestinal tract. Unlike a systemic primary response where the initial IgM response is followed by IgG antibody production, in this intestinal primary immune response to local antigenic stimulation, the early IgM response is succeeded by production of secretory IgA antibody.

Impressive evidence concerning the behaviour of secretory antibody has accumulated following studies on poliovirus infection and immunization. In a comparative study of immune responses following immunization with live attenuated (Sabin) or inactivated (Salk) polio vaccine, serum IgM, IgG, and IgA responses were found to be almost identical. Only oral immunization, however, produced a secretory antibody response in duodenal fluid, and this response, detectable two to three weeks after immunization, was limited to secretory IgA antibody. The absence of a secretory immune response in the duodenum following the administration of inactivated vaccine parenterally was interpreted as a failure of parenterally administered antigen to reach the intestinal mucosa where the immunocompetent cells mediating the secretory immune response are localized. Both the route of immunization and the non-replicating nature of the inactivated Salk poliovirus were probably implicated in this failure.

Further evidence of the local nature of the secretory immune response and its independence from systemic immunity is now available. A group of children with ‘double-barrel’ colostomies was immunized with live poliovaccine into the distal colonic segment and a specific IgA poliovirus antibody response was shown to be confined to this area. Little response was detected in proximal colon and no response in the nasopharynx. Further studies showed that the same phenomenon of localization occurred when inactivated poliovirus was used for immunization of the distal colon. In this instance, however, a higher dosage of vaccine was required to obtain a response.
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which was shorter in duration and more modest in titre, suggesting that the inability of inactivated vaccine to replicate reduces its capacity to immunize. Furthermore, the presence of poliovirus antibody in the alimentary tract afforded significant protection against reinfection with poliovirus even in the absence of circulating antibody and the degree of resistance was directly related to the titre of preexisting secretory antibody.

The enhanced resistance of secretory IgA to proteolysis and its capacity to form complexes with other proteins suggest that secretory IgA is well adapted to function as a protective antibody at mucosal surfaces. The mechanism by which secretory IgA antibodies exert their protective effect is not clear. There is no evidence that either 7S IgA or secretory IgA can fix complement. IgA antibodies, however, appear to require both complement and lysozyme to achieve maximum opsonizing activity. It is suggested further that secretory IgA antibodies are able to lyse E. coli in the presence of complement and lysozyme, all these components being required for the lytic action.

Conclusions

It is now clear that, in common with other mucosal surfaces, such as the vagina and respiratory tract, the intestine possesses a secretory immunoglobulin system which is functionally independent of the circulating antibody system. This local secretory mechanism plays an important protective role in local immune defence, and has several unique features. Secretory antibody is predominantly of the IgA class. It has a characteristic dimeric structure comprising two 7S IgA molecules linked by secretory piece which appears to protect the molecule from proteolysis in the intestinal lumen. While considerable progress has been made in elucidating intestinal immune mechanisms, many important questions remain unanswered. To the immunologist, the nature of central lymphoid tissue control over humoral immunity, the mechanism of secretory IgA assembly and secretion and its antibody function are especially fascinating. To the clinician, there are signs that recent progress in the understanding of intestinal immunity may shed some light on the pathogenesis of gastrointestinal diseases of obscure origin.

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