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IMMUNOLOGY OF THE GUT

Immunology of the Gut

Ciba Foundation Symposium 46 (new series)

In memory of the late Joseph Heremans

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Introduction

P. J. LACHMANN

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The area that this symposium is going to work over is an extensive one. I was taught at medical school that the absorptive area of the intestine alone was the size of a tennis court, though I cannot vouch for this being true. It is the overlapping fields of gastroenterology in its widest sense—to include even dental caries, in which perhaps not all gastroenterologists regard themselves as practitioners—that have been brought together with immunology in this symposium. Immunological mechanisms can be thought of as protecting the *milieu intérieur* from the *milieu extérieur*, at any rate as far as macromolecules are concerned. The mechanisms for doing this have to apply not only to the normal, sterile tissues of the body, which are protected from most macromolecules except for the insect's sting and the doctor's needle, but also to regions like the gut and to some extent the respiratory tract where the *milieu extérieur* has succeeded in getting inside the *milieu intérieur* and where foreign molecules in the form of both food and microorganisms exist in quantities which, compared with the quantities that immunologists usually deal in, are astronomical.

It is interesting here to draw a distinction between the respiratory tract and the gut. The respiratory tract is protected from antigenic material by a number of mechanical barriers, but once antigenic material gets down to the lung it is almost as antigenic there as when it is introduced parenterally. In the gut, on the other hand, there is no mechanical protection; and in parts of the gut, at any rate, foreign macromolecules are present in large quantities; but in this site they are not antigenic to any substantial extent. Therefore it is not surprising that specialized immunological mechanisms have evolved around areas like the respiratory tract and particularly the gut to deal with these special situations, and these special mechanisms are concerned intimately with the secretory immunoglobulins and especially with IgA. This is the particular immunoglobulin with the characterization of which Joe Heremans was in-

timately involved, and his absence, through his premature death, makes not only this meeting but the whole immunological community very much the poorer. Others are going to discuss IgA—its production, its functions and the cells that make it. The other limb of the immune response, cell-mediated immunity, will not be left out, and I am relieved to see that even complement will have a mention!

The interactions of immunology and clinical medicine have always been very much two-way. It is not just that work on immunology has expanded the field of medicine but also very much that studies of individual patients, the subtle experiments of nature, have made considerable contributions to our understanding of immunology. By no means the worst example of this is the role that studies of multiple myeloma and myeloma proteins have had in increasing our knowledge of immunoglobulin structure, function and genetics, and IgA and IgG, both of which are to be discussed, give good examples of this. It is still a very active field and we shall hear in some detail about the current status of the work on alpha chain disease, that extraordinary situation where partial immunoglobulin molecules are produced and secreted.

The study of immunity deficiency is a second good example of the two-way interaction, and here too work is still very active. We shall hear not only about the effects of primary immune deficiencies, of the sort where the immunodeficient child develops infections; but also about the paradoxical situation, which is now being increasingly appreciated, where minor forms of immune deficiency may become manifest not in increased sensitivity to infection as much as in increased liability to produce allergic diseases due to inappropriate immunological reactivity.

In their turn, studies of immunology have led to great advances both in the understanding and the prevention of disease. Perhaps prophylactic immunization against infection is still the major man-made change in the pattern of morbidity throughout the world, and it is to be hoped that in this area there is still much progress to be made. One could imagine, for example, that a vaccine against dental caries might have almost as much effect on morbidity in the world as a vaccine against cholera, though perhaps not as much as a vaccine against hookworm or malaria (although the latter is not directly relevant to the gut). To develop effective prophylactic immunization one has to understand the processes by which immunological mechanisms bring about immunity. This is a curiously complex topic in which much interest has been taken again in the past few years after a period when there was relatively little work devoted to it. The mechanisms seem to be different for all the major classes of pathogenic organisms. One might say that they are understood badly for bacteria, and worse for worms! We are going to hear about both these topics—about

immunity to the pathogenic variants of the normal gut flora, and about immune mechanisms directed towards nematodes, where there remains the long-standing question of whether the IgE system does have any useful function, and if it does, whether it is in relation to nematodes in the gut.

One major understanding which has come from the study of the immunology of infection is that the same mechanisms which give rise to immunity can also give rise to allergic tissue damage and can themselves contribute to the manifestations of the very infectious diseases against which they also provide protection. This is true both of overt infectious disease (it has, for an example in the gut, been claimed that the diarrhoea of shigella dysentery is largely allergic in nature) and also of diseases that are not obviously infectious at all. For example, brain damage in subacute sclerosing panencephalitis is now recognized as being due to allergic reactions to measles virus infection. The extent to which mechanisms of this kind may be involved in inflammatory bowel disease is a subject of great importance, and this is also to be discussed in the symposium.

Such allergic reactions are not restricted to antigens of infectious organisms, and a consideration of disease caused by allergic manifestations to dietary antigens will be a fitting conclusion to a meeting where I am sure there will be plenty for us all to mark, learn and inwardly digest!

The secretory IgA system of the gut

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Abstract Most commonly, humoral immunity manifested in the gastrointestinal tract of mammals is due to the presence of secretory IgA antibodies. Antibody specificities have been detected in the secretory IgA of gut secretions to a wide range of naturally occurring viral and bacterial components and to test antigens such as chemically modified proteins. Much of the IgA found in gut secretions is synthesized and secreted locally by the abundant plasma cells of the lamina propria. Development of methods for establishing local protective immunity in the gut requires knowledge of the origins of these plasma cells and of the whereabouts of their precursors when they are susceptible to antigen-driven proliferation and/or maturation.

The Peyer's patches have been shown to contain a population of B lymphocytes especially rich in precursors for IgA plasma cells and in cells which can repopulate gut lamina propria with such IgA plasma cells. The Peyer's patches also appear to 'sample' gut antigens, in that small amounts of antigens are passed intact through their dome epithelial cells.

Recent experiments bearing on the origins, differentiation and maturation, antigen sensitivity, migration and lodging of precursors for gut IgA plasma cells are discussed. We use the following three systems: (1) congenic transfer of cells from different murine lymphoid cell sources or mixtures of these (CB20 → BALB/c or BALB/c → CB20) and the use of allo-antisera to IgA allotypic determinants to assess their potential to impart an adoptive IgA antibody response to the recipient and to repopulate its histocompatible lamina propria with IgA plasma cells; (2) clonal precursor analysis (the method of Klinman) both to enumerate antigen-sensitive cells in different tissues of mice and to evaluate their potential to generate plasma cells making particular isotypes and idiotypes of antibodies; (3) use of pairs of Thiry-Vella loops in rabbits, each member either bearing or lacking a Peyer's patch, and quantitation of antibodies of each isotype and of total secretory IgA to assess the response of each loop with the time after local immunization. The results from all three systems provide strong evidence for the importance of Peyer's patches in supplying cells responsible for local humoral immunity and suggest both a differentiative pathway for IgA precursors and their whereabouts when antigen may cause the expansion of a population of specific cells.

Most commonly, humoral immunity manifest in the gastrointestinal tract of mammals is due to the presence of secretory immunoglobulin A (sIgA) antibodies. Thus, in order to devise effective immunization procedures leading to the establishment of local protective immunity in the gut, one must gain some knowledge of the following: (1) the origins and locations of the cells responsible for the synthesis and secretion of sIgA; (2) the stages at which they and/or their precursors are susceptible to antigen-driven proliferation and their whereabouts when such specific expansion may be possible after both primary and secondary challenge; (3) the interactions these cells must undergo with other cell types or with humoral factors—antigens, mitogens, hormones, etc.—before their maturation to IgA plasma cells; and (4) the migration routes and any tissues of temporary domicile favoured by the cellular progenitors of the gut IgA response and any selective lodging properties that they may develop *en route* to intestinal lamina propria. Observations to be presented by ourselves and by Dr Ahlstedt later in this symposium (Ahlstedt *et al.*, pp. 115–129) suggest that an understanding of these aspects of the development of humoral immunity in the gut may also be germane to the appearance of sIgA antibodies in other secretory (exocrine) tissue. Of course, another process relevant to the occurrence of sIgA antibodies in the gut that is not directly related to the generation of a local IgA response involves the passage of the IgA antibodies across an epithelial cell barrier into the intestinal or glandular lumen, and this will be considered later by Dr Brandtzaeg (Brandtzaeg & Baklien, this volume, pp. 77–108).

Our ability to formulate these particular areas of inquiry pertinent to the development of humoral immunity in the gut follows directly from a series of basic observations made during the past 17 years, many by Professor Joseph Heremans and his colleagues. The Heremans group isolated that isotype of immunoglobulin (Ig) from human serum which we now call IgA and defined some of its characteristic properties, such as its lower isoelectric point and higher sugar content relative to other Igs and its propensity to occur in a number of polymeric forms (Heremans *et al.* 1959). Using immunohistochemical methods we were then able to show the synthesis of the IgA isotype by a class of human or rabbit plasma cells different from those making IgG or IgM (Bernier & Cebra 1965; Cebra *et al.* 1966). This separate population of IgA cells assumed greater significance when considered with the finding by Hanson, Tomasi and colleagues from their two groups that the concentration of IgA in human milk and other exocrine secretions was considerably greater than that of any other isotype of Ig (Hanson, 1960, 1961; Tomasi & Zigelbaum 1963; Tomasi *et al.* 1965). The Tomasi group characterized the human sIgA as some sort of polymer of serum IgA containing 'extra' antigenic sites (Tomasi *et al.* 1965) which were later found to occur on a separate polypeptide now

called secretory component (SC) (South *et al.* 1966). Yet another distinct polypeptide, called J chain, was later found in sIgA, IgM and polymeric serum IgAs (Halpern & Koshland 1970). Shortly after the isolation of human sIgA (Tomasi *et al.* 1965) we were able to purify its homologue from rabbit milk (Cebra & Robbins 1966) and deduce from it the molecular weight and polypeptide chain composition of sIgA: four pairs of heavy (α) and light (L) polypeptides + one SC (mol.wt. = 60–70 000) + one J chain (mol.wt. = 15 000) (Cebra & Small 1967; O'Daly & Cebra 1971). In a comprehensive study the Heremans group went on to show that IgA either predominated over all other Ig isotypes in secretions or at least was more concentrated in secretions than in serum from all of many mammalian species examined (Heremans & Vaerman 1971). The rabbit represents a rather extreme case of IgA distribution since the concentration of this isotype, which is the major one in secretions, is about 20-fold higher in milk and 5–10 fold higher in intestinal secretions than in serum (Cebra & Robbins 1966; Robertson & Cebra 1976). An appreciation of the protective role of sIgA in the gut lumen has evolved in parallel with the molecular characterization. Although sIgA antibodies appear neither to react with Fc receptors of any cell type—and therefore do not 'opsonize'—nor to activate complement starting with Cl (Eddie *et al.* 1971), they do appear to be effective simply by complexing with antigen in the gut or at other mucosal surfaces. Thus sIgA antibodies can specifically neutralize toxins, prevent viral attachment to host target cells, and diminish adherence of bacteria to mucosal surfaces and hence the probability of colonization by them (see Ogra *et al.* 1975; Smith *et al.* 1966; Gibbons 1974; Freter 1970).

The local synthesis of much of the sIgA in secretions was inferred from the finding by Tomasi's group of many IgA plasma cells in the interstitium of human salivary glands (Tomasi *et al.* 1965) and by Heremans and his colleagues that human gut mucosa contained up to 200 000 IgA plasma cells per mm^3 in the lamina propria (Crabbé *et al.* 1965), or an estimated 7.5×10^{10} of such cells in the entire gut (Heremans 1975). The lamina propria of the rabbit intestine contains a similarly large number of IgA plasma cells (Crandall *et al.* 1967). Reflecting the 10–20 fold difference in IgA concentration between secretions and serum, these IgA cells are markedly compartmentalized in lamina propria and exocrine tissue—where they comprise 85% of all plasma cells—away from most IgG and IgM plasma cells which are found in spleen and peripheral lymph nodes in the company of only 2–5% IgA cells (Crandall *et al.* 1967; Cebra *et al.* 1966). In an effort to deduce how this compartmentalization of IgA plasma cells was achieved in the rabbit, we sought a source for cells which could repopulate the gut lamina propria of lethally irradiated animals among a variety of lymphoid tissues. Among the tissues tested were Peyer's

patches, which are situated in the mucosa of the small bowel and are quite distinct from the IgA plasma cell-rich surrounding lamina propria. The histology of mammalian Peyer's patches, especially those of the rabbit, has been thoroughly described (Faulk *et al.* 1971; Waksman *et al.* 1973). Large follicles of B lymphocytes, containing many dividing cells in the deeper and lateral regions of each, are characteristic of this lymphoid tissue. Smaller, thymus-dependent areas rich in T lymphocytes occur between the B cell follicles and closer to the dome epithelium.

Using the allotypic determinants present on the L chains of rabbit Igs as markers of cellular origin, we were able to show that Peyer's patches and appendix were enriched sources of cells which could repopulate the gut lamina propria of lethally irradiated rabbits with IgA plasma cells (Craig & Cebra 1971). Relative to peripheral lymph nodes, blood or spleen, Peyer's patches contained many more immediate precursors of IgA plasma cells as judged by the pokeweed mitogen-stimulated appearance of IgA plasma cells *in vitro* upon microculture of cells from the different sources and by the number of IgA plasma cells generated in spleens of recipients of the various cell populations (Craig & Cebra 1971; Jones *et al.* 1974; Craig & Cebra 1975). A sub-population of Peyer's patch lymphocytes was identified which bore no detectable *endogenous* membrane IgM but did carry as surface markers L chain and Fab (α) determinants associated with IgA (Craig & Cebra 1975; Jones & Cebra 1974). We were able to isolate this sub-population of cells, which usually comprised $<10\%$ of Peyer's patch lymphocytes, by fluorescence-activated cell sorting (Jones *et al.* 1974). Almost all of the immediate precursors for IgA plasma cells were found in this sub-population (Jones *et al.* 1974). A similar small minority of B lymphocytes with surface IgA has been detected in mouse Peyer's patches (McWilliams *et al.* 1974; Guy-Grand *et al.* 1974) although the potential of this sub-population has not been evaluated.

An 'antigen sampling' role has also been ascribed to the Peyer's patches, since macromolecules and even bacteria may pass through or by their dome epithelial cells and arrive intact in the midst of B lymphocyte areas (Bockman & Cooper 1973; Carter & Collins 1974). Heremans and his colleagues have made the very important observation that oral administration of either sheep erythrocytes or ferritin to germ-free mice results in the sequential appearance of IgA antibody-forming cells to the former in mesenteric nodes and then spleen and the appearance of ferritin-binding IgA cells after 30 days in gut lamina propria (Crabbé *et al.* 1969; Bazin *et al.* 1970). Gowans and his group and others (Guy-Grand *et al.* 1974; Gowans & Knight 1964; Griscelli *et al.* 1969; McWilliams *et al.* 1975) have shown that a small population of rapidly dividing lymphocytes in rat thoracic duct lymph or in mouse and rat mesenteric