The Mucosal Immune System*

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Mucosal surfaces are the main sites at which man and animals encounter their external environment. To defend against infectious agents and the absorption of dangerous macromolecules, the mucosae must have a complex defense system. Such a system includes specific immune system as well as a number of important non-immune systems. It is hoped that this review will provide a basic understanding on the function of the mucosal immune system, particularly with regard to the possibility of enhancing its function through immunisation. This is important in view of the fact that a number of common and serious infectious diseases, particularly in developing countries, originate at the mucosal surface and that current vaccines against these diseases are still far from being satisfactory.

The existence of a local immune system that is distinct from the systemic immune system has been known for several decades.[1,2] The major characteristic of the local immune system is the preponderance of secretory IgA (SIgA) that can be distinguished from serum IgA by physicochemical and immunological techniques. This fact parallels the predominance of IgA secreting plasma cells in the mucosae, although those secreting IgG, IgM, IgD and IgE have also been demonstrated. In IgA deficiency states, both IgM antibodies and IgM-secreting plasma cells are found in higher proportions. Gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT) represent the major mucosa associated lymphoid tissues (MALT) in man and many species of vertebrates that have been well characterised. In these tissues, lymphocytes may be organised into follicles (e.g., Peyer's patches of the intestine) or they may be dispersed or aggregated at random in the mucosal tissues.[3] Lymphocytes in the latter tissues include intraepithelial (IEL) and lamina propria (LPL) lymphocytes.[3,4] IEL may make up to as high as 30 per cent of the total cell count in the intestinal epithelium.[4]

Secretion and transport of immunoglobulins

Immunoglobulins in secretions can be grouped according to size into polymeric and monomeric categories. The former includes IgM, and dimeric or polymeric IgA, while the latter includes IgG, IgD, IgE and monomeric IgA. In most species, there is no evidence that these monomeric immunoglobulins have a specialised mechanism of transport from serum or interstitial fluid to the external environment. They appear to traverse intact epithelium by simple diffusion. However, such a process is rather inefficient, particularly in normal situations. In diseases involving the mucosae, such a passive transport mechanism may be enhanced, thus accounting for the increased levels commonly noted in these conditions.[5]

In contrast to monomeric immunoglobulins, polymeric immunoglobulins have a specialised mechanism of transport into external secretions.[2,5] However, the mechanism (s) involved varies from species to species and may vary even from secretion to secretion within a single species. Polymeric immunoglobulins differ from their monomeric counterparts by the presence of an extra peptide chain, the J chain, with a molecular weight around 20,000 daltons. Secretory polymeric immunoglobulins (SIgA and SIgM) also differ from their serum counterparts by the presence of a secretory component (SC). It is therefore logical to think that the specialised mechanisms of transport should somehow involve these 2 non-immunoglobulin components.

SIgA found in external secretions is the product of 2 different cell types, namely plasma cells producing dimeric or polymeric IgA together with the associated J chain,[6] and epithelial cells producing the secretory component (SC).[7] A number of studies reveal that the

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IgA molecule and the J chain are produced by plasma cells in juxtaposition to secretory or glandular tissues.[2] Different lines of evidence indicate that the secreted dimeric or polymeric IgA-J chain complexes are taken up by SC on the basolateral surfaces of the epithelial cells.[8,9] In other words, SC appears to act as a receptor molecule for polymeric IgA.[10] According to these investigators, the presence of the J chain on these polymers is a prerequisite for the SC binding.[8,11] The IgA-SC complexes are taken into the cells by endocytosis, transported within vesicles through the cytoplasm, and are finally discharged at the apical surface, probably following a cleavage of SC receptor from the membrane.[12] The discharged SIgA therefore contains a disulfide-bonded IgA-J-SC complex. The SC-mediated endocytic process just described appears to be a mechanism for secretion of both IgA and IgM from all secretory or glandular tissues of most animals, including man. However, the exact site and detailed intracellular events leading to the formation of disulfide bonds are yet to be elucidated.[13]

Although IgA in various external secretions is beleived to be synthesised largely by local IgA producing plasma cells, recent evidence indicates that a considerable portion of polymeric IgA in the bile is derived from circulating serum IgA (for review, see reference 14). This is particularly obvious in mice and rats whose serum IgA is predominantly dimeric.[15] In man a significant contribution comes from plasma cells in accessory glands of major bile ducts.[16] Ultrastructural study suggests that these IgA molecules are transported across the biliary epithelium by endocytosis.[16] The mechanism(s) involved in the selective transport of polymeric immunoglobulins from serum to bile is now being elucidated in several laboratories. Different lines of evidence including the detection of SC on the surface of hepatocytes suggest that these cells are actively involved in this process. Although the most likely mechanism is SC-mediated endocytosis, as with the mucosal epithelial cells described above, alternative explanations involving for example, a normal catabolic pathway of glycosylated proteins by the liver should not be disregarded. However, the preferential binding of polymeric but not monomeric IgA to hepatocytes argues against this possibility.

Functions of SIgA

Immune defense against infections occurring at mucosal surfaces correlates with antibodies in secretions more than antibodies in serum.[2] In the serum, protection against most bacterial and viral infections and against some parasitic infections is associated primarily with antibodies of the IgM and IgG classes; IgE antibodies have been shown to be protective against several parasitic infections. The role of serum IgA in systemic infections has never been clearly determined. On the other hand, IgA in secretions plays an important role not only in mucosal infections but also in systemic infections that originate at mucosal surfaces. There is an increased incidence of these infections in individuals with IgA deficiency states.[17]

SIgA antibodies protect mucosal surfaces by neutralizing toxins and viruses, and by agglutinating or coating infectious particles, thus preventing their adhesion to mucosal surfaces and facilitating their removal.[18] Native SIgA does not activate complement via either the classical or alternative pathway, although in aggregated form it can activate the latter. It was reported some times ago that the bacteriostatic capacity of SIgA antibody could be enhanced in the presence of complement and lysozyme.[19] These different lines of indirect evidence suggest that SIgA may participate in various complement mediated reactions including enhanced phagocytosis and cell lysis. Recently it has been demonstrated that a large proportion of oral polymorphonuclear (PMN) leukocytes express more receptors for the Fc portion of IgA per cell than blood PMNs.[20] Moreover, these oral PMNs, but not blood PMNs, are able to phagocytise target cells coated with IgA. In addition, the presence of IgA receptors on macrophages, blood monocytes and T and B lymphocytes has been reported.[21] These findings suggest that IgA may be involved in antibody-dependent cellular cytotoxicity (ADCC). However, the significance of both complement activation and ADCC in defense at mucosal surfaces remains to be determined, particularly in view of the fact that many secretions have anticomplementary activity and that the unphysiological environment may not favour optimal cellular functions.

SIgA antibodies can interfere with colonization of microorganisms on mucous surfaces of not only mothers but also their offspring via colostrum and milk. It has been amply demonstrated that a mother's milk is endowed with IgA antibodies to microorganisms residing in the intestine and can thereby reduce the incidence of infantile diarrhoea. This process is particularly important when one considers the fact that lactoferrin, an iron-binding protein, is also present in large quantity in milk and this protein has been shown to potentiate the bacteriostatic effect of SIgA.[22]

Besides being involved in defense against mucosal infection, SIgA antibody can also prevent absorption of macromolecules. Particularly important are those allergens in food and inhaled materials. Antigen-exclusion not only minimises the sensitisation process but also prevents allergic manifestations in sensitised individuals. However, mucosal sensitisation is known to induce tolerance against systemic responses. If this involves the systemic IgE antibody response, then

orally induced tolerance might be a possible therapeutic approach to allergic diseases. Lastly it should be mentioned that induction of the IgA antibody response and its presence in the circulation may serve as a means for the body to dispose of toxic antigens. This is accomplished by a selective transport of IgA immune complexes into bile via hepatocytes.[23]

For these various effector mechanisms to work efficienly, the presence of functioning IgA antibody is continuously required in secretions. Due to the presence of proteolytic enzymes in secretions, antibodies belonging to other immunoglobulin classes would be largely destroyed. SIgA, on the other hand, would have a better chance to survive because it is known to be considerably more resistant to proteolytic attack.[24] SC in SIgA is beleived to be the component that confers resistance to proteolytic attack. In recent years, however, several groups of investigators have reported that many bacteria produce proteases that specifically cleave IgA_1. The significance of this observation remains to be determined.[25]

Heterogeneity of mucosal lymphocytes

Mucosal lymphocytes, regardless of whether they are in the epithelial layer (intraepithelial lymphocytes or IEL) or in the lamina propria (LPL), are rather heterogeneous from the morphological, phenotypical or functional point of view.[4,26-28] While both T and B cells are found in both normal and diseased mucosae, their proportions may vary with the sites involved. For example, while most of the IEL are T cells, the lamina propria has, in addition to T cells, a large number of B cells in different stages of maturation. Radiolabelling study have shown that some of these cells are long lived while others are short lived.[4] Different lines of evidence indicate that only a small proportion of these mucosal lymphocytes proliferate and differentiate totally in the mucosae. Most of them have to migrate elsewhere before returning to the original or other more remote mucosal sites.

Functionally, IEL are predominantly large granular lymphocytes with cytotoxic-suppressor activity.[28] Using monoclonal antibodies, evidence is available indicating that these cells are relatively immature when compared with those normally encountered in spleen and other peripheral lymphoid organs.[27] The functional activities of these cells may vary from species to species. For example, there is good evidence showing that in guinea pigs both NK and K cell activities are present largely in IEL while in mice only the NK cell activity can be detected.[28] The characteristics of surface phenotype and NK function suggest that these lymphocytes belong to a different NK subpopulation than NK spleen cells. The significance of these IEL *in situ* has not been clearly determined, but from their location it is logical to expect that they must somehow be important in handling the antigens that get in. However, the exact mechanism of antigen handling remains to be investigated.

Compared with IEL, LPL are considerably more heterogenous not only with regard to B and T cells but also with regard to T cell subpopulations. For instance, not only are there T cells with distinct effector functions, but there are also various T cell subpopulations with immunoregulatory functions. The latter include T helper and T suppressor cells which may have important roles at the secretory sites as the immunoglobulins synthesised there are largely IgA which is T dependent. Recently there is evidence for the existence of a new type of immunoregulatory T cell in Peyer's patches. These switch T cells are able to induce B cells to ungergo class-specific switches from IgM to IgA production.[29] Data suggest that this process involves DNA recombination rather than cellular events resulting in terminal stage differentiation.

Differentiation and homing of mucosal lymphocytes

Although it has been recognised for a long time that most secretory or glandular tissues are populated predominantly by IgA-producing plasma cells, questions as to the origin of these cells and the mechanisms responsible for their selective homing to these mucosal sites have not been answered until very recently.[26] The studies designed to answer these questions must also consider the fact that some secretions, e.g., colostrum and milk, have antibodies against antigens present at remote mucosal sites even though these antibodies can not be readily detected in the serum. Different lines of evidence currently avaiable indicate that, following appropriate antigenic stimulation, both B and T cells, say, in Peyer's patches migrate from the original site for further differentiation and maturation.[26,30] On the way to thoracic duct, these cells migrate first to mesenteric lymph nodes. Then from the thoracic duct, they reach blood circulation, after which they would "home" not only to their original mucosal site but to a less extent also to remote mucosal sites.

The development of the secretory immune system is thymus dependent. Therefore, because of the peculiar compartmentalised phenomenon of IgA B cells as well as the differentiation and proliferation of these cells in secretory tissues, it is logical to expect that stimulation of the mucosal immune system must be well regulated, most likely by an array of immunoregulatory T cells. One of these regulatory T cells is suppressor T cell which, as discussed earlier, is found in high proportion in the mucosa. Like other mucosal lymphocytes, these cells migrate out of secretory sites and suppress systemic immune responses. The second type of immunoregulatory T cell associated

particularly with the mucosal immune response, is contrasuppressor-inducer T cells which have the ability to regulate suppressor T cells activity.[31] In addition, there is yet another regulatory T cell present in the mucosae, a switch T cell, which causes surface IgM positive (SIgM) B cells to differentiate preferentially to SIgA B cells. While these switch T cells switch SIgM B cells directly to SIgA B cells, they do not facilitate the terminal maturation of SIgA B cells into IgA secreting plasma cells.[29] The latter process requires an additional type of regulatory T cell, namely helper T cells which are present in many other lymphoid tissues outside Peyer's patches including mesenteric lymph nodes and spleen. In this aspect, switch T cells differ from conventional helper T cells which bring about the maturation of B cells already committed to a given isotype. The presence of these switch T cells in large numbers in Peyer's patches may explain why the latter are a major source of IgA precursor cells.

The last question to be dealth with in this section is why these IgA B cells return to mucosal sites. It should be mentioned at this point that the source of these large lymphocytes governs the site to which they will eventually migrate. For example, when injected into syngeneic recipients, donor cells from peripheral blood and peripheral lymph nodes preferentially migrate to peripheral lymph nodes while Peyer's patches, appendix and thoracic duct lymphocytes home largely on mucosal lymphoid tissues, particularly on those from which they originated.[32] Various factors have been proposed to explain this phenomenon but none are satisfactory.[30,33,34] Firstly, one may think of specific micro-environmental factors that preferentially attracts IgA blasts. Secretory component (SC) synthesised by epithelial cells has been frequently mentioned as a possible candidate. Another possibility is antigen present at the mucosal sites.[35] However, this, at least, is unlikely to be the main factor because these cells home to the gut of both conventional and germ-free animals.[35] Nevertheless, the presence of antigens may keep these cells from migrating away, thus allowing them to proliferate into IgA producing plasma cells.

Concept of a common mucosal immune system

Although the induction of immune responses in the mucosal or glandular tissues was originally thought to be entirely a local phenomenon, recent evidence suggests that this is not always the case. For instance, naturally occurring antibodies in many external secretions are directed against antigens not present at those sites. Furthermore, when antigen is applied to one mucosal site, the corresponding antibody may be found also at remote mucosal sites even though it is not always detectable in the circulation. Although the latter finding could mean that antibody synthesized at the original mucosal site is rapidly and selectively removed from the circulation by other mucosal or glandular tissues,[36-38] the recent demonstration[33,36] of cells producing specific IgA antibody at remote mucosal sites suggests an alternative explanation. That is that the immune systems in various mucosal or glandular tissues, while separated and distinct from the systemic immune component, are interconnected to one another, thus making up a common local immune system. Therefore, when the system at one site is stimulated, by virture of lymphocyte migration and the homing mechanism previously discussed, those at other mucosal sites would also be stimulated to mount appropriate immune responses. However, the magnitude of these responses might vary from one site to another, since evidence is also available indicating that lymphoblasts prefer to return to their original mucosal site. In addition to the migration of lymphoblasts from one site to another, IgA antibody produced at one mucosal site and found in the circulation may be rapidly secreted at another site. For example, hepatocyte secretion of IgA into the bile to reinforce intestinal immunity[37] is also consistent with this concept.

Stimulation and regulation of mucosal immunity

Undegraded macromolecules and antigenic particles can be absorbed across the intestine, largely via specialised epithelial cells overlying Peyer's patches (commonly referred to as "M" cells).[3] Following absorption, these antigenic materials are transported to MALT and subsequently disseminate to mesenteric lymph nodes. On several occasions, intact antigens have also been detected in the thoracic duct lymph as well as in the circulation. It is believed that macrophages in Peyer's patches and in the lamina propria handle these antigens similar to those found elsewhere in other tissues.

Although a mucosal IgA response occurs when antigen is presented locally to MALT, parenterally administered antigen may also prime or boost such a response.[2] For parenteral priming to be effective, the antigen must be given via the intraperitoneal route and often must be accompanied by an appropriate adjuvant. Attempts to evoke a mucosal IgA response entirely by parenteral immunisation have yielded unfavourable responses except when the antigen was given for a prolonged period in several repeated doses. However, to get optimal stimulation of a mucosal IgA response to commonly used antigens, prolonged or repeated local exposure is also required. Some antigens however are powerful mucosal stimulants, e.g., cholera toxin. The characteristics of an antigen that favour local production of a mucosal IgA response are poorly understood.[37] One

characteristic is that particulate or aggregated soluble antigens are more effective than soluble antigens in monomeric form, unless the latter can bind to cell membranes, thus facilitating antigen trapping. A second characteristic, though still rather controversial, is the ability to activate adenyl cyclase, an enzyme believed to enhance generalised lymphocyte functions including the immune response. It has been demonstrated by several groups of investigators that cholera toxin has both of these properties. It can stimulate substantial intestinal immune response when applied in trace quantity and often in the absence of adjuvant. As mentioned earlier, adjuvant is required with other antigens and the most commonly used adjuvant for experimental animals is complete Freund's adjuvant. However, it has been demonstrated recently that less toxic oral adjuvant can also be quite effective in stimulating both intestinal and extra-intestinal immune responses.[39] Whether or not such adjuvant can be safely used in human remains to be determined.

Both mucosal and systemic IgA responses are T-dependent, largely via immunoregulatory mechanisms of T lymphocytes in Peyer's patches and in other lymphoid tissues. It is intriquing that locally applied antigen can have an important influence on the systemic immune response, i.e., to either stimulate or depress it. The phenomenon of oral tolerance has been known for a long time, but the factors that determine whether mucosally applied antigens will evoke systemic immune tolerance or priming are not clearly understood. Following oral stimulation, antigen specific T suppressor cells can be demonstrated and shortly thereafter they appear in the circulation and spleen.[40] These suppressor cells are specific for IgM and IgG responses. However, whether or not they have any effect on the systemic IgA response remains to be determined. In contrast to its effect in suppressing the systemic response, the effect of mucosal antigen in inducing local tolerance has never been documented. Parenterally administered antigens, on the other hand, can exert an adverse influence on the mucosal IgA response. It is highly possible that such a mechanism involves the stimulation of antigen specific suppressor T cells or the production of high affinity IgG serum antibody, both of which can interfere with events occuring at mucosal sites.

While the mucosal IgA response is dependent on various T lymphocyte subpopulations, other factors can also modulate its process. For example, inadequate nutrition is known to interfere with the development of lymphoid tissues in general. Complicated protein-energy malnutrition (PEM), particularly in children, is known to be associated with a wide spectrum of immunological aberrations.[41] The defect most relevant to the present discussion is an impaired local immune response.[42] PEM and other deficiency states, particularly those involving vitamin A deficiency, can adversely interfere with the production and secretion of SIgA.[43] This may involve not only defective synthesis of the secretory component of SIgA by epithelial tissues, but also interference with proliferation and differentiation of antibody-producing cells and their subsequent migration to appropriate secretory or glandular tissues.[44-46] The one factor often purported to influence the migration of lymphoblasts to mucosal sites is the presence of SC membrane receptors on the basolateral surface of epithelial cells. If this is indeed an important factor for the homing of lymphoblasts, then factors which influence the production of SC must have an indirect influence on the homing process. The production and secretion of SC by some secretory tissues, e.g., the uterine epithelium, has been demonstrated to be hormonally dependent.[47] If this is also the case with other mucosal sites, then hormonal imbalance by whatever cause could have a negative influence on the mucosal IgA response. The latter is influenced not only by the presence of specific antigens at the tissue site, but also by unrelated environmental antigens, (e.g., lipopolysaccharide).[48] Infections caused by a variety of bacteria, viruses and parasites have been shown to suppress the systemic immune response but their influence on the mucosal IgA response is currently poorly understood.

The subject of mucosal memory has been a highly controversial issue and even now evidence available are not highly convincing. The controversy results largely from the discrepancy of data obtained from using different protocols of immunisation, different species, different antigens and different assay systems. In several studies, a secondary mucosal immune response has been reported and therefore memory must exist.[49] For example, a boostered IgA response to cholera toxin has been demonstrated in milk of people from endemic areas.[50] The biliary IgA antibody response in rats can be boosted under appropriate conditions.[51] Memory cells may remain in MALT at the site of priming, although some may circulate and lodge at remote mucosal sites.[36,37] The duration of IgA memory is not known for certain, but was demonstrated in some experimental animals to have been several months.

Vaccination of mucosal surfaces

One of the main objectives of research on mucosal immunity is to determine simple ways and means to manipulate the mucosal immune system in such a way that one can effectively immunise a population against important communicable mucosal diseases or against systemic diseases that originate at the mucosal surfaces. Because various mucosal sites are closely integrated, forming a common mucosal immune system, it is highly possible

to provide protection at one mucosal site by immunising another site that can be more readily approached. A prerequisite to vaccination is the presence of immunological memory against exposed antigens at these mucosal sites. Evidence currently avialable suggests that such a memory does exist, although it may be less distinct than that is known for the systemic counterpart. Granted that such a mechanism exists at the mucosal surface, one of the best ways to stimulate a mucosal immune response in animals is a systemic priming followed within an appropriate time interval by mucosal boosting. For example, to immunise the intestine, one should use intraperitoneal injection followed by oral boosting. Such a protocol is known to give excellent protection against infections by enteropathogenic bacteria. With a similar protocol, it is also possible to induce protection at remote mucosal sites through oral vaccination, a phenomenon made possible through the existence of the common mucosal immune system mentioned earlier. If such a procedure can be improved, it will not only benefit an individual host but in females it will also provide a newborn with an effective defense in the intestine at a time when it is urgently needed.

However, procedure just described is not yet suitable for human use. One major problem up till now has been the unavailability of a suitable adjuvant which should at the least be easily administered and relatively non-toxic. Recent developments in this field of investigation suggest that such an adjuvant for human use may become available in the near future. Another problem that one has to watch out for is a complication from concomitant immunosuppression following mucosal vaccination. As mentioned earlier, many common antigens can not only induce mucosal IgA response but they can also simultaneously suppress a systemic response. Moreover, many of these antigens are poor mucosal antigens. The question that is asked is whether it is possible to alter or to add special features to these antigens to make them more effective in stimulating the mucosal immune system. It has been shown, for example, that aggregation of soluble antigens into larger molecular weight complexes is one way to make them more effective mucosal antigens. Along this line of thinking, it should be possible to add to the existing antigens special properties that can make the whole complex adhere to mucosal surfaces and/or activate the enzyme adenyl cyclase. It should be possible to chemically conjugate the antigens under study to a carrier with self adhering properties. Another alternative approach to oral vaccination is to genetically provide avirulent strains of microorganisms that colonize the intestine with protective antigens. I am rather optimistic that very soon new approaches to vaccination will be developed and the obstacles discussed in this paper will be overcome.

Conclusion

It must be clear from this brief overview of the subject that much remains to be learned about how mucosal immune responses are induced and regulated, even though we have begun to understand in the recent past how the mucosal immune system operates. The movement of cells among various mucosal tissues, the regulation of their proliferation at particular sites, and the possibility that various subclasses of T, B and other cells may have selective mucosal specificity deserve further investigation. We now know, for example, that vaccines may be given orally to induce immune responses that can be expressed in other secretions and thus protect those mucosal sites. With further understanding of the properties of antigens that optimally stimulate the local immune response in the presence of new and effective adjuvants, I am optimistic that new approaches to vaccination will become available in the near future. Furthermore, it will be possible to use these approaches in the regulation of the systemic response, thus alleviating tissue damages and diseases that might otherwise develop as a result of undesirable responses. With more information that will be available in the near future, it may be possible to manipulate these responses by altering diets, by using biologically active agents like hormones or by using other biological response modifiers to control the movement and differentiation of the various cell types. When we get a clear understanding of these points, it will be possible to provide a mass vaccination of a population, particularly in developing countries, against many common cummunicable diseases. Both the basic research and the practical applications of its advances in this area deserve further exploration.

Summary

It is now accepted that there exists among most higher vertebrates that have been well analysed a common mucosal immune system which is distinct from the well-known systemic counterpart. The discussion deals largely with the differentiation and homing of mucosal lymphocytes which by themselves are highly heterogeneous and how these cells are stimulated and regulated. It describes also the mechanism of secretion and transportation of IgA antibodies, including those synthesised by the glandular tissues and those in the circulation, into various external secretions. Finally, the last portion of the review includes suggestions for improved ways and means to stimulate a local immune system such that it would be possible in the near future to have a mass vaccination program against some