Antibody-dependent passive protection of mucosal surfaces

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ABSTRACT
Extensive experiments performed mostly in a variety of animal models convincingly demonstrated the protective effect of polyclonal or monoclonal antibodies administered by the mucosal route. Because of the independence of the mucosal and systemic compartments of the immune system, antibodies from the circulation are not effectively transported in sufficient quantities into external secretions. Nevertheless, local application of antibodies of the desired specificity to mucosal membranes of the respiratory, gastrointestinal, and female genital tracts protected experimental animals from the subsequent challenge by corresponding viral or bacterial pathogens. Thus, generation of monoclonal antibodies of desired specificity and the selection of delivery systems to extend their otherwise short survival on some mucosal surfaces are essential aims of their usability in humans for the effective prevention of mucosally acquired infectious diseases.

Introduction
The protective effect of passively administered antibodies present in and specific for plant-derived toxins, ricin or abrin, was convincingly demonstrated by Ehrlich in 1892.1 Milk of lactating females previously immunized with ricin or abrin protected suckling pups from the lethal challenge when given by the oral route. These studies for the first time demonstrated that the postnatal application of antibodies protects the naive offspring. Subsequent extension and application of these pioneering experiments to other systems revealed that polyclonal antibodies specific for a variety of antigens participate in the effective protection of other mucosal surfaces as extensively reviewed.2,3 Thus, the postnatal administration of preformed polyclonal or monoclonal antigen-specific antibodies of major Ig isotypes provide protection against the mucosal challenge with relevant infectious agents, including the viruses (HIV/SIV, influenza virus) or bacteria (Salmonella and Escherichia coli).2-11 These antibodies, acting in concert with humoral factors of innate immunity – such as mucin, lactoferrin, and the peroxidase system – prevented the penetration of microorganisms through the mucosal surfaces of the respiratory, gastrointestinal, and genital tracts and neutralized biologically active antigens resulting in the efficient protection.2-11 However, there are significant differences related to the species-specific properties and mechanisms involved in the origin and selective transport of antibodies of the IgA, IgM, and IgG isotypes, due to the usage of Ig-specific receptors and their expression on various populations of epithelial cells present in individual mucosal organs.12,13 In human external secretions, with the exception of the female genital tract, IgA is the dominant Ig isotype and it is produced by plasma cells found in high numbers in mucosal tissues.14 In contrast, murine, rabbit, and rat hepatocytes extremely effectively transport polymeric IgA (pIgA) from circulation into the bile and subsequently into the gut lumen because the corresponding receptor for polymeric Ig (pIgR) is copiously expressed on hepatocytes in these species but not in humans and other vertebrates.12,13 Furthermore, the survival of passively administered antibodies to various mucosal surfaces is highly dependent on the possible degradation of such antibodies by exogenous bacterial or endogenous proteases.15 In addition, the outflow of endogenous mucosal secretions, such as saliva or intestinal fluid, is likely to dilute exogenous antibodies and reduce their functional activity, thus requiring their frequent administration. Nevertheless, exploration of alternative administration possibilities as described in the ensuing chapters may, at least partially, extend their effectiveness on mucosal surfaces.

Independence of systemic and mucosal IgA compartments
The systemic and mucosal IgA compartments display a high degree of mutual independence with respect to the antibody structure, function, maturation, distribution, and antigenic specificity of IgA produced by cells involved in biosynthesis in different tissues (Table 1).14,16,17 In humans, IgA is present in plasma dominantly (95–99%) in its monomeric (m) form with two heavy (H) and two light (L) chains linked by interchain disulfide bonds. Detailed studies of plasma IgA, which appeared to exist in the polymeric (p) form, revealed that these are either aggregates of mIgA or immune complexes, due to the absence of the J chain found only in pIgA and the inability to bind pIgR in vitro.18 Furthermore, plasma IgA occurs dominantly in the IgA1 subclass (~85%).16,19 In sharp contrast, external secretions contain IgA almost exclusively in its
Polymeric form as dimers (d) and tetramers (t), (~60% d vs. 40% t) associated with the J chain present in pIgA and IgM and the secretory component (SC), the extracellular segment of pIgR which remains covalently associated with pIg and is responsible for the selective transport of pIgA through the mucosal epithelial cells. Only trace amounts of pIgA, devoid of SC, and mIgA are detectable in some secretions. The proportion of IgA1 to IgA2 varies in individual external secretions and reflects the distribution of IgA-producing cells in mucosal tissues. Various molecular forms of IgA also display distinct biological functions. In general, plasma mIgA exhibits strong anti-inflammatory activity through the interference with the complement-mediated and phagocytosis-promoting functions of IgG and IgM antibodies of identical specificities. However, mIgA can effectively neutralize biologically active antigens and perhaps participate in the intracellular neutralization of viruses. On the other hand, S-IgA of external secretions displays broader biological activities including the effective inhibition of absorption of inert antigens in their soluble or particulate forms from mucosal surfaces. In addition, S-IgA inhibits by antigen-specific or glycans-mediated mechanisms adherence of bacteria to mucosal epithelial cells, and interferes with their uptake by epithelial cells. S-IgA also neutralizes biologically active antigens such as toxins, enzymes, and viruses either free in external fluids or internalized by epithelial cells. Importantly, the pIgA displays the significantly higher ability, even by several orders of magnitude, to neutralize viruses thanks to the presence of 4 or 8 antigen-binding sites on dIgA or tIgA, respectively, exploiting the bonus-effect of multivalency. Through the interaction of S-IgA with the innate humoral factors of immunity, IgA significantly enhances their biological activities. Thus, the binding of mucin to S-IgA results in the significant enhancement of biological activities of both substances in the inhibition of adherence and probably also neutralization, and thus the complexes found between S-IgA and mucin further potentiate the biological protective activities of both components.

Furthermore, S-IgA may also enhance antibacterial activities of the lactoperoxidase system, lactoferrin, and lysozyme present in external secretions thus contributing to the more effective defense of the large surface area of mucosa.

To further document the mutual independence, the maturation of systemic and mucosal IgA compartments in humans’ displays highly diverse patterns. Depending on the adequate environmental antigenic exposure, S-IgA in external secretions is present from the early stages of development. Thus S-IgA can be detected in saliva and numerous IgA-producing cells are present in mucosal tissues within several weeks or months after birth. This is not the pattern of maturation of IgA in plasma. For so far unexplained reasons, the adult levels of IgA in plasma are reached in adolescence. This fact is of enormous functional significance in the induction of systemic and mucosal IgA-mediated immune responses and thus immunity to environmental antigens and vaccines delivered by the diverse immunization routes with an ensuing beneficial effect in mucosal protection or undesired inhibition effect for the systemic IgG-and/or IgM-mediated defense mechanisms.

The tissue distribution of cells producing mIgA or pIgA of the IgA1 or IgA2 subclasses is directly correlated with the presence of corresponding IgA forms in different body fluids indicating their local origin. Plasma mIgA, mainly of the IgA1 subclass, is produced by long-living plasma cells in the bone marrow as demonstrated by immunofluorescence and analyses of supernatants of cells kept in tissue culture. Using analogous experimental approaches it was confirmed that the phenotypes of IgA-producing cells correspond to their product present in individual external secretions. Thus, in the nasal mucosa mainly pIgA1-producing cells are found while in the large intestine the IgA2-producing cells dominate. Furthermore, IgA antibodies present in plasma or external secretions which are specific for the same antigen, recognize diverse antigenic epitopes and may differ in their IgA subclass association, thus providing additional evidence for the independence of the systemic and mucosal IgA compartments.

### Origin of IgA in plasma and excreta secretions

Based on the studies of IgA metabolism, it became clear that in humans the daily production of IgA by far exceeds the combined synthesis of IgS of other isotypes. We produce per kg of body weight per day ~70 mg IgA, ~25 mg IgG and 7 mg IgM. Two thirds of IgA is selectively transported into external secretions especially of the intestines. Lower levels of IgA than IgG in the circulation are due to the differences in their half-lives, which are ~4-5 days for IgA as compared to ~21 days for IgG. IgA present in plasma is produced mainly by numerous long-living plasma cells in the bone marrow and also in the spleen and lymph nodes. IgA enters the circulation predominantly in the form of mIgA of the IgA1 subclass. In mice and primates, circulatory IgA is catabolized in the liver with participation of the asialoglycoprotein receptor expressed on hepatocytes. Only trace amounts of
mIgA of circulatory origin enter external secretions. S-IgA is produced by plasma cells present in large numbers in various mucosal tissues, especially in the gut, with a surprising long life-span of several years. IgA is secreted by these cells in the form of dIgA and tIgA associated with the J chain which is involved in IgA polymerization and is also essential for the binding of plgA by the plgR expressed on surfaces of various populations of mucosal and glandular epithelial cells. This receptor remains attached to plgA with the J chain as S-IgA. The plgR expression on epithelial cells is regulated by hormones, cytokines, vitamins, and selected bacterial components. There are, however, marked species-dependent differences in the form of circulatory IgA and its transport into external secretions. For example, in mice, rats and rabbits, circulatory IgA is present dominantly in the polymeric form and hepatocytes express plgR responsible for an effective hepatocyte-mediated transport of plgA into the bile and then into the gut fluid. As a matter of fact, in these species, most of the intestinal S-IgA consists of plgA produced in mucosal tissues, enters the circulation as plgA, and subsequently appears as S-IgA due to the effective hepatocyte-mediated transport in the bile and then in the gut secretions. Importantly, systemically injected murine plgA or plgA produced by hybridoma cells subcutaneously injected in mice is effectively transported into the bile and then into the gut fluid but not into other secretions including saliva and milk. Thus, plgA produced by hybridoma cells with plgA of desired specificity is effectively transported into the gut lumen, where it effectively displays S-IgA-mediated protective activity. This is not the case in humans and other species in which plgR is not expressed on hepatocytes. Convincing evidence for the ineffective transport of plgA as well as IgG or IgM from plasma into the human external secretions was provided in several mutually complementary studies. Using radiolabeled mIgA, plgA, IgM, and other proteins injected intravenously, it was clearly demonstrated that these Igs penetrated into the intestinal secretions and saliva only in trace amounts. Similarly monoclonal plgA, IgG, and IgM proteins present in gram quantities in plasmas of patients with IgA or IgG multiple myeloma or IgM Waldenström’s macroglobulinemia were detected in their saliva in minimal quantities (~1%) using reagents specific for the idiotypic determinants of individual monoclonal myeloma proteins. These findings are of great importance because it is obvious that systemically injected monoclonal Igs, including plgA of selected specificity – for example, against HIV, influenza virus, or SARS-CoV-2 – would not be transported into external secretions and thus would not provide desired protection. The failure to transport plgA from plasma into the external secretions of mucosal tissues and glands is most likely due to the effective competition of locally produced plgA by plasma cells infiltrating mucosal tissues with plasma-derived plgA for the epithelial plgR.

What is next?

Studies mostly performed in animal models convincingly demonstrate the protective effects of mucosally applied polyclonal or monoclonal antibodies as mediators of protection. However, there are several promising improvements to further enhance their protective activities. To extend the presence of antibodies on mucosal surfaces of upper respiratory or genital tracts, the application of antibodies with mucosa-adhesive components such as carboxymethylcellulose would prolong their local presence. To preserve and selectively deliver such antibodies to the desired locations of the gastrointestinal tracts, their packaging in capsules which release their content based on the variable pH in selected compartments – be it the stomach or small and large intestine – offer interesting possibilities. The selective delivery of antibodies or vaccines to the upper or lower respiratory tract is dependent on the size of aerosol particles. Therefore, the use of delivery appliances that generate small particles to reach the lower respiratory tract versus those which would preferentially remain in the upper respiratory tract, represents approaches deserving further studies. Furthermore, the selection of antibodies according to their IgA subclass may be of importance due to the higher sensitivity of IgA1 than IgA2 to proteolysis, which is likely to extend the protective functions of IgA2 – associated antibodies. Undoubtedly, the use of monoclonal antibodies in the protection of mucosal surfaces provides an important stimulus for further advances.

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