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Mucosal Immunity: Role of Gut-Associated Lymphoid Tissue (GALT) in IgA Response

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INTISARI

Marsetyawan H. N. E. Soesatyo – *Imunitas mukosal: Peran jaringan limfoid usus dalam respon IgA.*

Imunitas mukosal ditandai dengan adanya sekresi imunoglobulin (Ig)A pada permukaan mukosa dan kelenjar sekretorik, dikenal sebagai *secretory (s) IgA*. Sistem imun mukosal berbeda dengan dan tidak tergantung pada sistem imun sistemik sebab IgA diproduksi oleh sel plasma lokal, berbentuk polimerik, dan kadar sIgA tidak berkaitan dengan kadar IgA monomerik di dalam sirkulasi darah. *Secretory* (s)IgA berperan melindungi mukosa tubuh terhadap kuman patogen, toksin bakterial, enzim-enzim, dan sejumlah besar antigen asing termasuk antigen protein dari makanan. Selain menggumpalkan bakteri, menetralisasi virus dan toksin, sIgA mampu menghalangi perlekatan mikroorganisme pada pemukaan sel epitel usus, sehingga invasi kuman ke dalam jaringan bisa dicegah. Proses ini dikenal sebagai *immune exclusion*. Selain itu sIgA tahan terhadap enzim proteolitik yang dihasilkan bakteri Gram (-).

Saluran pencernaan sepanjang usus halus dan usus besar mengandung jaringan limfoid yang tersebar di dalam epitel, lamina propria, atau tersusun sebagai agregat seperti lempeng Peyer *(Peyer's patch, PP)*. Jaringan limfoid ini dikenal sebagai *gut-associated lymphoid tissue* (GALT). GALT, khususnya PP berfungsi sebagai tempat induksi respon IgA, sedangkan lamina propria sebagai tempat efektor respon imun mukosal. Induksi antigen pada PP akan mengaktifkan sel B yang diprogram untuk menghasilkan IgA atas bantuan *T helper* (T_H), interleukin (IL-4, IL-5 dan IL-6) dan *follicular dendritic cells* (FDC). Selanjutnya sel B spesifik bersama T_H akan bermigrasi ke limfonodi mesenterial, ductus thoracicus, sirkulasi darah dan beredar ke seluruh tubuh. Akhirnya sel B mengalami pemasakan menjadi sel plasma IgA yang siap mensekresi IgA ke permukaan mukosa, seperti saluran pernafasan bagian atas, saluran genital, dan saluran pencernaan. IgA juga dicurahkan ke dalam kelenjar-kelenjar sekretorik, seperti lakrimal, ludah dan kelenjar susu. Peristiwa migrasi sel B dan sel T dari GALT dan kembali (*homing*) ke jaringan mukosa, baik yang letaknya dekat maupun jauh dari tempat induksi, merupakan dasar dari imunitas mukosal. Konsep ini dikenal sebagai sistem imun mukosal umum.

Key Words: mucosal immune system – sIgA – Peyer's patch – M cells – oral immunization.

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INTRODUCTION

A protective local and mucosal immune system seems to function independently of systemic immunity. The concept of local immunity was initially proposed by Besredka in 1919 (*cit.* Brandtzaeg, 1989), that rabbits after being immunized orally with killed Shiga bacillus, were protected against fatal dysentery irrespective of the serum antibody titer. In 1922 the human mucosal immunity was first reported by Davies (*cit.* Brandtzaeg, 1989) that bacterial agglutinins could appear in dysentery stools several days earlier than in the blood. The molecular basis for local immune system was established when Tomasi *et al.* (1965) confirmed that external secretions contain a unique immunoglobulin (Ig), which is called secretory IgA (sIgA). This antibody which is the predominant Ig class in human external secretions provides specific immune protection for the mucosal tissues (McGhee & Mestecky, 1990; Holmgren *et al.*, 1992).

BIOLOGICAL SIGNIFICANCE OF THE MUCOSAL IMMUNE SYSTEM

The role of mucosal IgA in protection against infections is illustrated by the fact that most infectious agents enter the host via the mucous membranes (McGhee & Mestecky, 1990; Hanson & Brandtzaeg, 1993). For instance, the great majority of the 40.000 children under the age of five years that die everyday has been due to mucosal infections in the respiratory and gastrointestinal tracts (UNICEF, 1992). In addition, the protection for the breastfed infants provided by sIgA in milk has been demonstrated for cholera, enterotoxigenic *E. coli* (ETEC) and *Campylobacter* infections. Moreover, breast feeding is 18-fold better at protecting against neonatal septicaemia when compared to infants who are not breast-fed and the risk of dying from diarrhoea in infancy is almost 24 times higher without breast feeding compared with exclusive breast feeding (UNICEF, 1992). These illustrate the importance of mucosal immunity for the well being of the young as well as the adult.

CONTRIBUTIONS OF GALT TO THE MUCOSAL IMMUNITY

The mucosal surface of the gut has an enormous exposure area to exogenous agents including microorganisms, toxins, enzymes and dietary antigens. It is not surprising, therefore, that mucosal tissue is equipped with local immune system capable in initiating and effecting a wide variety of immunologic reactions.

The intestine contains the so-called gut-associated lymphoid tissue (GALT) (Bienenstock & Befus, 1984), which represents the largest immunological organ of the body. More than 80% of all immunoglobulin (Ig)-producing cells (approximately $10^{10}m^{-1}$ Ig- producing cells) are found along the gut mucosa. These cells produce more sIgA (50-100 mg/kg body weight/day) (Brandtzaeg, 1989; Mestecky *et al.*, 1991). Although GALT is frequently associated with Peyer's patch(es) (PP), its name designates for all lymphoid cells and structures along the gut mucosa (Soesatyo *et al.*, 1990; Soesatyo, 1992). This includes lymphocytes within the epithelium (intraepithelial lymphocytes, IEL), lamina propria, isolated lymphoid follicles, appendix, and colonic lymphoid patches (Soesatyo *et al.*, 1993). In addition, the gut-draining mesenteric lymph nodes (MLN) are considered to be part of GALT (Gebbers & Gebbers, 1992).

STRUCTURAL AND FUNCTIONAL FEATURES OF PP

Peyer's Patch, an organized lymphoepithelial structure, comprises B cell-dependent follicles, interfollicular T cell-dependent areas, and follicle-associated epithelium (FAE) overlying the dome of lymphoid nodule (FIGURE 1). The FAE is characterized by the presence of modified epithelial cells, called M (membranous/microfold) cells, located interspersely among absorptive cells of the lymphoid follicles.

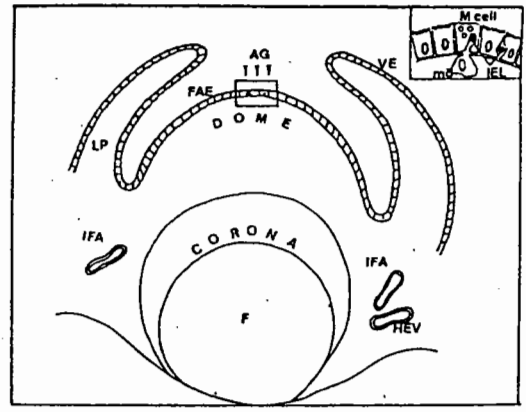

FIGURE 1. – Schematic drawing of Peyer's patch and its compartments. Note: the follicle-associated epithelium (FAE) with M cell (see inset); VE = villous epithelium; IFA = interfollicular T cell area; LP = lamina propria; F = follicle; AG = antigen, IEL = intraepithelial lymphocyte; HEV = high endothelial venule.

M cells are unique both in morphology and functional properties (Sneller & Strober, 1986; Trier, 1991). Unlike the absorptive epithelial cells, these cells have small numbers and short microvilli, a poorly developed glycocalyx, remarkable cytoplasmic processes, few lysosomes, and low acid phosphatase content. These special structures of M cells are instrumental for the transepithelial transport of potentially antigenic materials from the gut lumen. M cells are ideal gateways for the presentation of enteric antigens to the underlying immunocompetent cells of the lymphoid nodule. In addition to lymphoid cell populations, nonlymphoid accessory cells as macrophages and interdigitating dendritic cells (IDC) are found in the dome and T cell areas, respectively.

It is generally agreed that the induction for IgA immune reactions occur in the PP (Dahlgren *et al.*, 1989; Bjerke & Brandtzaeg, 1990; Weinstein & Cebra, 1991). In addition, this lymphoid organ serves as a source for IgA plasma cell precursors that populate mucosal surfaces of the body (Weisz-Carrington *et al.*, 1987; Bjerke & Brandtzaeg, 1990). Luminal antigents will be taken up by M cells, and are subsequently processed by the dome macrophages and presented to T_H ($CD4^+$) cells which in turn activate B cells. The expression of membrane (m)Ig-bearing B cells in the PP germinal centers (GC) undergoes switching, *ie.* from mIgM/IgD B cells to mIgA B cells with the help of T_H (Tswitch), follicular dendritic cells (FDC) and interleukin (IL-4). This preference for switching to mIgA B cells is likely due to the local microenvironment within PP GC (Cebra *et al.*, 1991; Weinstein & Cebra, 1991). Committed B- and T-cells will then

leave the PP, migrate to MLN, and enter the blood circulation through the thoracic duct. These B cells expand, undergo proliferation and differentiation, and home to the effector sites, *ie.* in the gut lamina propria and migrate to remote mucosal tissues. These cells mature to become IgA plasma cells, and produce sIgA antibodies in the secretions, such as salivary, tears, milk, gut- and vaginal washings. The final specific maturation of IgA B cells is influenced by IL-5 and IL-6 (McGhee *et al.*, 1989; McGhee & Mestecky, 1990). The migration of specifically primed T and B cells from GALT and then homing to the mucosa (mucosa-associated lymphoid tissue, MALT) and the external secretions, is an important basis for mucosal immunity (Brandtzaeg, 1989). The concept of a common mucosal immune system is illustrated in FIGURE 2.

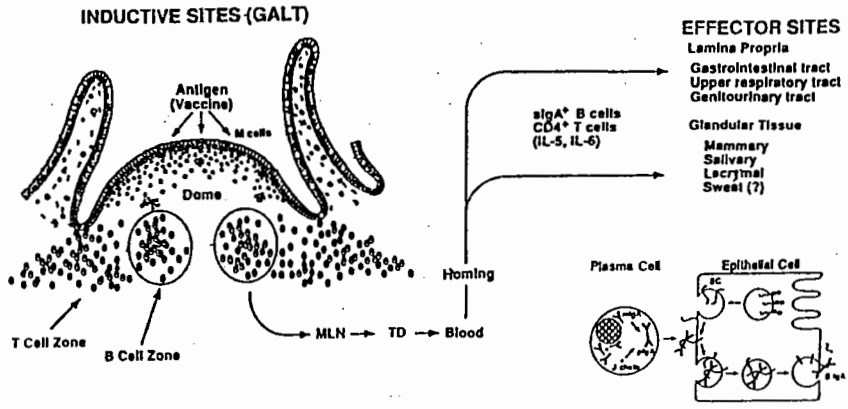

FIGURE 2. – Concept of common mucosal immune system. The initial induction of mucosal response occurs in the GALT following a lumenal antigen sampling by M cells. Antigen-sensitized, precursor IgA $^{+}$B cells and CD_4 T_H cells leave via efferent lymphatics and migrate to the mesenteric lymph nodes (MLN), into thoracic duct (TD) and to the blood circulation. Migrating cells enter and homing to the effector sites for production and transport of pIgA into mucosal surfaces or secretions. The pIgA with J-chain interacts with SC on the basolateral side of the epithelial cells, is endocytosed, transported across the cytoplasm, and then released at the apical membrane (McGhee & Mestecky, 1990).

MECHANISMS FOR SIgA TRANSPORT

As other Ig isotypes, IgA is composed of four polypeptides chain subunits: two α heavy chains and two light chains (either κ or λ) forming two identical antigen-combining sites. Each α heavy chain has one variable region domain and three constant region domains. A distinctive feature of IgA is its ability to form polymers by means of having a special constant region (C_H) terminal extension with an extra cystein residue, which can participate in cross linking monomeric subunits together via disulfide bridges. While IgA monomer is found in the circulation, IgA polymers are produced by plasma

cells locally in the tissue. Polymeric (p)IgA usually occurs as dimers (11 S) (Childers *et al.*, 1989; Mestecky *et al.*, 1991). The polymerization occurs as these two monomeric forms are joined together by a small glycoprotein (MW 15 kd), called joining (J)-chain. The J-chain that enables pIgA to bind to membrane secretory component (SC), is synthesized in the cytoplasma of B cells at various stages of differentiation and in plasma cells. IgA antibodies containing J-chain could increase the avidity for antigen. The SC is a transport secretory antibodies (sIgA and also sIgM), because it protects the polymeric form against proteolytic enzymes. Molecular structure of SC comprises a single polypeptide chain folded into five immunoglobulin-like domains, which shares sequence homology with Ig light-chain variable regions. The secretory component also contains large amounts of carbohydrates, in which the function(s) is not yet known, possibly involved in the interaction of SC with pIgA.

Transport of pIga into secretions is mediated by SC. The SC is synthesized by and expressed on the surfaces of epithelial cells in the gastrointestinal and respiratory tracts, acinar and ductal cells in the gastrointestinal and expressed on the surfaces of epithelial cells in the gastrointestinal and respiratory tracts, acinar and ductal cells in exocrin tissue, and cells lining the uterine. In the small intestine, SC is found primarily in the columnar cells of the crypts on the surface of epithelial cells. It should be noted that in rats, mice and rabbits, SC is also expressed on the hepatocyte membrane and constitutes an additional receptor and transport mechanisms for the secretion of pIgA into bile. This transport mechanism is not seen in human, because SC is not produced in the hepatocytes (Mestecky *et al.*, 1986). IgA that is found in human bile is synthesized locally in minor lymphoid areas of the liver in the vicinity of large hepatic ducts, and thus, it is not transported from the blood (Childers *et al.*, 1989). In the cytoplasm of epithelial cells, the transmembrane form of SC is synthesized on the rough endoplasmic reticulum (RER) and then moves through the cells to the Golgi apparatus, where terminal glycosylation occurs. Finally, SC is transported to the basolateral side of the cell, and is then inserted into the membrane. The inserted SC molecule as a reseptor is about 100 kd, whereas SC form in secretions as a cleavage product is 71-75 kd. The pIgA with J-chain bound by SC at the cell surface is internalized into vesicles and transported to the cell apex. The vesicles fuse with the membrane, and the entire complex (only small SC portion remains on cell membrane) is released as sIgA (FIGURE 2). Proteolytic cleavage is thought to be the mechanism of release. However, the site of cleavage on SC molecule and the enzyme(s) responsible are at present unknown.

BIOLOGICAL ACTIVITIES OF SIgA

IgA shares with other antibody isotypes the ability to interact with an enormous spectrum of antigens of viral, bacterial and parasitic origin with a high degree of specificity. IgA in external secretions occurs predominantly in dimeric or tetrameric forms (with four or eight antigen-binding sites, respectively), and thus, displays greater avidity than monomeric IgA. The pIgA has been shown to neutralize viruses more efficiently than monomeric IgA (Taylor & Dimmock, 1985), and its multivalence also enables Ig polymers to agglutinate bacteria better than corresponding monomers. In addition to viruses, sIgA antibodies also neutralize other biologically active antigens such as bacterial toxins and enzymes (Kilian *et al.*, 1988). For example, studies on cholera toxin in a rat model have shown that specific IgA antibody efficiently prevents the dehydrating secretory effects of cholera toxin in the intestine. It was proposed that IgA

may allow *Vibrio cholera* to be present without causing disease until the organism is cleared by other immune and nonimmune mechanisms. Some organisms, e.g. certain *Streptococcus, Neisseria, Haemophylus, Clostridium*, are able to counteract the effects of IgA by producing specific IgA_1 proteases (in general IgA_1 is proteases resistant). These enzymes cleave IgA_1 in the hinge region into one Fc and two F_{ab} residues. It is not clear whether cleavage of IgA_1 by these proteses is sufficient to inhibit the protective effects of IgA, because human myeloma IgA_1 molecules of known antitoxin activity were not significantly inactivated by IgA_1 protases from several organisms. Besides direct actions of sIgA against pathogens (direct killing, agglutination, neutralization), invasion of mucosal sites. The role of antibodies in immune exclusion has been well documented that, antibody-coated bacteria cannot attach the epithelial cells. Previous enteric exposure to foreign soluble antigens diminishes the absorption of the same but not of unrelated antigens owing to the presence of specific sIgA antibodies (Kilian *et al.*, 1988; Mcghee & Mestecky, 1990). Through this immune exclusion mechanisms, IgA limits further absorption of undigested antigenic material and the formation of potentially harmful circulating immune complexes that contain predominantly IgG antibodies. Among the best evidence for immune exclusion is mucosal immunity to *Vibrio cholerae*, which produces an enterotoxin that binds to a ganglioside receptor (GM_1) on intestinal epithelial cells. sIgA antibodies purified from serum and colostrum of Indian patients convalescing from cholera inhibited adherence of *Vibrio cholerae* to sections of rabbit intestinal tissue (Majundar & Ghose, 1981; Majundar & Ghose, 1982). However, antiadherence activity of sIgA from colostrum of Swedish women (cholera is not endemic in Sweden) was absent. This was in contrast to sIgA collected from colostrum of Indian women which has a high antiadherence activity against cholera.

CONCLUSION

Although sIgA is quite versatile and has been able to adapt the extensive challenge of the mucosal ecosystem, its optimal effects might require interactions with other spesific and nonspesific host defence factors. For instance, interactions with mucosal phagocytes, lymphocytes involved in the antibody dependent cell-mediated cytotoxicity (ADDC) with CD_{3+}, CD_{4+} or CD_{8+} T cell subsets, should not be ruled out. In addition, as the initiation of an IgA response occurs in PP, it would be beneficial if oral immunization and vaccination could be targetted to the GALT in order to obtain an optimal mucosa-binding carriers, such as cholera B subunit, biodegradable microspheres or live vectors, by using mucosal adjuvants.

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