Mucosal Immunosenescence in the Gastrointestinal Tract: A Mini-Review

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Key Words

Mucosa · Aging · Dendritic cells · M cells · Secretory IgA

Abstract

It has been shown that pathogen-specific secretory IgA (SIgA) antibody (Ab) is the major player at mucosal surfaces for host defense. However, alterations in the mucosal immune system occur in advanced aging, which results in a failure of induction of SIgA Abs for the protection from infectious diseases. Signs of mucosal senescence first appear in the gut immune system. Further, changes in the intestinal microbiota most likely influence mucosal immunity. To overcome the immunological aging decline in mucosal immunity, several adjuvant systems including mucosal dendritic cell targeting have been shown to be attractive and effective immunological strategies. Similarly, microfold (M) cells involved in the antigen (Ag) uptake are ideal targets for facilitating Ag-specific mucosal immune responses. However, the numbers of M cells are reduced in aged mice. In this regard, Spi-B, an essential transcription factor for the functional and structural differentiation of M cells, could be a potent strategy for the induction of effective mucosal immunity in aging.

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The Mucosal Immune System

The mucosal immune system in higher mammals is sophisticated and consists of an integrated network of tissues, lymphoid and mucous membrane-associated cells, and effector antibody (Ab) molecules. Along with cytokines, chemokines, and their receptors, these effector Ab molecules, which are primarily of the IgA isotype, are key players in mucosal immunity and appear to function in synergy with innate host factors [1, 2]. Thus, in order to induce antigen (Ag)-specific immune responses at these mucosal barriers, one must consider the common mucosal immune system, which supports the concept of distinct mucosal IgA inductive and effector tissues [1, 2]. The mucosa-associated lymphoid tissue (MALT) serves as the major mucosal inductive site. MALT is covered by a lymphoepithelium containing microfold (M) cells and well-organized regions, the subepithelium with enriched Ag-presenting cells, a B cell zone with germinal centers, and adjacent T cell areas including an equal distribution of naïve and memory T cell phenotypes [1, 2]. Upon Ag activation, memory B and T cell populations then emigrate from the mucosal inductive environment via lymphatic drainage, circulate through the bloodstream, and

home to mucosal effector sites, where abundant IgA-producing plasma cells are present. The effector sites for mucosal immune responses include the lymphoid cells in the lamina propria of the gastrointestinal (GI), upper respiratory, and reproductive tracts as well as secretory glandular tissues [1, 2]. The Ag-specific mucosal effector cells include IgA-producing plasma cells as well as mature B and T lymphocytes. Secretory IgA (SIgA) is the primary Ig involved in protecting mucosal surfaces and is locally produced by plasma cells in mucosal effector tissues [1, 2]. In this regard, the majority of T and B cells in effector tissues are activated and express a memory phenotype [1, 2]. Thus, it is generally agreed that mucosal immune responses are initiated in mucosal inductive [e.g., gut-associated lymphoid tissue (GALT) and nasopharyngeal-associated lymphoid tissue (NALT)] but not effector tissues.

Despite recent achievements in the understanding of the complexities within the mucosal immune system, only limited information is available in terms of age-associated changes that occur in mucosal immune responses. In this short review, we will mainly focus on GI immune responses in aging in order to shed light on the unique cellular and molecular changes that occur in mucosal immunosenescence.

Mucosal Immunosenescence

Immune functions are known to deteriorate with age in several species. In humans, the elderly are at a higher risk for infections, especially severe infections, as well as for certain autoimmune diseases and cancer, and their immune responses to vaccination are diminished [3]. It has been accepted that aged humans exhibit a loss of naïve T cells and a more restricted T cell repertoire [4]. Further, aging results in decreased human CD8⁺ cytotoxic T lymphocyte responses, restricted B cell clonal diversity, failure to produce high-affinity Abs, and an increase in memory T cells [5–7]. It has been suggested that although certain dendritic cell (DC) populations are fully functional in aging [8–11], both foreign Ags and self-Ags induce enhanced proinflammatory cytokines [12, 13]. This enhancement of inflammation can be detrimental; however, very old individuals with a more balanced pro- and anti-inflammatory phenotype may be the most fortunate [14, 15]. The association of inflammation in aging has been termed ‘inflamm-aging’ [16]; however, we still do not have direct evidence that inflamm-aging occurs in and therefore influences the mucosal immune system.

Studies have provided extensive evidence of a dysregulation of and an overall decline in mucosal immunity especially in the GI tract of the elderly [3]. The most common method for evaluating mucosal immune responses is perhaps to test the external secretions for the presence of SIgA Abs. In humans, GI lavages taken from either aged or young subjects were shown to contain similar total Ig levels [17]. Our group has shown that early development of aging occurs in the GI tract immune system [18]. Fecal extract samples from 1-year-old mice contained low levels of Ag-specific SIgA Abs; however, total IgA levels were essentially the same as those seen in young adult mice [19]. Similar results have also been reported for total IgA levels in the serum of aged mice, rats, and humans [18]. These studies indicate an absence of age-associated impairment in total IgA synthesis in external secretions. However, Ag-specific IgA Ab responses in elderly humans, mice, and rats are markedly lower than those seen in the young. Further, our previous studies showed that the GALT inductive immune system is affected by immunosenescence earlier than the NALT-based and systemic immune systems [19, 20]. In addition, it has been suggested that maturation into adulthood, from 8 to 24 weeks of age, significantly influenced the induction of oral tolerance in various strains of mice [21]. However, oral tolerance established at an early age could be maintained even during the aging process, while the induction of oral tolerance to new or virgin Ags was impaired in aged mice. These results clearly show that Ag-specific mucosal SIgA Ab responses and oral tolerance are diminished in aged mice, especially those supported by the GALT immune system.

The Potential Role of the Intestinal Microbiota in Mucosal Immunosenescence

The mammalian large intestine contains up to 10¹² bacteria per gram of intestinal contents [22, 23]. Indeed, the human gut microbiota harbors more than 50 genera/several hundred species, which represent more genes in the gut microflora than are seen in the human genome [24]. The normal microbiota is essential to maintain appropriate homeostatic conditions, providing energy in the form of short-chain fatty acids, nutrients, and protection against colonization with potential pathogenic bacteria by the production of antimicrobial peptides [23, 25]. In addition to these functions, the intestinal microbiota plays a major role in the maturation of the host immune system including intestinal SIgA Ab production and intraepithelial lymphocyte development [22, 26]. For ex-

ample, germ-free mice have an immature GI mucosal immune system, which includes hypoplastic Peyer's patches (PPs) as well as diminished numbers of IgA-producing cells and CD4⁺ T cells [22, 27]. Cells of germ-free mice exposed to cells of normal mice or monoassociated with *E. coli* resulted in the maturation of the mucosal immune system [28, 29]. Further, it was reported that bacterial stimulation of human intestinal epithelial cells supported IgA2 subclass switching [30]. Conversely, the lack of intestinal IgA Ab responses altered the intestinal microbiota by allowing bacterial population changes to occur. Thus, the aberrant expansion of segmented filamentous bacteria was noted in activation-induced cytidine deaminase (AID)-deficient mice, which lack an appropriate molecular environment for IgA class switching [31]. Further, opportunistic bacteria, mostly *Alcaligenes* species, specifically inhabit GALT or PPs as well as isolated lymphoid follicles with an associated preferential induction of Ag-specific SIgA Abs in the GI tract [32]. The absence of a microflora in the GI tract also affects oral tolerance induction [33]. Thus, one cannot readily induce oral tolerance in germ-free mice [34]. Indeed, human microbiome analyses have revealed significant changes in the intestinal microflora in the elderly (<65 years) [35, 36]. However, others have shown that the change in the microbiota was seen only in centenarians with increased inflammatory cytokine responses, but not in the elderly (average age 70 ± 3 years) [37]. Nevertheless, these findings would indirectly suggest that the alterations in the intestinal microflora and the decline in the gut immune system are major changes associated with aging.

Induction of Mucosal Immune Responses in Aging

Elderly individuals are in general much more susceptible to infections usually acquired via mucosal exposures. The GI tract in the elderly is particularly susceptible to infectious diseases, suggesting that a poor mucosal immunity is a major factor leading to higher mortality from infections in aging [38, 39]. Further, Ag-specific mucosal IgA Ab responses are diminished in aged animals, especially those seen in the GI tract-associated immune system [3, 18]. Moreover, the severity and mortality caused by influenza virus and the bacterial pathogen *Streptococcus pneumoniae* (the pneumococcus) are sharply increased in humans of advanced age [40, 41]. Although it has been shown that an effective protection can be provided by pathogen-specific systemic IgG without mucosal IgA responses [42], pathogen-specific SIgA Ab responses are a

necessary component for providing a first line of effective immunity against these respiratory pathogens at their entry site [8, 43]. However, it has proven difficult to induce de novo vaccine-specific mucosal immunity in the elderly using current vaccine approaches. Indeed, it has been shown that the tri- and tetravalent live attenuated influenza virus nasal vaccines are ineffective in the elderly (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6332a3.htm> and <https://www.flumistquadrivalent.com/consumer/index.html>). This could be due to the pre-existing influenza-specific Abs including respiratory SIgA in older individuals, which may influence the uptake of the nasal influenza vaccine.

The induction of mucosal immune responses requires the use of either mucosal adjuvants and/or live attenuated microbe delivery systems [1, 2]. In addition, the co-administration of adjuvant(s) offers the advantage of eliciting Ag-specific parenteral immune responses [1, 2]. In this regard, adjuvant systems have provided significant improvements in the development of influenza vaccines in the elderly [44, 45]. Thus, an H5N1 vaccine with the MF59 adjuvant induced a rapid rise in broadly crossreactive Abs as well as long-lived human memory B cells [44]. More recently, the AS03 adjuvant system (squalene, DL- α -tocopherol, and polysorbate 80; GlaxoSmithKline) improved the immune response to inactivated 2009 H1N1 influenza vaccine in both healthy adults (18–64 years) and older adults (>65 years) [45]. Despite this advance, a recent study showed that the nasal vaccination of mice with detergent split influenza Ag [A/Uruguay/716/2007 (H3N2)] given with purified monophosphoryl lipid A (MPL) in liposomes promoted detrimental Th17-mediated immunity [46]. The vaccine induced both mucosal and plasma-derived Abs; however, the mice lost weight but eventually recovered [46]. These findings illustrate the point that adjuvant selection and the delivery method must be carefully considered in order to develop effective and safe vaccines. Since it has been shown that resident memory T cells play key roles in the protection from influenza virus infection [47], reactivation of these T cell populations may represent an additional approach to induce Ag-specific immunity.

DC-Targeting Mucosal Immunization for Restoring Aging

To explore new avenues for effective mucosal immunization strategies, investigators have begun to target mucosal tissues and immune cells for vaccine delivery. To

Fig. 1. Unique features of M cells and their differentiation. **a** M cells exhibit short and sparse villi when compared with other surrounding FAE cells. Dogma suggests that this feature contributes to an easy access to the M cells for numerous external materials including commensal bacteria. In addition, the shape of M cells looks like ‘arms’ that are able to ‘hold’ lymphocytes and Ag-presenting cells. **b** Like other intestinal epithelial cells, M cells develop from intestinal epithelial stem cells (IESC). Enterocytes located in the FAE possibly receive RANKL stimulation from subepithelial stromal cells of PPs via RANK, which is expressed on most intestinal epithelial cells. This stimulation triggers the induction of Spi-B, which is an essential transcription factor for mature M cells. The target genes of Spi-B contribute to the final development and functional expression of M cells. For example, GP2, which is one of the Spi-B targets in M cells, acts as a scaffold receptor for FimH protein of *E. coli* and *Salmonella* spp.

this end, mucosal DC-targeting Ag delivery systems have been shown to induce Ag-specific SIgA responses [48–50]. CpG oligodeoxynucleotides (ODN) as vaccine adjuvants have been shown to restore Ag-specific immune responses to ovalbumin (OVA), diphtheria toxoid, hepatitis B, pneumococcal polysaccharides, amyloid β , and tumor cells in aged mice and rats [3]. In addition, it has been shown that IL-15 treatment also restored impaired DC functions in mesenteric lymph nodes of aged mice [51]. When normal 3-month-old versus 18-month-old mice were orally immunized with the weak immunogen OVA plus CpG ODN as adjuvant, both groups of mice showed high and equivalent levels of OVA-specific systemic IgG and mucosal SIgA Ab responses [52]. Furthermore, our studies showed that when a combined nasal

adjuvant consisting of a plasmid encoding the Flt3 ligand cDNA (pFL) and CpG ODN was given with OVA or with pneumococcal surface protein A (PspA) or hemagglutinin (HA) to aged mice, significant levels of Ag-specific SIgA Ab responses were induced in the external secretions with full protection from pneumococcal or influenza virus infection [8, 9, 48]. Importantly, this double adjuvant system elicited balanced Th1- and Th2-type cytokine responses without increasing potential inflammatory IL-17 responses [8, 9, 48].

Potential for an M Cell-Targeting Strategy in Aging

As emphasized thus far, MALT (NALT and GALT) plays an important role in mucosal immunity. In this regard, an Ag sampling system that takes up luminal Ags from the mucosal environment into the tissue is mediated by M cells. M cells have unique morphological features, such as relatively short irregular microvilli on their apical surfaces and a pocket structure that enfolds lymphocytes and Ag-presenting cells including DCs (fig. 1a). These unique features allow M cells to transport luminal Ags from the gut or nasal lumen to underlying MALT lymphocytes more efficiently. Thus, a strategy for targeting these M cells for the induction of mucosal immunity in aging is highly attractive. Reoviruses use their own protein sigma one ($\sigma 1$) to initiate infection through M cells [53, 54]. In this regard, M cell-targeting DNA vaccine complexes consisting of plasmid DNA and the covalently attached reovirus $\sigma 1$ to poly-L-lysine (PL) induced significant mucosal SIgA Ab responses in addition to systemic immunity [54]. Further, a novel M cell-specific monoclonal Ab (NKM 16-2-4) has been used as a carrier for M cell targeting with a mucosal vaccine to elicit protective immunity against lethal challenge with botulinum neurotoxin [55]. Oral immunization of Ag fused with M cell-targeting peptide ligand (Co1) resulted in enhanced Ag-specific immune responses [56]. Although there is little information about the molecular mechanisms for Ag sampling in M cells due to the difficulty in their in vitro culture, it has been reported that glycoprotein 2 (GP2) is specifically expressed on M cells and acts as a binding receptor for FimH-expressing bacteria (e.g., *E. coli* and *Salmonella* spp.) to induce effective uptake of and specific immune responses to such bacteria [57, 58]. Interestingly, it has also been reported that the *Salmonella enterica*, serovar Typhimurium (*S. Typhimurium*) type III effector protein SopB induces a transition of follicle-associated epithelium (FAE) enterocytes into M cells [59].

Recently, it has been reported that one of the E26 avian leukemia oncogene transformation-specific (Ets) family transcription factors, Spi-B, is essential for the functional and structural differentiation of M cells [60–62]. Like all other intestinal epithelial cell lineages, M cells develop from leucine-rich repeat-containing G protein-coupled receptor 5-positive (Lgr5⁺) intestinal epithelial stem cells [62]. Some M cell precursor cells receive receptor activator of nuclear factor- κ B ligand (RANKL) stimulation from the subepithelial stromal cells in the FAE region. This signal triggers the expression and activation of Spi-B and subsequently the upregulation of several Spi-B-target genes including GP2, which is considered to be a matured M cell marker (fig. 1b). Of importance, it has been shown that GP2⁺ mature GALT M cells are significantly decreased in aged mice [63]. Although the expression of RANKL and RANK and their signaling are not altered in aged mice, Spi-B-positive cells are significantly diminished in their FAE region through an unknown mechanism [63]. In agreement with the reduction in mature M cells, the uptake of latex particles into the PPs is severely impaired in aged mice. Furthermore, in the absence of M cell-intrinsic Spi-B, the activation of T cells toward orally inoculated *S. Typhimurium enterica* is severely diminished [61]. Therefore, a reduced M cell number may be one of the causes of immunosenescence in the elderly. Although M cells are also found in NALT FAE, there is

no report describing a change in the density of mature M cells in NALT FAE with aging so far. Therefore, it is possible that one of the reasons for the delay in immunosenescence in NALT is the maintenance of high numbers of mature functional M cells on NALT FAE of aged mice. From the view of vaccine development, forced Spi-B activation and/or expression may be a good target for an oral vaccine adjuvant used in the elderly.

In conclusion, there is significant evidence showing that the GI tract immune system is altered by advanced aging; however, the precise cellular and molecular mechanisms that lead to mucosal immunosenescence remain to be elucidated, especially in humans. A clear understanding of the mucosal immune system in aging could lead to the development of new strategies for vaccines specifically tailored for the elderly.

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