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REVIEW ARTICLE

Impact of Aging on Gastrointestinal Mucosal Immunity

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There is considerable evidence that the mucosal or secretory immune response in the gastrointestinal tract is compromised by aging. The generation of a mucosal immune response is an extremely complex process that involves antigenic stimulation of a specific subpopulation of immunologically competent cells in the Peyer's patches, differentiation and migration of these cells to the small intestinal lamina propria, initiation and regulation of local antibody production in the intestinal wall, and mucosal epithelial cell receptor-mediated transport of antibodies to the intestinal lumen. Available data suggest that gastrointestinal mucosal immunosenescence reflects deficits in: (1) the differentiation and/or migration (homing) of immunoglobulin A immunoblasts to the intestinal lamina propria, and (2) the initiation and/or regulation of local antibody production. The significant age-related increases in the incidence and severity of gastrointestinal infectious diseases, coupled with the potential for immunopharmacologic manipulation of the mucosal immune compartment, substantiate the merit of studies designed to resolve the etiology of mucosal immunodeficiency in the elderly.

KEY WORDS: aging; gut mucosal immunity; secretory immunity; immunoglobulin A; IgA; immunosenescence.

An extensive data base suggests that aging is associated with systemic immunodeficiency. The evidence points towards age-related dysfunctions of B and T lymphocytes and age-related impairments in the regulation of the immune response by cytokines and other factors (see refs. 1 and 2 for reviews). Mucosal surfaces are anatomically associated with a discrete compartment of the immune system, which is autonomous from the systemic system by virtue of: (1) a different major immunoglobulin isotype, (2) a unique process for initiating an immune response, and (3) independent lymphocyte populations. Among mammalian organ systems with a mucosal surface, the gastrointestinal tract represents the largest single immunological organ, contains >70% of the organism's immunoglobulin-producing cells, and produces more immunoglobulin A (IgA) than the organism's total production of immunoglobulin G (IgG). Despite the facts that mucosal surfaces are directly exposed to potential pathogens and constitute the first line of immune defense, the question of age-related perturbation of the mucosal or secretory immune system has not been studied extensively (see ref. 3 for a review). Furthermore, much of the data concerning mucosal immunity in old animals and old humans is contradictory. The gastrointestinal tract in the elderly is particularly susceptible to infectious and inflammatory diseases, suggesting that mucosal immune defenses are compromised (4, 5, see ref. 6 for a review). For example, statistics from the World Health Orga-

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nization demonstrate a 400-fold increase in mortality attributed to gastrointestinal infections in the elderly in comparison to young adult populations (see ref. 2 for a review).

The medical problems of the elderly and the associated health care costs should encourage research on mucosal immunodeficiency in this age group, as well as on its relationship to the incidence, prevalence, and severity of age-associated diseases. Such research efforts have merit since the mucosal immune compartment is accessible to manipulation by immunopharmacological agents that enhance the secretory immune response.

EVIDENCE FOR GASTROINTESTINAL MUCOSAL IMMUNODEFICIENCY IN THE ELDERLY

Infectious diseases are the fourth leading cause of death and constitute a significant cause of morbidity in the elderly. This aged subpopulation is more susceptible to gastrointestinal infection and exhibits considerably higher rates of progression of infections than do younger age groups. Furthermore, the efficacy of certain vaccines, such as those directed against influenza viruses and pneumococcae infections, is markedly diminished in elderly individuals (see ref. 6 for a review; 7–9). Strassburg et al have shown that elderly subjects exhibit a 70% efficacy for influenza vaccine in mortality reduction, but only a 30% efficacy in preventing the clinically defined disease (10). Epidemiological studies have suggested that the rate of progression of HIV/AIDS is greater in elderly persons than in younger subjects (11–15). Many elderly individuals are afflicted with debilitating diseases that compound their immunosenescent state and may further influence their responses to vaccines or pathogens (16). Geriatric populations destined for study should be characterized with respect to health and nutritional status, because both of these variables have been shown to influence immune responsiveness (17–19).

Despite evidence of mucosal immunodeficiency, several studies have reported increased serum IgA levels in old animals and humans compared with corresponding levels in younger individuals. Ebersole et al reported an eightfold increase in serum IgA in rats between 1 and 20 months of age (20). It should be noted, however, that 20-month-old rats are usually not beyond the 50% survivorship level and, thus, should not be considered senescent. Amman et al measured human serum IgA levels and found that these were 50% higher in older (>57 years) than in younger (<42 years) subjects (21). Other clinical studies have reported similar increases or no change in serum IgA levels as a function of aging (22–24).

The total IgA level in intestinal lavage fluid increased approximately twofold in nonimmune rats between 4 and 20 months of age (20). Arranz et al found no age-related differences in total or secretory IgA levels in intestinal lavage samples of young and elderly human subjects and concluded that the intestinal IgA response is unaffected by age (24). Although serum and intestinal lavage IgA levels increase or remain unchanged with age in rodents, monkeys, and humans, other studies suggest that nonspecific immunoglobulin levels in intestinal secretions are poor indicators of mucosal immunity and that specific antibody titers represent a more critical measure of mucosal immune responsiveness (20, 25–28). Furthermore, Senda et al suggested that age-enhanced IgA levels in the intestinal lavage reflect monomeric IgA rather than the polymeric form, which undergoes receptor-mediated epithelial transport to mucosal surfaces (29). Steffen and Ebersole reported that the avidity of IgA antibodies for *Mycoplasma pulmonis* antigen actually increased with age in mice (30). However, it should be noted that the oldest mice examined in this study were only 16 months of age and, thus, were not senescent.

Several groups of investigators have measured intestinal IgA antibody titers in response to selected antigens as a function of age. For example, it has been shown that the intestinal IgA antibody response to intraduodenal cholera toxin diminishes with increased age in rats and mice (31, 32) (Figure 1). Intraduodenal immunization of rhesus macaques with cholera toxin elicited a substantial intestinal anti-cholera toxin IgA response in young, but not in old, animals (25). In contrast, such immunization led to increased serum IgA antitoxin titers in young and old macaques. Anti-cholera toxin immunoglobulins G (IgG) and M (IgM) antibody titers in the intestinal secretions were higher in old than in young macaques, perhaps indicative of a compensatory response to the failure of IgA production or abnormal immunoregulation (25, 33).

Although several clinical studies have documented an age-related decline in specific mucosal IgA antibody responses, there have been conflicting reports. Amman et al, as well as other groups of investigators, observed age-related deficits in the IgA antibody responses to various antigens in human subjects (see refs. 6 and 7 for reviews, 21). Despite an age-related

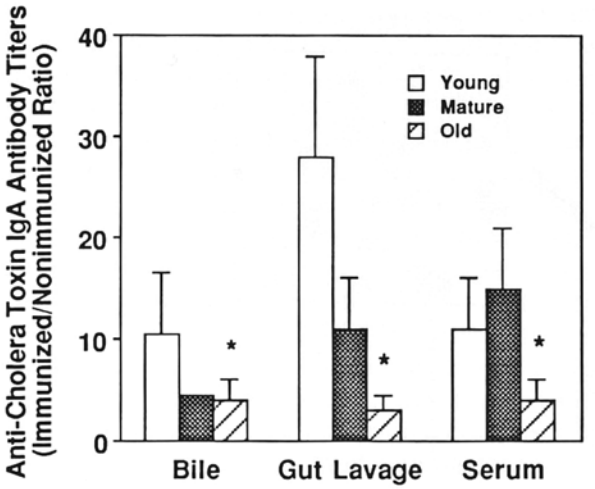


Fig 1. The ratio of anti-cholera toxin IgA antibody titers in cholera toxin immunized and nonimmune young, mature, and old rats. Animals were immunized intraduodenally with 50 μg of cholera toxin in PBS with 2% gelatin or sham-immunized with 2% gelatin in PBS alone, on days 0 (primary) and 14 (boost), killed on day 19, and the antibody titers measured in bile, intestinal secretions (gut lavage), and serum by capture ELISA. The ratio of anti-cholera toxin IgA antibody titers in immunized rats to those in naive animals in all three samples declined significantly during aging (*$P < 0.01$ compared with young animals). Each bar represents the mean ± SEM of data from three to five animals. (Data derived from Ref. 30.)

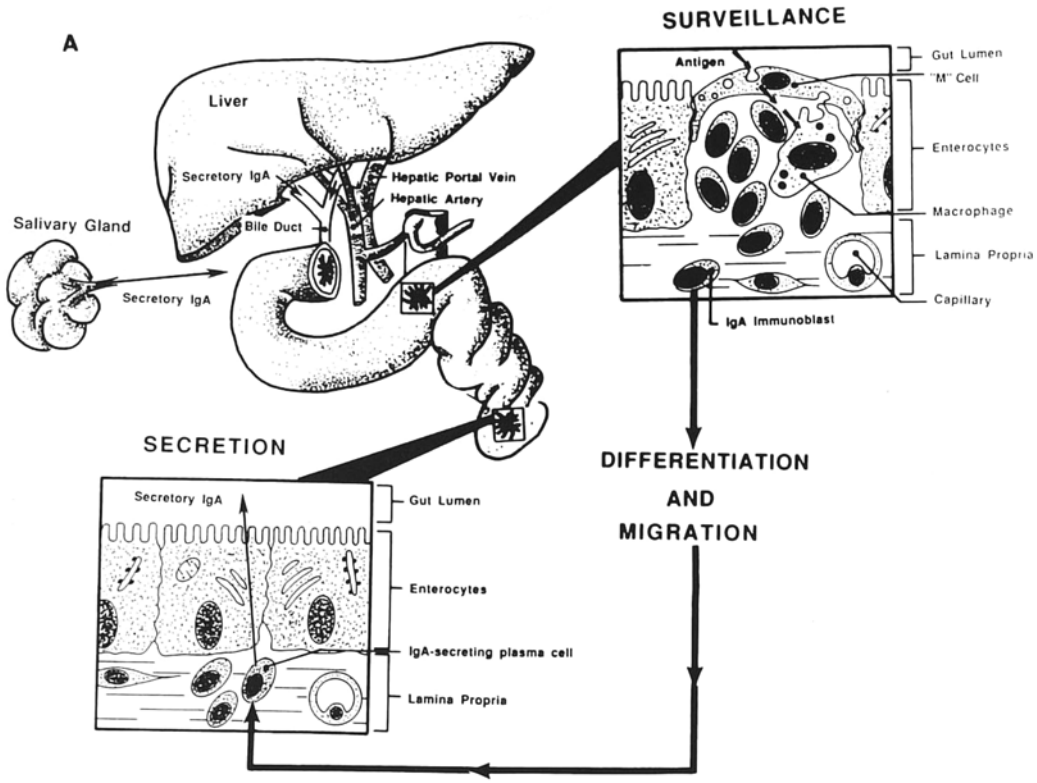
decline in antibody titers in the lung lavage of mice orally immunized with influenza virus, Waldman et al reported that the antibody responses in the nasal lavages of immunized young and old humans were similar (8). Similarly, Ganguly et al measured equivalent nonspecific IgA titers in the saliva of nonimmunized young (33 years) and elderly (72 years) volunteers (9). However, most studies to date suggest that the mucosal immune response is compromised in the geriatric population. Major issues that remain to be resolved include: (1) how aging impairs the secretory immune response, (2) whether or not immunosenescence predisposes the elderly to infectious diseases, and (3) whether immunodeficiency associated with aging might be reversible.

EVIDENCE FOR AGE-RELATED SHIFTS IN COMPOSITION OF GUT-ASSOCIATED LYMPHOID TISSUE (GALT)

The mucosal immune system depends on the cooperation of lymphoid and epithelial cell components to initiate and maintain an immunological response. An effective response in the gastrointestinal tract involves: (1) antigen uptake at the mucosal surface via specialized epithelial cells (M cells), which overlie nodules of the Peyer's patches; (2) transport of antigen across M cells, and its presentation to immunologically competent cells in the Peyer's patches; (3) differentiation and migration (homing) of antigen-stimulated Peyer's patch IgA B immunoblasts to the intestinal lamina propria; (4) regulation of antibody production by mature IgA plasma cells in the lamina propria, and (5) transport of polymeric IgA and IgM antibodies across the intestinal epithelium to the mucosal surface (Figure 2). These secretory antibodies neutralize toxins on the mucosal surface, block the adherence of bacteria to the epithelium, and reduce the penetration of antigens across the mucosa. Diminished intestinal IgA antibody titers in elderly subjects might, theoretically, reflect age-associated diminutions in: (1) uptake and transport of luminal antigens by M cells, (2) maturation and migration of Peyer's patch B immunoblasts to the intestinal lamina propria, (3) local antibody production, or (4) epithelial transport of polymeric immunoglobulins from the lamina propria to the intestinal lumen. Individually or collectively, diminished activity of these processes might impair the intestinal IgA response to antigens. The following is a summary of current knowledge of the effects of aging on the sequential steps involved in the initiation of an intestinal mucosal immune response.

We are unaware of any qualitative or quantitative evidence of an age-related impairment in the uptake and/or transport of antigens by M cells; most studies have focused on steps subsequent to antigen presentation. Kawanishi and Kiely reported that the number and distribution of mouse Peyer's patches remain unchanged, but that the follicle weight and yield of Peyer's patch lymphocytes decline substantially in aging mice (34). Furthermore, studies from our laboratory demonstrated that aging does not influence the number of Peyer's patches per small intestine in rats or the yield of lymphocytes per patch (31). In the 1960s, Cornes reported that the number of Peyer's patches in the human small intestine decreases with age (35). The interpretation of this early work is tempered by the fact that Peyer's patches are extremely difficult to visualize in fresh human or monkey tissue.

Several investigators have suggested that losses in specific B- and/or T-lymphocyte subpopulations, as well as shifts in the distributions of lymphocyte subsets, in gut-associated lymphoid tissue (GALT) may contribute to an age-related decline in mucosal immune competence (34, 36–39). For example, Kawanishi and Kiely observed a decline in the $T_{suppressor/cytotoxic}$ cell subpopulation in Peyer's patches of old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Enterocytes
Macrophage
Lamina Propria
Capillary
IgA Immunoblast
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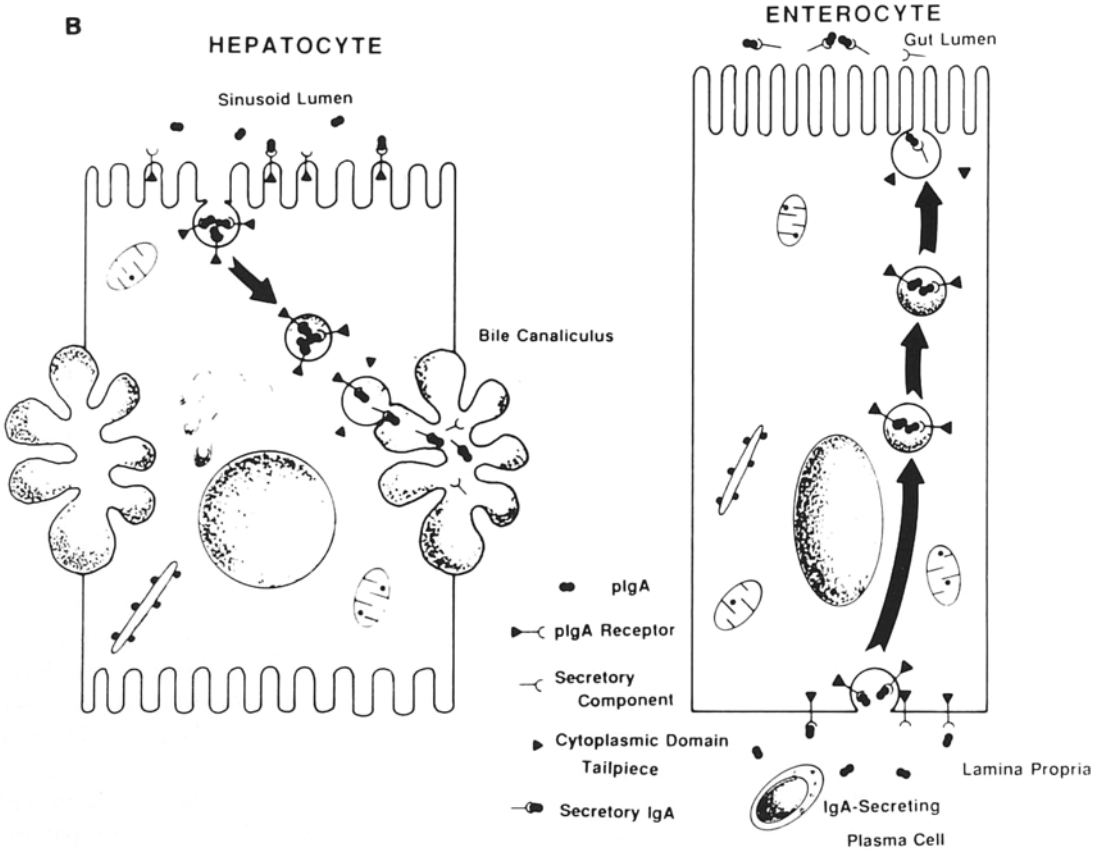
B
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Gut Lumen
pIgA
pIgA Receptor
Secretory Component
Cytoplasmic Domain Tailpiece
Secretory IgA
Lamina Propria
IgA-Secreting Plasma Cell

mice (34, 40). Ebersole et al did not detect any changes in mature T-lymphocyte subpopulations, but reported that the Peyer's patches of old mice contained fewer immature and IgA^+ cells than those of young mice (41). Quantitative studies from our laboratory, involving flow cytometry, did not reveal any age-related shift in the relative proportion of Peyer's patch $T_{suppressor/cytotoxic}$ ($CD8^+$) cells in rats (42). However, subsequent immunohistochemical staining revealed age-related differences in the anatomical distribution of $CD8^+$ lymphocytes in rat Peyer's patches. In young rats, discrete zones of densely stained $CD8^+$ cells were seen in the interfollicular areas, and weakly stained cells were present within the follicles of the patches. In old rats, the frequency of intensely stained $CD8^+$ lymphocytes between the follicles was markedly reduced, and cells of this phenotype were evenly distributed throughout the patches. Interestingly, the frequency of $CD8^+$ lymphocytes in the intestinal lamina propria of the rats in this study increased 2.5-fold between 3 and 29 months of age. However, the relative percentages of Peyer's patch and mesenteric lymph node total T lymphocytes ($W3/13^+$) and $T_{helper/inducer}$ ($W3/25^+$) cells were found to remain stable during aging (42). These data suggest that suppressor/cytotoxic T-cell distribution in the inductive and effector sites of the GALT exhibits an age-related shift, and they clarify the apparent contradiction between immunohistochemical and flow cytometric data concerning the effect of aging on the relative abundance of $CD8^+$ T lymphocytes in rodent Peyer's patches (34, 40, 41, 43).

The proliferative capacity of GALT T lymphocytes from young and old animals has been studied fairly extensively and, in general, has not exhibited an age-related decline. Concanavalin A elicited a greater proliferative response in Peyer's patch T lymphocytes isolated from old mice than in similar cells from young animals (44). However, these data must be tempered by those of Kawanishi and Kiely, which indicate that concanavalin A-induced proliferation of Peyer's patch $T_{suppressor/cytotoxic}$ lymphocytes is markedly impaired in old mice (40). Systemic lymphocytes undergo well-documented age-related declines in their proliferative response to a variety of mitogens, but this response is postponed or less evident in cells isolated from the mesenteric lymph nodes of old mice, especially those expressing the T_{helper} phenotype (45, 46).

All surface immunoglobulin-positive isotypes and IgA^+ cells account for 60–70% and 6–17%, respectively, of Peyer's patch cells in all age groups of naive and immunized rats (42). Ebersole et al reported a decline in the number of IgA^+ cells in rat Peyer's patches and salivary glands during aging and suggested that the maturation of B immunoblasts into surface IgA-bearing cells is compromised in old animals (20). Again, the oldest rats examined were only 20 months of age, ie, not truly senescent. Haaijman et al observed that the number of cytoplasmic immunoglobulin-containing cells in GALT declined with age in mice but that there was no significant shift in the number of surface immunoglobulin-positive lymphocytes (39). These data appear to suggest that there is an age-related impairment in the differentiation of surface IgA-bearing immunoblasts into mature antibody-secreting plasma cells. Kawanishi and Kiely reported that old mice are characterized by a decline in the total number of IgA^+ cells in Peyer's patches and mesenteric lymph nodes (34). Studies from our laboratory have shown that the total population of surface immunoglobulin positive lymphocytes in GALT remains unchanged in rats during aging but that there is a concomitant twofold increase in IgA^+ cells in the Peyer's patches (31). The increase in Peyer's patch IgA^+ cells, coupled with quantitative immunohisto-

Fig 2. (A) Schematic diagram of the gastrointestinal mucosal immune system. Secretion across the acinar cells of the salivary glands represents an important route for entry of IgA into the oral cavity and proximal gastrointestinal tract. In certain rodents, the hepatobiliary pathway accounts for much of the IgA that enters the intestinal lumen, whereas in most other species examined, including humans and primates, the major transport of this immunoglobulin occurs across the intestinal epithelium. Surveillance of the intestinal lumen for antigens, and initiation and regulation of the secretory immune response, involve the Peyer's patches. Specialized epithelial cells (M cells) on the dome of the Peyer's patch transport antigens to underlying macrophages and lymphocytes. The precursors of IgA-secreting plasma cells, presumably IgM^+–IgD^+ double-positive lymphocytes in the Peyer's patches, undergo isotype switching to IgA expression, migrate to the mesenteric lymph nodes for further T-lymphocyte-dependent maturation, and "home" to the lamina propria of the intestine via the systemic circulation. In the intestinal lamina propria, mature plasma cells serve as the primary source of IgA antibodies. (B) Schematic diagram of receptor and vesicle-mediated translocation of IgA across hepatocytes and small intestinal enterocytes. In rats and other rodents, polymeric IgA (pIgA) binds to the polymeric immunoglobulin receptor (pIgR) on the sinusoidal membranes of hepatocytes, and the entire complex (pIgR + pIgA) is endocytosed into vesicles and transported to the bile canaliculus via a microtubule-dependent mechanism. During this transit, the pIgR is cleaved and the portion of the receptor (secretory component) complexed to pIgA is secreted into the bile, along with the attached pIgA. This molecular complex of secretory component and pIgA is termed secretory IgA. Free secretory component (without attached pIgA) is also secreted into the bile; the pIgR is not recycled. The subcellular pathway in enterocytes is similar, except that pIgA is endocytosed at the basolateral membrane and transported to the apical surface, where the secretory IgA is released into the intestinal lumen. (Revised from Ref. 2.)

chemical evidence of an age-related decline in this surface isotype in the intestinal lamina propria, supports the hypothesis that aging compromises the maturation of Peyer's patch IgA^+ immunoblasts and/or their migration to the intestinal lamina propria.

EFFECT OF AGE ON MIGRATION OF IgA IMMUNOBLASTS TO INTESTINAL LAMINA PROPRIA

There is evidence that aging perturbs the differentiation of Peyer's patch IgA^+ immunoblasts and their migration to the intestinal lamina propria, with consequent diminution in local antibody production. Several groups of investigators have reported a loss or no change in the number of IgA^+ cells in the intestinal lamina propria during aging in rodents (30, 36, 47, 48). The loss of IgA^+ plasma cells in the intestinal wall of old animals coincides with reduction in the titer of IgA antibodies in the intestinal secretions (30, 34, 36). Bianchi et al reported a fivefold decline in the population density of IgA^+ cells in the intestinal lamina propria of nonimmune mice between 12 and 20 months of age (36). Quantitative immunohistochemical analysis of B-cell isotypes, following intraduodenal immunization of young, mature, and old rats with cholera toxin, revealed significant age-related reductions in the numbers of IgA^+ (>60%) and cholera toxin-positive (>50%), ie, antibody-containing, cells in the intestinal lamina propria (31) (Figure 3). In cholera toxin-immunized mice, Green-Johnson et al reported that the number of antibody-containing cells in the intestinal wall was significantly lower in old than in young animals (32). In contrast, there is no evidence for an age-related loss of antibody-synthesizing cells in the mesenteric lymph nodes of mice immunized orally with trinitrophenylated bovine γ globulin, or of rats immunized intraduodenally with cholera toxin (31, 49).

In the only relevant human study to date, Arranz et al reported that there were twice the number of IgA^+ cells in the intestinal lamina propria of elderly versus young subjects (24). These data conflict with the majority of those obtained in rodents. In a study from our laboratory, flow cytometry was used to quantify cholera toxin-positive and IgA^+ cells in the peripheral blood of nonimmune and cholera toxin-immunized rhesus macaques (25). It was assumed that the relative numbers of these two cell phenotypes would serve as an index of antigen-stimulated IgA immunoblast migration. The work showed that both populations of mononuclear cells were reduced three

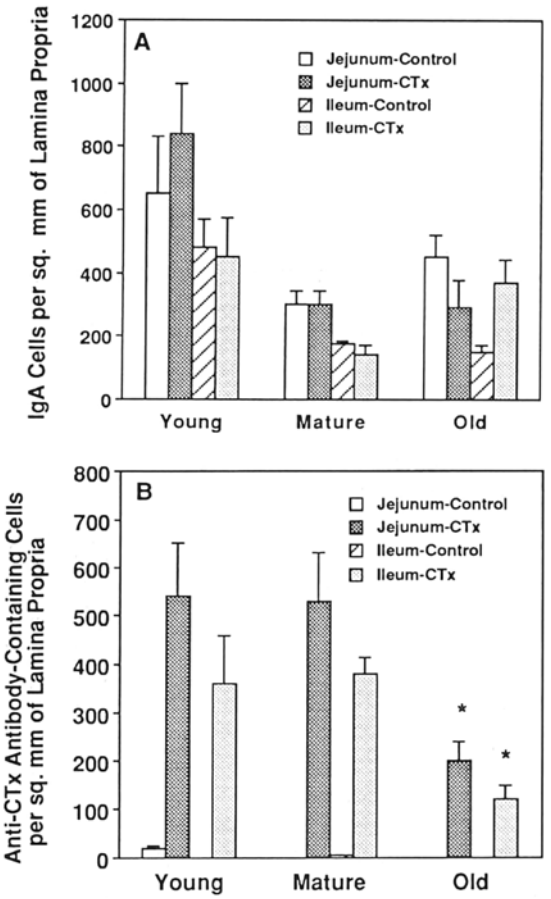

Fig 3. Numbers of IgA^+ (A) and anti-cholera toxin antibody-containing cells (B) in the lamina propria of the small intestines of young, mature, and old cholera toxin immunized (CTx) and nonimmune (control) rats, measured by quantitative immunohistochemistry. (A) With the exception of the ileums of old cholera toxin-immunized animals, the number of IgA^+ cells in the lamina propria declined significantly as a function of increasing age ($P < 0.01$). (B) No anti-cholera toxin antibody-containing cells were detected in the lamina propria of the jejunum or ileum of the nonimmunized rats regardless of age. Although the response to cholera toxin was similar in young and mature rats, the intestinal lamina propria of old cholera toxin-immunized animals contained significantly fewer antibody-containing cells than that of corresponding young animals ($^*P < 0.01$). The values are expressed as the mean number of cells per square millimeter of lamina propria ± SEM. (Data derived from Ref. 30.)

to fourfold in the blood of immunized old macaques, in comparison to young animals (Figure 4). This observation, coupled with a decline in the number of cholera toxin-positive plasma cells in the intestinal lamina propria, suggests that the homing of IgA immunoblasts to the intestinal wall is compromised in old animals and that this contributes to reductions in antibody-secreting plasma cells, local antibody production, and mucosal immune responsiveness.

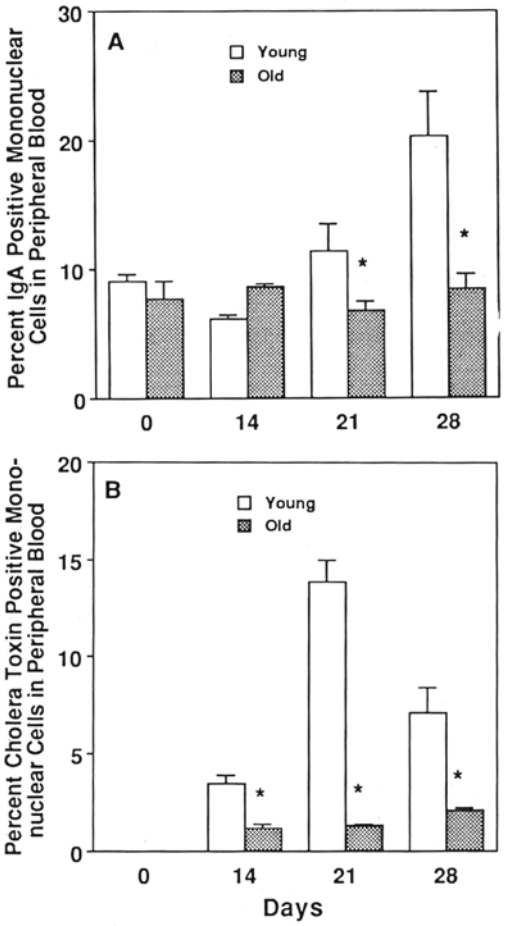

Fig 4. Relative percentages of IgA^+ (A) and cholera toxin-positive (B) cells in the mononuclear cell population in the peripheral blood of young and old rhesus macaques, before (day 0) and after primary immunization (day 14) and boosting (days 21 and 28) with cholera toxin. Blood samples were collected on day 0, the animals were immunized intraduodenally with 500 μg of a mixture of cholera toxin and toxoid on days 0, 14, and 21, and blood samples were collected on days 14, 21, and 28. Mononuclear cells were prepared from heparinized blood by Ficoll density gradient centrifugation, the GM1 ganglioside receptor for cholera toxin was blocked, and the cells were incubated with fluorescein-conjugated cholera toxin plus biotinylated rabbit anti-monkey IgA, followed by streptavidin–phycoerythrin. The relative number of each cell population was then determined by flow cytometry. (A) The number of IgA^+ cells increased significantly in young, but not old macaques, during the course of the study. (B) No cholera toxin-positive cells were detected in the preimmune samples, and the number of cells expressing this surface phenotype was significantly greater in young versus old animals at each subsequent time point. The values represent the mean percentage of cells stained ± SD, for five to six animals ($^*P < 0.01$). (Data derived from Ref. 24.)

EFFECT OF AGE ON LOCAL ANTIBODY PRODUCTION IN THE INTESTINE

The age-associated decrease in titers of intestinal IgA antibody might, theoretically, reflect deficits intrinsic to plasma cells, or alterations in the immediate environment of these cells. Kawanishi and Kiely reported a 40–70% decline in T-cell-dependent immunoglobulin production by Peyer's patch and mesenteric lymph node B cells from old mice in comparison to young or mature animals (34). In contrast, Rivier et al observed an age-related increase in the production of anti-α Dextran B1355 IgA antibodies by mouse mesenteric lymph node cells (50). Daniels et al measured antibody production by lymphocytes isolated from young and old rats following intraduodenal immunization with cholera toxin (51). Five days after primary immunization, antitoxin production by spleen and mesenteric lymph node lymphocytes was greater in the case of young rats than of old animals. Peyer's patch cells from toxin-primed old rats produced significantly more IgA, IgG, and IgM antibodies than did corresponding cells from young animals, suggesting an age-related delay in the egress of antigen-stimulated B lymphocytes from the Peyer's patches. Finally, lymphocytes isolated from the spleens, mesenteric lymph nodes, and Peyer's patches of toxin-boosted rats produced similar or smaller amounts of antibody in the case of young than of old animals. These data suggest that while the intestinal antibody response to intraduodenal cholera toxin is impaired in senescent rats, this impairment does not result from any inability to initiate a response. Rather, the apparent impairment of intestinal antibody production in old animals might reflect: (1) reduced numbers of plasma cells in the lamina propria, (2) suppression of local antibody synthesis, or (3) impairment in epithelial cell transport of antibodies to the intestinal lumen.

The differentiation of IgA^+ B lymphocytes is regulated by cytokines, for example, IL-1 and IL-6, and local IgA production in the intestinal lamina propria appears to be under the regulation of IL-6, $CD4^+$ T-cells, $T_{suppressor/cytotoxic}$ cells, and gastrointestinal neuropeptides, eg, vasoactive intestinal peptide (VIP) and substance P (32, 52–56; see refs. 57 and 58 for reviews). A recent review contains the suggestion that serum IL-6 levels rise with increasing age, as part of an "inflammatory response" (59). Conceivably, numerical reduction in IgA plasma cells in the intestinal lamina propria of old animals might reflect impaired B-lymphocyte differentiation consequent to reduced sensitivity of these cells to IL-6 (53). Alternatively, or in addition, the increased population of $T_{suppressor/cytotoxic}$ lymphocytes ($OX8^+$) in the intestinal lamina propria of old rats might impair terminal differentiation of B lymphocytes or suppress local antibody production (42).

EFFECT OF AGE ON EPITHELIAL CELL TRANSPORT OF IgA TO INTESTINAL LUMEN

Polymeric immunoglobulin A and M (pIgA, pIgM) antibodies produced by plasma cells in the small intestinal lamina propria are transported from the basal to the luminal surface of intestinal enterocytes by a receptor-mediated vesicular translocation mechanism (see ref. 60 for a review). Practically all mucosal epithelial cells, including salivary and respiratory epithelial cells, express the polymeric immunoglobulin receptor (pIgR) on their basolateral surfaces. Furthermore, in several mammalian species, including rats, mice, and rabbits, pIgR is expressed on the sinusoidal surfaces of hepatocytes and is responsible for transporting pIgA from the blood to the bile. The hepatobiliary pathway is a major route for the secretion of IgA antibodies into the intestinal lumen in these species, but seems to be relatively unimportant in humans (61). However, since the expression of pIgR in hepatocytes is highly regulated, and the hepatocellular transport of pIgA is identical to that in enterocytes, the hepatocyte can serve as a model for pIgR-dependent pIgA transport (62, 63). The pIgR is synthesized on the rough-surfaced endoplasmic reticulum, where its molecular mass is 105 kDa, and is translocated to the Golgi where it undergoes terminal glycosylation to the transmembrane form (116 kDa). After phosphorylation of the serine at position 664 in the tail region (120 kDa), and binding of IgA, the pIgR–pIgA complex is internalized via endocytic vesicles and transcytosed to the bile canalicular membrane (64–66). The pIgA binding portion of the pIgR is cleaved, and the resulting molecular complex (pIgA and the binding domain of the pIgR) is secreted as secretory IgA (67, 68) (Figure 2B).

Much of the research on the effects of aging on pIgR expression and pIgA transport in the liver and intestine has been performed in the authors' laboratories. Approximately 10 years ago, we observed a four to sixfold decline in the transport of pIgA from blood to bile in rats between the ages of 3 and 25 months (69). A quantitative autoradiographic analysis of the distribution of radiolabeled pIgA in the hepatocytes suggested that the vesicular translocation of the ligand–receptor complex from the sinusoidal membrane to the bile canaliculus was impaired in old animals. The vectorial movement of pIgA-containing vesicles, as well as the transport of nascent pIgR to the canalicular membrane, is dependent on the integrity of the microtubule system (70). Recent work from our laboratory demonstrated a 70% decline in the concentration of polymerized α and β tubulin by 12 months of age, and a 50% loss of total tubulin by 24 months, in rat liver (71). These shifts in critical cytoskeletal elements raise the possibilities that there is a decrease in the number or length of microtubules and that the microtubule-dependent translocation of pIgA is compromised during aging. We are unaware of any data concerning the effect of aging on the vesicular translocation of pIgA in small intestinal enterocytes.

Studies using ligand binding assays and Scatchard analysis demonstrated that the number of hepatic pIgA receptors declined three to fourfold between 3 and 25 months of age in rats, whereas the binding affinity remained unchanged (72). This age-related loss of hepatic receptor expression has been demonstrated *in vivo* and *in vitro* (72, 73). Compared to hepatocytes isolated from young rats, the binding and uptake of ^{125}I-labeled pIgA by cultured hepatocytes from old animals was found to be reduced approximately 2.5-fold (73). The pIgR mRNA steady-state level declines only 20% during the same age span and, thus, does not correlate with the three to fourfold loss of receptors from the hepatocyte surface (74). *In vitro* studies using cultured rat hepatocytes revealed an age-dependent lag in the incorporation of [^{35}S]cysteine into newly synthesized pIgR, suggesting that aging may affect this receptor posttranscriptionally (73). Furthermore, there is evidence for an age-related decline in the secretion of pIgR by cultured rat hepatocytes (73). In summary, the age-related decline in the hepatobiliary transport of pIgA in rats may reflect diminished synthesis of pIgR molecules and impaired intracellular translocation of the receptor–ligand complex.

Several years ago, work in our laboratory demonstrated that human and rhesus macaque hepatocytes do not express pIgR on their surfaces; this finding supports the concept that hepatobiliary secretion of pIgA is minimal or nonexistent in primates (61). Very little is known about the possible effects of aging on pIgR expression by small intestinal enterocytes. Basolateral plasma membranes of rat enterocytes bind pIgA, with binding characteristics identical to those of the rat liver pIgR (75). Membranes from rat small intestinal crypt cells exhibit greater pIgA binding (320 fmol/mg protein) than membranes isolated from villus tip enterocytes (105 fmol). This crypt-to-villus tip gradient of pIgA binding is identical in young and old rats, and a similar pattern is seen in rhesus macaques (25, 76). The fact that pIgR functional activity in the small intestinal epithelium correlates with cellular

rather than donor age suggests that diminished intestinal antibody titers in old animals reflect a deficit proximal to the terminal steps of the intestinal immune response, ie, prior to pIgR–pIgA transport and secretion.

CONCLUSIONS

Currently available data suggest that the intestinal immune response is compromised in old animals and elderly humans. Information concerning the possible effects of aging on the initial steps in this response, ie, antigen uptake and presentation, is nonexistent. There is some evidence of shifts in the relative distributions of certain GALT B- and T-lymphocyte subpopulations during aging in rodents. Furthermore, the relative abundance of certain lymphocyte subsets, for example, $T_{suppressor/cytotoxic}$ lymphocytes, or precursors of IgA plasma cells may influence subsequent steps, such as the production of intestinal antibody. Although a marked decline in the expression of the pIgR contributes to age-related reduction in transport of pIgA across rat hepatocytes, the absence of a similar correlation between donor age and receptor expression in small intestinal enterocytes suggests that this deficit is hepatocyte-specific. Intestinal antibody production may be sensitive to age-related shifts in T-cell distribution or to changes in the levels of (or plasma cell sensitivity to) critical cytokines (6). Future studies may help to answer the question of whether aging influences the intestinal mucosal immune response independently of any age-related perturbation of systemic immunity.

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