Human Intestinal IgA Response Is Generated in the Organized Gut-Associated Lymphoid Tissue but Not in the Lamina Propria

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Background & Aims: **The intestinal lamina propria has traditionally been viewed as the effector site of mucosal immune responses. However, this view has been challenged with the identification, in the murine lamina propria, of an in situ class switch DNA recombination pathway to IgA. In this study, we tested the hypothesis that in situ class switching occurs in the human lamina propria.** ***Methods:*** **Immunohistochemistry was used to analyze tissue microenvironments and RT-PCR to look for molecular evidence of Ig class switching and to track clonally related cells of B lineage.** ***Results:*** **We found no evidence of proliferation of either lamina propria $CD20^+$ or $CD19^+$ cells or evidence of activation-induced cytidine deaminase mRNA expression outside the organized gut-associated lymphoid tissue, although Iα-Cα immunoglobulin germ-line gene transcript expression could be identified in the lamina propria. We identified clonally related cells, including IgA and IgM isotype-switched variants, in multiple samples known to be free of activation-induced cytidine deaminase, organized lymphoid tissue, or cellular proliferation. For 4 groups of cells, the patterns of somatic mutations on the rearranged IgV_H5 gene segment were more similar between cells from distant sites than from their immediate neighbors, implying dissemination of cells from a common set of precursors.** ***Conclusions:*** **Our data are inconsistent with a model in which precursors of human IgA-secreting plasma cells are induced or expanded in the lamina propria. The human lamina propria is therefore likely to solely be an effector site of intestinal secretory IgA responses that originate from the organized gut-associated lymphoid tissues.**

The secretory immunoglobulin A (S-IgA) antibody response provides an important line of defense against mucosal pathogens. Its main extracellular functions are considered to be agglutination and immune exclusion, although it may also have an intracellular anti-inflammatory action during transport through intestinal epithelial cells.[1,2]

In mice, there are 2 precursor populations of intestinal plasma cells; bone marrow–derived B-2 cells from Peyer's Patches (PP) and the self-replenishing B-1 cells, mainly located in the peritoneal and pleural cavities.[3,4] Three years ago, Fagarasan et al[5] proposed that both the organized GALT and the intestinal lamina propria (i-LP) were sites of generation and diversification of plasma cell precursors.[5] They proposed that, with the help of LP stromal cells and dendritic cells that have the capacity to sample antigen across the epithelial barrier,[6] B cells (presumably B1 cells) would respond and class switch to IgA in situ, independently of T cells. A recent study did not confirm these findings; Shikina et al[7] found that IgA-class switch recombination (CSR) molecules were selectively detected in organized mucosa-associated lymphoid structures but not in the diffuse i-LP.[7] It is important to note that murine and human mucosal B-cell systems are not the same. Humans do not have a peritoneal B-cell compartment that is likely to function as a significant precursor of intestinal plasma cells.[8,9] So far, only the PP have been identified as precursors of human IgA plasma cells by the detection of clonally related cells in the germinal center (GC) of PP and adjacent i-LP by analysis of Ig gene sequences.[10] Clonally related human plasma cells can be widely disseminated, presumably

Abbreviations used in this paper: AID, activation-induced cytidine deaminase; CSR, class switch recombination; GALT, gut-associated lymphoid tissue; GC, germinal center; GLT, immunoglobulin germ-line gene transcripts; H-CDR3, third complementarity-determining region of the immunoglobulin heavy chain; I, intervening exon; IgV_HDJ_H-C_H, Ig heavy chain transcript; ILF, isolated lymphoid follicle; i-LP, intestinal lamina propria; PP, Peyer's patch(es).

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dispersed via the blood and returned to the mucosa by homing receptors and chemokines.[11,12]

There is evidence to support the concept that human plasma cell precursors can diversify by somatic hypermutation and class switching of their Ig genes in mucosal microenvironments outside organized GALT. Clonally related plasmablasts/plasma cells, including Ig isotype-switched variants, in some cases have been identified within small samples of mucosa from colon, ileum and duodenum/jejunum,[13] salivary gland,[14] lung,[15] and nasal mucosa.[16] Activation-induced cytidine deaminase (AID) messenger RNA (mRNA) expression has been identified in nasal mucosa, although the precise microenvironment from which the AID expressing cells were derived was not studied.[16] Therefore, we sought evidence that the human IgA response is generated and diversified in the i-LP, using an immunohistochemical and RT-PCR-based analysis of human ileal and colonic mucosa.

If the human plasma cell response is diversified in the i-LP, we would expect to observe expression of Iα-Cα immunoglobulin germ-line gene transcripts (GLT) and AID mRNA in intestine in the absence of organized lymphoid tissue since CSR is absolutely dependent on AID expression.[17] We would also expect to see evidence of proliferation of cells of B lineage in i-LP because CSR is dependent on cell proliferation[18] and also because the local accumulation of related cells as observed in human tissues would require local cell division. We therefore investigated these factors in detail in tissue sections of human colon and ileum. We also investigated immunoglobulin gene sequences from clonally related cells in the context of AID expression and local cellular proliferation. To observe related cells in an overall polyclonal background,[19,20] we used primers specific for the small family IgV_H5, which has only 2 functional members: IGHV5-51 and IGHV5-a.[21]

In this study, AID expression was restricted to organized gut-associated lymphoid tissue (GALT). Proliferating B cells were restricted to the GC and marginal zones of organized GALT. We identified related plasma cells/plasmablasts in lamina propria from local and distant sites. Examples of cells that were more closely related to cells from distant sites than their immediate neighbors were observed implying dissemination from a distant common set of precursors.

Materials and Methods

Human Intestinal Tissues

Details of patients and blocks of intestinal mucosa used are summarized in Table 1. All tissue samples were taken from uninvolved areas of resected tissues at least 10 cm from visible tumor, and all were confirmed as histologically normal. Seven duodenal biopsy specimens were also used. They were taken as

Table 1. Ileal and Colonic Specimens From 29 Patients Undergoing Surgical Resection

	Age (*y*)	Sex	Surgical procedure	Diagnosis	Site(s) studied	Tissue processing
Case 1	78	Female	Right hemicolectomy	Duke's stage B adenocarcinoma of the ascending colon	1 block of ileal mucosa 1 block of colonic mucosa	C[a]/I[b]/R[c] C/I/R
Case 2	73	Male	Partial resection of the sigmoid colon	Duke's stage B adenocarcinoma	3 blocks of sigmoid colonic mucosa (located 5 cm apart)	C/I/R
Case 3	63	Male	Right hemicolectomy	Duke's stage C1 adenocarcinoma of the caecum	1 block of colonic mucosa	C/I/R
Case 4	74	Female	Right hemicolectomy	Duke's stage B adenocarcinoma in ascending colon		S[d]
Case 5	69	Male	Abdominoperineal resection	Duke's stage B rectal adenocarcinoma	Colonic tissue samples	S
Case 6	79	Male	Total colectomy	Synchronous Duke's stage B caecal and Duke's stage C1 sigmoid adenocarcinomas		S
Case 7–19	Unknown	Unknown	Colectomy		1 block of colonic mucosa from each case	C/I
Case 20–29	Unknown		Right hemicolectomy		1 block of ileal mucosa from each case	C/I

NOTE. Samples used for the preparation of [a]cryostat sections for [b]immunohistochemistry and [c]RT-PCR analysis.
[d]Samples used for the immunomagnetic isolation of i-LP $CD138^+$ cells from single mononuclear cell suspensions.