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Generation of Gut-Homing IgA-Secreting B Cells by Intestinal Dendritic Cells

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Normal intestinal mucosa contains abundant immunoglobulin A (IgA–secreting) cells, which are generated from B cells in gut-associated lymphoid tissues (GALT). We show that dendritic cells (DC) from GALT induce T cell–independent expression of IgA and gut-homing receptors on B cells. GALT–DC–derived retinoic acid (RA) alone conferred gut tropism but could not promote IgA secretion. However, RA potently synergized with GALT–DC–derived interleukin-6 (IL-6) or IL-5 to induce IgA secretion. Consequently, mice deficient in the RA precursor vitamin A lacked IgA–secreting cells in the small intestine. Thus, GALT–DC shape mucosal immunity by modulating B cell migration and effector activity through synergistically acting mediators.

The gut harbors the large majority of IgA–secreting cells found in the body. After activation of naive B cells by the B cell receptor (BCR), newly generated IgA-producing cells leave the GALT, enter the blood, and home to the intestinal lamina propria, where they are required for optimal protection against intestinal pathogens (1, 2). However, BCR stimulation in nongut mucosal tissues produces few gut-homing IgA–secreting cells, which suggests that specific mucosa-associated differentiation signals induce both class switching to IgA and targeting of the ensuing effector B cells to the gut. Previous studies have shown that gut-homing T cells acquire their tissue tropism in response to imprinting signals from GALT–DC (3, 4). Moreover, recent findings indicate that DC can present unprocessed antigens to B cells (5, 12) and influence the differentiation and survival of antibody-secreting cells (ASC) (7). Here, we asked whether DC might contribute to the acquisition of tissue-specific functional properties of B cells, particularly their migration and/or effector activity in the gut.

Lymphocytes that infiltrate the gut mucosa display a distinct set of adhesion molecules (2). In particular, gut-tropic T cells and ASC express the integrin α4β7 and the chemokine receptor CCR9, which are essential for lymphocyte migration to the small intestine (2). GALT–DC selectively induce these traffic molecules in activated T cells (3, 4) because GALT–DC, unlike DC from other lymphoid tissues, synthesize RA. The presence of this vitamin A metabolite is sufficient to induce gut-homing receptors on activated T cells, even in the absence of GALT–DC (8).

Given the impact of GALT–DC–derived RA on cells, we hypothesized that this mechanism might also target B cells to the gut. Indeed, addition of DC from Peyer’s patches (PP–DC) or RA to activated murine spleen B cells induced high levels of α4β7 and maintained robust CCR9 expression on B cells, whereas pooled DC from inguinal, axillary, and brachial peripheral lymph nodes (PLN–DC) or RA-free media induced α4β7lowCCR9low B cells (Fig. 1, A to D, and figs. S1 and S2). Using optimized culture conditions (5), we generated two populations of CCR9high cells that were then performed in vivo homing experiments. As expected, RA substantially boosted B cell migration to the small bowel (Fig. 1, E and F).

Next, we asked whether human GALT–DC operate similarly. Naive (CD45+CD27+) and antigen-experienced (CD45+CD27−) human spleen B cells were activated with antibody to IgM together with autologous DC from human spleens, livers, or mesenteric lymph nodes (MLN–DC). Similar to the murine system, autologous DC also induced the proliferation of viable human B cells (figs. S3, C and D, and fig. S4D), and activated B cells that were exposed to liver- or spleen-derived DC expressed much less α4β7 and CCR9 than B cells that were activated together with MLN–DC or liver/spleen–DC plus RA (Fig. 2, A and B). Imprinting of gut tropism by MLN–DC depended on RA, because α4β7 and CCR9 induction was abrogated by LE540, which blocks the RAR family of RA receptors (9). Thus, the cellular and molecular mechanisms that imprint gut-homing B cells are conserved across species.

Gut tropism was not acquired only by naive human B cells. CD27−, antigen-experienced B cells, initially α4β7lowCCR9low, also became α4β7highCCR9high upon activation in the presence of RA or MLN–DC (Fig. 2, A and B), suggesting that memory B cells retain migratory plasticity, analogously to memory T cells (10, 11). To explore this more rigorously, we stimulated B cells with or without RA and then restimulated under reversed imprinting conditions. B cells that were activated without RA expressed few gut-homing receptors but became α4β7highCCR9high upon restimulation with RA (Fig. 2, C and D). Conversely, B cells first exposed to RA and then restimulated without RA down-regulated CCR9 but remained α4β7high (Fig. 2E and SOM Text 2). Thus, effector B cells adjust their homing commitment to changing microenvironmental conditions. Accordingly, in repeatedly immunized humans the ASC homing phenotype depends on the route of antigen entry during the most recent immunization, irrespective of previous immunizations (12).

In vivo, human and marine IgA–ASC express the chemokine receptor CCR10, which has been implicated in ASC migration to mucosal tissues (13). However, neither RA nor PP–DC induced B cell responsiveness to CCR10 agonists, even though B cells migrated toward the CCR9 ligand CCL25/TECK (fig. S1B and SOM Text 3). Having established that GALT–DC–derived RA induces gut-tropic B cells, we asked whether GALT–DC also induce IgA secretion, the hallmark effector activity of mucosal B cells. Indeed, activated B cells cultured with PP–DC secreted much more IgA than B cells activated without DC or with PLN–DC (Fig. 3A and fig. S5). This activity required only PP–DC but not T cell help or other environmental factors. Previous studies had reported induction of IgA secretion by B cells cocultured with PP–DC, but only in the presence of T cells (14). However, T cell–independent IgA induction has been observed in vivo (15), but the factors that induce IgA–ASC in this setting were not identified.

Because only PP–DC, but not PLN–DC, induced IgA–ASC, we asked whether this tissue-specific difference was due to differential cytokine production. In particular, IL-6 induces IgA responses in vivo and in vitro (14, 16). However, adding antibody to IL-6 to B cell/PP–DC cocultures had only a modest, nonsignificant effect on IgA production (Fig. 3B). IL-5 is also implicated in IgA responses (17), but antibody to IL-5 did not decrease PP–DC–driven IgA production (Fig. 3B).

Because no single candidate cytokine was essential for PP–DC–induced IgA–ASC generation, we hypothesized that RA might be involved. This prediction seemed reasonable because the addition of RA to LPS–activated splenocytes promotes IgA secretion (18). Moreover, vitamin A deficiency impairs intestinal IgA responses, and vitamin A supplementation restores IgA levels in malnourished mice (19, 20). Indeed, RA receptor blockade by LE540 decreased IgA levels in B cell cocultures with PP–DC (Fig. 3B). Concomitant inhibition of both RA and IL-6 had an additive effect, suggesting that these agents cooperate for optimal IgA induction. These observations were reproduced with V110/Yen B cells expressing a switchable transgenic vesicular stomatitis virus (VSV)–specific BCR; exposure of bona fide naive V110/Yen B cells to ultraviolet-
**Fig. 1.** GALT-DC and RA imprint gut tropism in mouse B cells. Naive B cells were activated with antibody to IgM in the presence of PP-DC or PLN-DC (A and B) or D1-DC with or without RA (C and D). After 4 days, B cells were analyzed for (A) and (C) α4β7 and (B) and (D) CCR9 expression. Nonactivated naive B cells are shown for comparison. MFI, mean fluorescence intensity. ***, P < 0.01; ****, P < 0.001; n = 6 to 8. (E and F) Activated CD45.2⁺ B cells were incubated with or without (control) RA plus D1-DC and labeled with green (carboxyfluorescein-succinimidyl-ester) and red (chloromethyl-benzoyl-aminotetramethylrhodamine) fluorophores, respectively, and mixed and injected into CD45.1⁺ congenic mice. After 18 hours, the homing index (ratio of CFSE⁺/CMTMR⁺ DC45.2⁺ cells in each tissue, corrected for input ratio) was determined. (E) Representative fluorescence-activated cell sorting (FACS) plots illustrate that RA-treated B cells, but not control cells, accumulated in the small intestine. (F) Homing indices in recipient tissues. BM, bone marrow; MLN, mesenteric lymph node. *, P < 0.05; **, P < 0.01 versus homing index = 1; n = 4. Error bars, mean ± SEM.

**Fig. 2.** Gut-homing receptor regulation on human B cells. Human spleen–derived B cells (95% CD19⁺) from six donors were separated into CD27⁻ (naive) and CD27⁺ (antigen-experienced) subsets. (A and B) Cells were stimulated with antibody to IgM in the presence of liver-DC or spleen-DC with or without RA (100 nM), or with MLN-DC with or without LE540 (1 μM). After 7 days, CD19⁺ cells were analyzed for (A) α4β7 and (B) CCR9 expression. ***, P < 0.001 versus naive B cells or B cells plus liver/spleen-DC; #, P < 0.001 versus MLN-DC. (C and D) Naive B cells from three donors were cultured with spleen-DC and antibody to IgM with or without RA (1st stimulation). On day 7, B cells were analyzed for (C) α4β7 and (D) CCR9, reactivated with or without RA or LE540 (2nd stimulation), and reanalyzed on day 14. ***, P < 0.001 versus concomitant α4β7⁺ or CCR9⁺⁺ sample; #, P < 0.001 versus spleen-DC. Error bars, mean ± SEM.
inactivated VSV plus PP-DC elicited notable IgA secretion (fig. 56), indicating that PP-DC induced de novo IgA class switching rather than expansion and/or survival of already committed IgA-ASC.

To determine whether RA plus IL-5 and/or IL-6 are sufficient to induce IgA secretion, we stimulated B cells with or without PLN-DC and different combinations of RA and cytokines (Fig. 3C and fig. S7). Each factor by itself or together with PLN-DC induced negligible IgA production, and combined IL-5 plus IL-6 was also ineffective. However, RA plus IL-5 and/or IL-6 and PLN-DC substantially enhanced IgA production. This synergistic effect of RA plus IL-5/IL-6 with PLN-DC was much more pronounced than the effect of B cell coculture with PP-DC. Indeed, PP-DC–induced IgA production was also boosted by RA plus IL-5/IL-6 (Fig. 3D and SOM Text 4). Given that IL-5 and IL-6 synergized with RA but not with each other, their effect on IgA secretion might require a shared signaling pathway, possibly involving JAK2 and STAT3 (21, 22). Although this apparent redundancy of IL-5 and IL-6 (and possibly other cytokines) awaits further in vivo exploration, this effect could explain why genetic deficiency in IL-6 does not abrogate antigen-specific IgA responses (23). Transforming growth factor–β (TGF-β) has also been implicated in IgA switching/secretion (24, 25), but TGF-β1 did not induce IgA secretion in this setting, even in combination with RA and/or IL-5/IL-6 (fig. S8 and SOM Text 5).

Interestingly, RA plus IL-5/IL-6 did not induce IgA-ASC in the absence of DC, which suggests that DC contribute additional essential factor(s). This effect was observed not only with murine DC1-DC, PP-DC, and PLN-DC but also with human DC from liver, spleen, and MLN (Fig. 3E, fig. S7, and SOM Text 6). Human MLN-DC, but not liver-DC or spleen-DC also possessed a potent intrinsic capacity to promote human IgA-ASC (Fig. 3E). Human MLN-DC–driven IgA secretion depended on DC–derived RA and IL-6, and supplementation of RA plus IL-6 to human B cells augmented IgA secretion in cultures containing liver-DC, but not in the absence of DC.

Having established that GALT-DC–derived RA is essential to induce gut-homing IgA-ASC in vitro, we investigated the physiological role of RA using vitamin A–deficient mice, in which GALT-DC lack the prerequisite substrate for RA production (8). Compared with control mice, most secondary lymphoid organs in vitamin A–deficient animals contained fewer α4β7– antigen-experienced (i.e., B220–/IgD+) B cells (Fig. 4A). By contrast, the frequency of all B220+/IgD– cells (which include memory B cells) in vitamin A–deficient mice was decreased only in Peyer’s patches but was normal or increased.

![Figure 3](https://www.sciencemag.org)
in other lymphoid organs (Fig. 4B). Thus, vitamin A deficiency does not compromise antigen-experienced B cell numbers per se but affects preferentially the intestinal effector/memory repertoire. Indeed, as reported previously in rats (26), IgA+ cells were substantially reduced in the lamina propria of vitamin A−/− mice (Fig. 4, C and D). Vitamin A deficiency also affected IgA−cell frequencies in GALT and spleen (figs. S9 and S10), suggesting that the paucity of intestinal IgA-ASC was caused not only by the inability of GALT-resident B cells to acquire gut-homing receptors but also by defective differentiation of IgA-ASC in lymphoid tissues.

Despite the lack of intestinal IgA-ASC, vitamin A−/− mice had normal serum IgA levels (Fig. 4E), indicating that IgA-ASC do not obligatorily depend on RA. Extraintestinal IgA-ASC express traffic molecules distinct from gut-homing receptors (2), whose induction is probably RA-independent.

We conclude that GALT-DC induce gut tropism and IgA secretion in B cells by overlapping, yet distinct, mechanisms. The characteristic ability of GALT-DC to synthesize RA is sufficient to imprint gut tropism. However, to generate IgA-ASC, RA must synergize with cytokines produced by the DC and/or other cells. Although there are probably alternative mechanisms generating extraintestinal IgA-ASC, RA appears critical for IgA-ASC to populate the small bowel. The clinical relevance of this tissue-restricted mechanism is underscored by the observation that vitamin A supplementation decreases the incidence and severity of diarrhea in malnourished children (27), resulting in mortality reduction by as much as 34% (28).

References and Notes


30. A. Sommer et al., Lancet 327, 1169 (1986).
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References
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