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*J Immunol* 2015; 194:606-614; Prepublished online 5 December 2014;
doi: 10.4049/jimmunol.1402383
http://www.jimmunol.org/content/194/2/606

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**Supplementary Material**
http://www.jimmunol.org/content/suppl/2014/12/05/jimmunol.1402383.DCSupplemental

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Natural Anti-Intestinal Goblet Cell Autoantibody Production from Marginal Zone B Cells

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Expression of a germline V_{H}3609/D/J_{H}2 IgH in mice results in the generation of B1 B cells with anti-thymocyte/Thy-1 glycoprotein autoantibody by coexpression of V_{L}21-5/J_{L}2 L chain leading to production of serum IgM natural autoantibody. In these same mice, the marginal zone (MZ) B cell subset in spleen shows biased usage of a set of Ig L chains different from B1 B cells, with 30% having an identical V_{L}19-17/J_{L}1 L chain rearrangement. This V_{H}3609/V_{L}19-17 IgM is reactive with intestinal goblet cell granules, binding to the intact large polyanalyte form of mucin 2 glycoprotein secreted by goblet cells. Analysis of a μκ B cell AgR (BCR) transgenic (Tg) mouse with this anti-goblet cell/mucin2 autoreactive (AGcA) specificity demonstrates that immature B cells expressing the Tg BCR become MZ B cells in spleen by T cell–independent BCR signaling. These Tg B cells produce AGcA as the predominant serum IgM, but without enteropathy. Without the transgene, AGcA autoreactivity is low but detectable in the serum of BALB/c and C.B17 mice, and this autoantibody is specifically produced by the MZ B cell subset. Thus, our findings reveal that AGcA is a natural autoantibody associated with MZ B cells. The Journal of Immunology, 2015, 194: 606–614.
Materials and Methods

Mice

The VH3609/DH72 targeted insertion (knock-in) mouse line (V13609h) was made by homologous recombination in ES cells as previously reported (19), with a slight modification to the targeting construct, by extending the short (right side) homology arm from 0.8 kb to 2.5 kb, transfecting the R1 ES line (129Sv × 129/Sv-CF1 origin). The selected NeoR gene was eliminated by crossing the knock-in founder to CMV-Cre-Tg.BALB mice. V13609h transgenic (Tg) mice have been described previously (9). To establish V13609h/V19-17 μkTG mouse lines, we cloned a V19-17/Jk1 rearranged k L chain gene from a V13609h* MZ B cell line generated by hybridoma, P16-8F5, by long-distance PCR. V19-17 is identical to GenBank MUSGLAFE. Generation of μkTG mouse lines was carried out as previously described (20), by injection into (C3H × C57Bl/6)F1 eggs. One μkTG founder line, EP67, was used in this study as representative of six anti-globet cell/mucin2 autoantibotic (AGA-μk) mice (all showed increased MZ B cell generation and IgM production in serum). The V13609h/V19-17 μk Tg (EP67 μkTG) mouse line and the V13609h mouse line were backcrossed to C.B17 mice more than six generations before use in experiments. Btk mutant Xid and Rag-1 knockout mice were both on a BALB/C background. Muc2 and Muc1 knockout mice were on a C57BL/6 background. C.B17, BALB/cAnN, C57BL/6 ICR, and C.B17 SCID were bred and maintained in our laboratory animal facility. Germ-free (Swiss Webster) mice and Sprague Dawley rats were purchased from Taconic. All animal experiments were conducted under a protocol approved by the Fox Chase Cancer Center Institutional Animal Care and Use Committee.

Flow cytometry analysis and reagents

Multicolor flow cytometry analysis, sorting, and mAb reagents have been described previously (21). RA anti-mouse IgG1 Abs used in this study were made as previously described, by using SM6C10 IgM (V13609h/V19-25) as immunizing Ag (20). The anti-V13609h* IgMab (EP67) is P9-19A4, a rat IgG2a. The P9-13H8 Ab (IgG1) is P9-10C7, a rat IgG2a. The P9-13H8 Ab 13H8k, a rat IgG1, reacts with Vk19-17. The P9-19A4 anti-thymocyte/thy-1 glycoprotein autoantibodies (ATA) idotype Ab (20) does not react with VH3609/Vk19-17. Single-cell Igk chain sequencing

For V13609h Tg mouse data in Supplemental Table I, cDNA was prepared and amplified from individual sorted B cells, MZ B cells (CD21CD23 AA4 V13609h) and follicular (FO) B cells (CD21-CD23AA4 V13609h) from spleen and immature B cells (AA4CD21CD23) cells from bone marrow (BM) and spleen, for K gene sequencing by nested PCR as described previously (9). The V1k-Ck PCR product was cloned using TA vector (Invitrogen, Carlsbad, CA); then individual plasmid clones were made as previously described, by using SM6C10 IgM (V13609h/V19-25) as immunizing Ag (20). The second-round PCR fragments were purified and then sequenced using a nested C primer. Electrophoresis and Western blotting

Isolation of colonic crypts

After opening the colon longitudinally and washing in Dulbecco’s PBS without Ca2+ and Mg2+, specimens were incubated with 0.04% sodium hypochlorite for 15 min at room temperature, then incubated with 3 mM EDTA for 90 min at room temperature, with moderate shaking throughout the procedure. Then samples were vigorously shaken for 2 min, removing stromal materials, and colonic crypts/mucus granules were pelleted by centrifugation at 480 g for 3 min at 4°C.

Electrophoresis and Western blotting

Cross-linking of Ab and immunoprecipitation

RagKO mouse colonic crypts were incubated with MK19 or MK21 IgM for 1.5 h at 37°C, washed with PBS, then incubated with PBS containing 1 mM cDNP cross-linker, diobisuccinimidyl propionate (DSPI Dermo Scientific), for 30 min at 37°C, and finally quenched by adding 20 mM Tris- HCl for 5 min. For immunoprecipitation, cells were lysed in NP-40 lysis buffer, and soluble lysate was incubated with rat anti-mouse IgM Ab (331.12) bound protein G-Dynabeads (Invitrogen) for 1 h. Beads were washed four times in 0.5% NP-40 lysis buffer. Bound material was eluted with 1% NP-40 lysis buffer
containing Laemmli buffer with 2-ME, boiled for 5 min; then supernatant was applied to SDS-PAGE, for IgM, Muc2, and DBA lectin blotting.

**Experimental colitis**

Mice were treated by oral administration of 4% dextran sulfate sodium (DSS; 36–50 kDa molecular mass; MP Biomedicals, Solon, OH) in drinking water for 5 d, which was thereafter replaced by normal distilled water. Examination of the intestine was carried out 3 (the declined body weight), 8 (recovered body weight), and 14 d after cessation of DSS water.

**Results**

**BCR associated with the MZ B cell subset**

Expression in mice of a germline VH3609/D/JH2 μ H chain as a transgene (VH3609m Tg) promotes generation of B1 B cells with anti-Thy-1 autoreactivity (9). ATA recognizes highly glycosylated Thy-1(CD90) that is predominantly expressed on the membrane of immature thymocytes (24), in both native and also dying cells. ATA IgM is secreted into serum as a natural autoantibody by B1 B cells (9, 20). This ATA BCR uses a Vk21-5/Jk2 κ L chain, generated by rearrangement of the Ig L chain locus (9). In targeted VH3609/D/JH2 insertion mice (knock-in, VH3609h), as shown in Fig. 1A, among CD5+ B cells (B1a) in the peritoneal cavity, VH3609h B cells expressing the engineered IgH are present (VH3609idh) with a large proportion being 13H8khi+. This anti-κ Ab recognizing limited set of κ L chains including Vk21-5 and Vk19-17 (“13H8k”), which we developed by immunization with ATA IgM, reacts with a restricted set of mouse κ L chains at various levels, including Vk21-5/Jk2 κ. Vk21-5/Jk2 κ expression in 13H8khi+ B1a B cells, as ATA B cells, was confirmed by single-cell sequence analysis (31/31 = 100%). In contrast, the majority of CD5- B220hi B cells (B2) in the peritoneal cavity show less staining by 13H8k than ATA B cells (Fig. 1A) and do not express Vh21-5/Jk2 κ.

In the spleen of the same mouse, an alteration of the 13H8k binding pattern diversity is seen in VH3609idh B cells from the transitional immature stages (CD21hiCD23lo) that can progress to become either mature FO B cells or MZ B cells. In contrast with VH3609idh FO B (13H8kmed/hi and 13H8k), MZ B cells, including CD21hiCD23+ pre-MZ B cells, show predominant 13H8kstimmed cells (arrow in Fig. 1A, Spl B/MZ B). Single-cell sequence data from four individual VH3609t mice showed frequent usage by MZ B cells of an identical κ chain, Vh19-17/Jk1, followed by Vkba9/Jk5 and Vh19-25/Jk1 (Fig. 1B). Further single-cell Igκ sequence analysis using VH3609m Tg mice, comparing VH3609idh MZ B cells (CD21hiCD23−) with immature and FO B cells, is shown in Supplemental Table I; the selected Igκ set associated with MZ B is shown in Fig. 1C. These results revealed that ~30% of MZ B cells have an identical Vh19-17/Jk1 rearrangement (CDR3 region is shown in Fig. 1C), in contrast with much lower and the lowest frequency in FO B and immature B cells, respectively. This Vh19-17/Jk1 Igκ is another L chain strongly recognized by 13H8k.

To assess whether the accumulation of B cells with this BCR in the MZ B subset occurs directly from immature transitional stage cells or a secondary outcome of mature FO B cell activation, we made the μκ Tg mouse line, EP67, expressing this VH3609/Vk19-17 BCR. In EP67 mice, the majority of newly generated immature B cells express Vh3609idh and are 13H8khi+. In contrast, the majority of CD5− B cells express 13H8kmed/hi. This suggests that the 13H8k expression in immature B cells is not due to activation of mature FO B cells, but rather due to a primary process of BCR repertoire selection in immature B cells. These results are consistent with previous reports that show that the majority of MZ B cells in the peritoneal cavity express Vh3609idh and are 13H8khi+. In summary, our results indicate that the accumulation of MZ B cells in the peritoneal cavity is due to a primary process of BCR repertoire selection in immature B cells, which is independent of mature FO B cell activation.
B cells in BM express this Tg BCR. At 3 wk of age, early generated \( \mu \kappa Tg^{\text{CD21-CD24}} \) immature B cells progressed to become CD21hi (and CD1d CD95) in spleen without intermediate generation of FO B cells (Fig. 2A, asterisk), comprising a significant fraction of the CD21hiCD23+ MZ B cell pool (Fig. 2A). In adult (2 mo) animals, \( \mu \kappa Tg^{\text{B}} \) B cells became both MZ B cells and FO B cells when competitor \( \gamma ^{\text{B}} \) B cells were absent as assessed by surface phenotype by flow cytometry analysis, colocalization by immunohistochemistry staining, and expression of MZ B cell–specific genes (Fig. 2B). However, the addition of non-Tg B cells in chimeric BM transferred mice restricted \( \mu \kappa Tg^{\text{B}} \) B cells to predominantly assume a CD21hi MZ B cell fate (Fig. 2C, asterisk). Furthermore, introducing Btk deficiency in the context of \( \mu \kappa Tg^{\text{A}} \) VH3609/Vk19-17 BCR Tg mice. However, Thy-1 reactivity was the first explanation considered for MZ B cell generation in 13H8khi VH3609/Vk19-17 BCR Tg mice. However, anti-goblet cell autoantibody to Muc2 previously was found that Thy-1 expressed at aberrantly low levels that is Thy-1 dependent (20, 21). Therefore, to investigate its specificity, we cotransfected the VH3609 m and Vk21-5 with ATA VH3609 into the SP2/0 hybridoma line to produce secreted IgM, referred to \( \kappa \) Mk19 B cells. When we used ethanol-fixed cryosection panels and cell/tissue/cell line staining and ELISA. Although MK19 binding was below detection in most assays used, including assessment of reactivity to the cytoplasm and nucleus of various tissues (Supplemental Table IIA), it intensely stained in intestinal mucosal cell suspensions. This is in sharp contrast with thymocyte-restricted staining by MK21, an Ab that shares the same IgH (Fig. 3A). When we used ethanol-fixed cryosection staining, MK19 bound to intestinal goblet cells, together with contiguous luminal mucus, with staining distinctively highest in colon, followed by small intestine, but absent in stomach tissue (Fig. 3B, 3C).

The major constituent of intestinal goblet cell granules is mucin, and Muc2 is the predominant secretory mucin found in colon, small intestine, but not stomach (25). Therefore, we hypothesized that \( \kappa \) Mk19, testing for possible autoreactivity associated with MZ B generation, was first assessed by standard procedures, including cell/tissue/cell line staining and ELISA. Although MK19 binding was below detection in most assays used, including assessment of reactivity to the cytoplasm and nucleus of various tissues and cell lines (Supplemental Table IIA), it intensely stained intestinal mucosal cell suspensions. This is in sharp contrast with thymocyte-restricted staining by MK21, an Ab that shares the same IgH (Fig. 3A). When we used ethanol-fixed cryosection staining, MK19 bound to intestinal goblet cells, together with contiguous luminal mucus, with staining distinctively highest in colon, followed by small intestine, but absent in stomach tissue (Fig. 3B, 3C).

In the AGcA \( \mu \kappa Tg \) line EP67, Tg+ B cells (recognized by staining for the Tg IgM\( ^{\text{A}} \) allotype) become plasma cells (PCs) in red pulp (Fig. 5A, PC). Secreted transgene IgM comprises most of the IgM in serum, and this secretion is T cell independent (Fig. 5B). Anti-goblet cell autoreactivity by IgM\( ^{\text{A}} \) in EP67 mouse serum was confirmed by colon staining, in comparison with ATA \( \mu \kappa Tg \) 3369 mouse serum, where thymocyte-reactive IgM\( ^{\text{A}} \) is predominant, produced by B1 B cells (20) (Fig. 5C). Thus, AGcA B cells differentiate into cells secreting autoantibody into serum, as with ATA B cells.

In both mice and humans, the major fetal colonic mucin is Muc2, as in adults (30). Thus, AGcA-reactive granules, predominantly Muc2, are normally present in gut from the fetal/neonatal stage throughout life as an abundant self-antigen. Because of this natural abundance, to assess whether AGcA B cell generation is also occurring in non-Tg normal mice, LPS mitogen-stimulated culture
supernatant from different B cell subsets was tested for AGcA activity. Colon staining analysis showed that IgM(s) with goblet cell reactivity, similar to VH3609/Vk19-17 AGcA, is produced by MZ B cells of BALB/c (and C.B17) mice, but not by B1 or FO B cells (Fig. 5D). Furthermore, AGcA autoantibody was detectable in serum, particularly in BALB/c, followed by CB17 (Table I). In contrast with BALB/c or CB17 strains, the AGcA reactivity was below detection limits in C57BL/6 mice, both in LPS-stimulated B cell subset supernatant (data not shown) and in serum (Table I). However, generation of AGcA MZ B cells and autoantibody production is not blocked in C57BL/6 mice. This was shown in an experiment backcrossing EP67 μTg mice, originally on a CB17 background, for four generations to C57BL/6, whereupon similar AGcA MZ B cell generation and autoantibody production occurred (Supplemental Fig. 1B). Thus, generation of AGcA-autoreactive B cells can occur if the germ-line BCR repertoire is appropriate. In conclusion, AGcA belongs to the class of strain-biased natural autoantibodies that is produced by MZ B cells.

Function of natural AGcA autoantibody

The presence of natural IgM autoantibody has been shown to play a protective role (5–7). However, it also carries a risk to initiate injury in conjunction with complement (31). Because auto-reactivity to goblet cell granules was originally found in children with colitis (17), the question naturally arises whether the presence of AGcA, normally produced as a natural autoantibody, carries a risk for initiating disease. Although AGcA B cells preferentially differentiate to become MZ B cells in the spleen environment, some B cells with AGcA BCRs circulate and are found in intestinal tissue, where the Muc2 autoantigen is present.

In adult EP67 μTg mice, without competing non-Tg B cells, a significant fraction of AGcA B cells become FO B cells in addition to MZ B cells (Fig. 2B). These B cells circulate to form follicles in both peripheral and mesenteric lymph nodes, and AGcA B cells are also found in intestinal tissue, including the colon, forming isolated lymphoid follicles, located nearby epithelial goblet cells that contain the target Ag (Fig. 6A, upper panel). AGcA (IgMα) PCs also appear in the intestinal lamina propria with abundance of AGcA IgM in serum. However, the secreted multimeric IgM does not permeate intact epithelial walls to reach the goblet cells. This is clear from a lack of staining of goblet cell sections by anti-IgMα reagent, unless goblet cells exposed by sectioning are first incubated with AGcA-containing autoserum from an EP67 mouse, as shown in Fig. 6A (lower panel). Thus, even abnormal excessive production of natural

**FIGURE 2.** Btk-dependent MZB cell development by VH3609/Vk19-17 BCR B cells. (A) Spleen B cell FACS analysis of the VH3609/Vk19-17 μTg mouse line, EP67, at 3 wk of age. The CD21hi B cell area is marked by red dotted line. Predominance of CD21lo pre-MZ B and MZ B cells in Tg+ mice (starred) relative to Tg− littermate. (B) FACS analysis of 2 mo adult EP67 spleen B cells. TgμTg+ B cells, the predominant population, comprise 40–50% in spleen, and half become either FO B or MZ B cells. MZ B cell phenotype cells are colocalized in the MZ with SIGNR1+ macrophages (ER-TR9+), and FO B cell phenotype cells are present in B220+ follicles with CD21hi FO DCs (20× objective lens). Quantitative PCR analysis of selected genes was compared in sorted VH3609idid MZ B and FO B cells from EP67 mice (n = 3 each, mean and SE) with wild type C.B17 mouse FO B cells. (C) Mixed cell transfer of adult mouse BM hematopoietic stem cell–enriched fractions using a 1:10 TgμTg EP67/non-Tg ratio. Six weeks after transfer, spleen B cell FACS analysis was performed, gating for VH3609id (Tg−, IgMβ+) cells (9% of total B cells) and VH3609idid (Tg−, IgMβ+) B cells. Representative data of four recipients (n = 4) from two separate transfer experiments are shown. All mice showed predominant MZ B cell generation by the Tg+ B cells. Transfer of EP67 BM alone generated both MZ B and FO B cells, as found in intact adult EP67 mice (data not shown). (D) Analysis of BM AA4+ immature B and spleen B cells in 2-mo-old EP67 μTg mice without or with Xid (Btk mutant) background. In contrast with BM, in spleen there is a reduction of total B cell numbers (2-fold lower in Xid), together with reduction of μTg B cell frequency, and an absence of VH3609/Vk19-17 MZ B cells (red ellipse region) in EP67.Xid mice. Representative data are shown from analyses of four mice.
AGcA IgM autoantibody in the EP67 Tg mice does not result in goblet cell loss, and mucosal pathology is not enhanced over a 15-mo period (data not shown).

To further assess how AGcA plays in mucosal immunity/disease, we treated EP67 Tg mice, in comparison with 3369 Tg mice, with an oral administration of DSS. DSS is toxic to intestinal epithelial cells, causing an epithelial barrier integrity defect. Body-weight decrease occurred 3 d after a 5-d DSS treatment was discontinued, followed by recovery after 3 more days, in both mouse lines. Three to 8 d after ceasing DSS treatment, whereas 3369 mice normally produce natural ATA as the predominant IgM in serum (20), there was a decrease in IgM normally found in the intestine of DSS-treated 3369 mice, including ATA IgM PCs (Fig. 6B, lower panel). In contrast, DSS-treated EP67.CB17 mice showed microvascular dilation with increased red blood infiltration throughout the intestine as occurred during ongoing DSS treatment, together with an increase in AGcA PCs in the lamina propria (Fig. 6B, upper panel). In the colon, there was no sign of accelerated colitis; rather, we observed that most goblet and epithelial cells regenerated 8 d after ceasing DSS treatment. AGcA PCs surround the degenerated epithelial area, in contrast with the intact area in EP67 mouse colon (Fig. 6C, left side versus upper right side), suggesting clearance of damaged goblet cells. Surveying for an additional 2 wk showed that chronic ulcerative colitis (UC) was not induced in DSS-treated EP67.CB17 mice (data not shown). In this DSS colitis model, strain differences are well-known, such that chronic colitis occurs more often in C57BL/6 than BALB/c backgrounds (32). The EP67 Tg BCR on BALB/c IgM allotype-congenic C.B17 mice had abundant serum AGcA and also showed similar resistance to chronic colitis development as BALB/c mice.

Discussion

We show in this study that B cells with AGcA become MZ B cells in spleen and produce natural autoantibody in serum. This AGcA binds predominantly to intact large Muc2 glycoprotein. In the same mouse, expression of a BCR with anti-thymocyte autoreactivity results in B1 B cell generation. Both AGcA and ATA react with highly glycosylated proteins, specific to murine determinants, showing no binding to corresponding rat glycoproteins (27). Accumulation of AGcA MZ B cells occurs from the transitional immature stage and is dependent on Btk. Therefore, a BCR-ligand signal is required for AGcA MZ B cell development, as with ATA B1 B cells where again functional Btk is critical (9). How immature AGcA B cells are exposed to Ag, thereby generating MZ B cells and not B1 B cells, remains to be determined. However, in AGcA MZ B cell generation, DCs and blood vessels in spleen seem likely to play roles in presenting self-antigen, followed by the Notch 2 signal that is key in MZ B cell development (33). Goblet cells continually secrete Muc2 into the intestinal mucosa, providing a mucosal barrier against bacterial invasion. As with bacteria in the mucosal lumen, DC dendrites also continually internalize Muc2 from mucus (14, 34). If these DCs move into the lymphatic vessels and enter blood circulation, then transport of Muc2 to the spleen can occur. This may explain the early initiation of AGcA MZ B cell development in spleen, filtering the blood.

**FIGURE 3.** AGcA by MZ B cell–associated V_{H}3609/V_{L}19-17 IgM (MK19). (A) Colon mucosal cell suspension was stained by MK19, in contrast with thymus cell suspension that was stained by MK21. (B) Cryosection staining of colon. Goblet cells and contiguous luminal mucus were stained with MK19, but not MK21. IB4 (BSI-B4) using a 10× objective lens. (C) MK19 staining of transverse sections of intestines and stomach using a 20× objective lens. (D) Colon goblet cells (horizontal section) were shown to be PAS+ (paraffin section staining). MK19-stained granules in the goblet cells, which were distinct from PNA and IB4 bound supranuclear areas and nuclei stained by DAPI, as assessed using a 20× objective lens. (E) Apical goblet cell staining by MK19 was similar to DBA lectin binding in colon. MK19 staining (bottom middle) was merged with PNA and WGA lectins (top middle). (F) MK19 staining of Muc1- and Muc2-deficient mouse colon demonstrates predominantly Muc2-dependent binding of MK19. (G) Absence of MK19 staining of rat colon goblet cells. (E)–(G) using a 10× objective lens.
promoted by the presence of Muc2 in neonates as well as adults. Thus, differences in BCR signaling because of variation in affinity (21) and/or the site of Ag availability may generate MZ rather than B1 B cells.

Our data also demonstrate genetic influences on AGcA B cell generation. Variation of MZ B–associated natural AGcA autoantibody levels between mouse strains resembles B1 B cell–associated natural autoantibody, T15 idiotype positive anti-phosphorylcholine natural autoantibody (T15id+ anti-PC) that is higher in BALB/c compared with C57BL/6 (35, 36). However, generation of T15id+ anti-PC B cells does occur in C57BL/6 mice, although at a much lower frequency (37). This IgM is one of the most studied natural autoantibodies in mice. Binding to apoptotic cells leads to the initial generation of T15id+ anti-PC B cells, with anti-PC autoantibody production, and cross-reactivity of this Ab to oxidized low-density lipoprotein helps to prevent atherosclerosis (38). Furthermore, re-

![FIGURE 4. Predominant MK19 AGcA binding to intact Muc2 glycoprotein. (A) Western blot of colon NP-40 lysate. Mucus released from crypts by centrifugation was suspended in 1% NP-40 and applied to 3.3% SDS-PAGE with a 2.5% stacking gel for detection of glycosylated colon mucin(s). Comparison of wild-type B6 versus Muc2KO.B6. Anti-Muc2 (N terminus peptide). (B) Colonic crypts from Rag1 null mice were incubated with MK19 (or MK21), cross-linked (CL) with DSP, then suspended in NP-40 buffer for SDS-PAGE. Left set is Western blot of IgM H chain band using soluble and insoluble lysate, showing the presence of MK19 IgM CL colon granules detected by anti-IgM. Right set of the same lysates preincubated with MK19 shows copresence of Muc2. MK19 IgM and Muc2 were both predominantly in the insoluble fraction. (C) Soluble material from MK19 IgM prebound (MK21 as a control) colon granules, followed by cross-linking, was incubated with anti-IgM–bound protein G Dynabeads for 18 h, then washed, eluted, and applied to SDS-PAGE analysis. Presence of MK19 bound to ~600 kDa DBA*Muc2 is shown. IB, immunoblot; LB, lectin blot.

![FIGURE 5. Natural AGcA IgM production from MZ B cells. (A) The presence of AGcA PCs (high IgMa+ and CD19\(^+\)) in EP67 mouse spleen. CD19\(^+\) B cells are also stained with IgM\(^{++}\) at a lower level than PCs. (B) Predominant AGcA IgM in serum of EP67 mice was independent of T cells (EP67.RagKO). \(\mu\times Tg\) IgM is IgM\(^{++}\) on a C.B17 (IgMb) mouse background. \(n = 9\) each. (C) Colon and thymus cryosection staining comparison between EP67 AGcA \(\mu\times Tg\) mouse and 3369 ATA \(\mu\times Tg\) mouse sera, both with Tg IgM\(^{++}\) predominance in serum. (D) AGcA reactivity by IgM produced from normal mouse MZ B cells. Colon from Rag1 null mice stained by LPS-stimulated B cell culture supernatant IgM (adjusted to 2–10 \(\mu\)g/ml) from BALB/c mice. B1 (B1a) B cells in the peritoneal cavity and MZ B cells, and FO B cells in spleen were purified from the same mouse. Representative of three experiments (two with BALB/c, one with C.B17). (A), (C), and (D) using a 20X objective lens. RP, red pulp; WP, white pulp.]
activity to phosphorylcholine expressed by various microorganisms plays a protective role against pathogens (35). We show in this study that MZ B–associated AGcA is also higher in BALB/c than C57BL/6, again demonstrating the importance of genetic background in generation of the natural autoantibody repertoire. One possibility is that the VH or VL segment required for the AGcA BCR is different enough in C57BL/6 mice to reduce its affinity below that required for selection into the MZ B cell pool. Alternatively, the frequency of B cells with an appropriate AGcA BCR is lower in C57BL/6 mice, as found with T15id+ anti-PC B cells.

Understanding the presence of natural autoantibodies in vertebrates, their roles, and genetic influences have been a subject of continuing study. As with B1 B cell–derived natural autoantibodies, the production of AGcA IgM may normally function as a scavenger natural autoantibody, as suggested by experiments with DSS-treated mice. Because cells of the intestinal tract are constantly sloughing off and renewed by intestinal stem cells, the presence of AGcA IgM in animals with appropriate genetic background may help to promote clearance of damaged cells. When B cells producing natural AGcA switch to IgA in intestine, the secreted Ig will reach the mucosal lumen and bind to Muc2. This may enhance the physical barrier provided by Muc2 and aid in preventing microbial invasion, an interesting possibility to describe enables such further study of the significance of natural AGcA.

In human pathology, the relevance of anti-goblet cell autoantibody to UC has been an issue for decades, as a potential disease–inducing autoantibody (17). Sera from children with UC contain Ab that reacts with fetal colon cells (17, 18), and anti-Muc2 reactivity in UC patient serum has been demonstrated (39). The presence of anti-goblet cell autoantibody among first-degree relatives of UC patients without obvious sign of disease has been interpreted as indicating a genetic risk for development of UC (40). However, a role for AGcA in enteropathy has not been clearly established; rather, AGcA is often detected in the serum of healthy individuals (41). As demonstrated in this study with mice, anti-goblet cell autoantibodies in relatives of UC patients may in part be composed of natural Igs at genetically regulated levels, as found for BALB/c versus C57BL/6 strains. The mouse model we describe enables such further study of the significance of natural autoantibodies, testing whether it functions to suppress inflammatory disease, but carrying a risk for promoting disease. In addition, in considering reactivity to the highly glycosylated Muc2 molecule, potential cross-reactivity to pathogenic bacteria or viruses to enable defensive immunity by MZ B cells is another area that merits further investigation.

![FIGURE 6. Natural AGcA autoantibody in intestine.](http://www.jimmunol.org/)

### Table I. Strain difference of natural AGcA IgM levels

<table>
<thead>
<tr>
<th>Serum from</th>
<th>AGcA Reactivity</th>
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<tbody>
<tr>
<td>EP67 Tg,CB17</td>
<td>100% (8/8)</td>
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<tr>
<td>BALB/cAnN</td>
<td>0%</td>
</tr>
<tr>
<td>C.B17</td>
<td>0%</td>
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<tr>
<td>C57BL/6</td>
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Analysis of sera from individual 2- to 4-mo-old mice (both male and female, 5–8 mice/group) for capacity to stain Rag KO colon goblet cells. All reactivity compared at 1/100 dilution with similar total IgM levels, and also in comparison with 1/100, 1/500, and 1/1000 diluted one EP67 µgTg serum as a standard sample. Reactivity levels by mouse sera marked as (+) were around 8- to 10-fold less than EP67 µgTg sera (+++), although still detectable. Undetected at 1/100 dilution was (−). All sera show no reactivity to rat colon goblet cells.
Acknowledgments
We thank Gunner C. Hansson for advice on analysis of Muc2, Sandra Gendler for Muc1-deficient mice, Li-Jun Wen for V_{P}360P9 mouse generation, Anthony Yeung for advice on colon cryopreservation, Catherine Renner for help with histologic analysis, Yue-Sheng Li for supervising single-cell sequencing, and Kerry Campbell for discussion and comments on the manuscript.

Disclosures
The authors have no financial conflicts of interest.

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