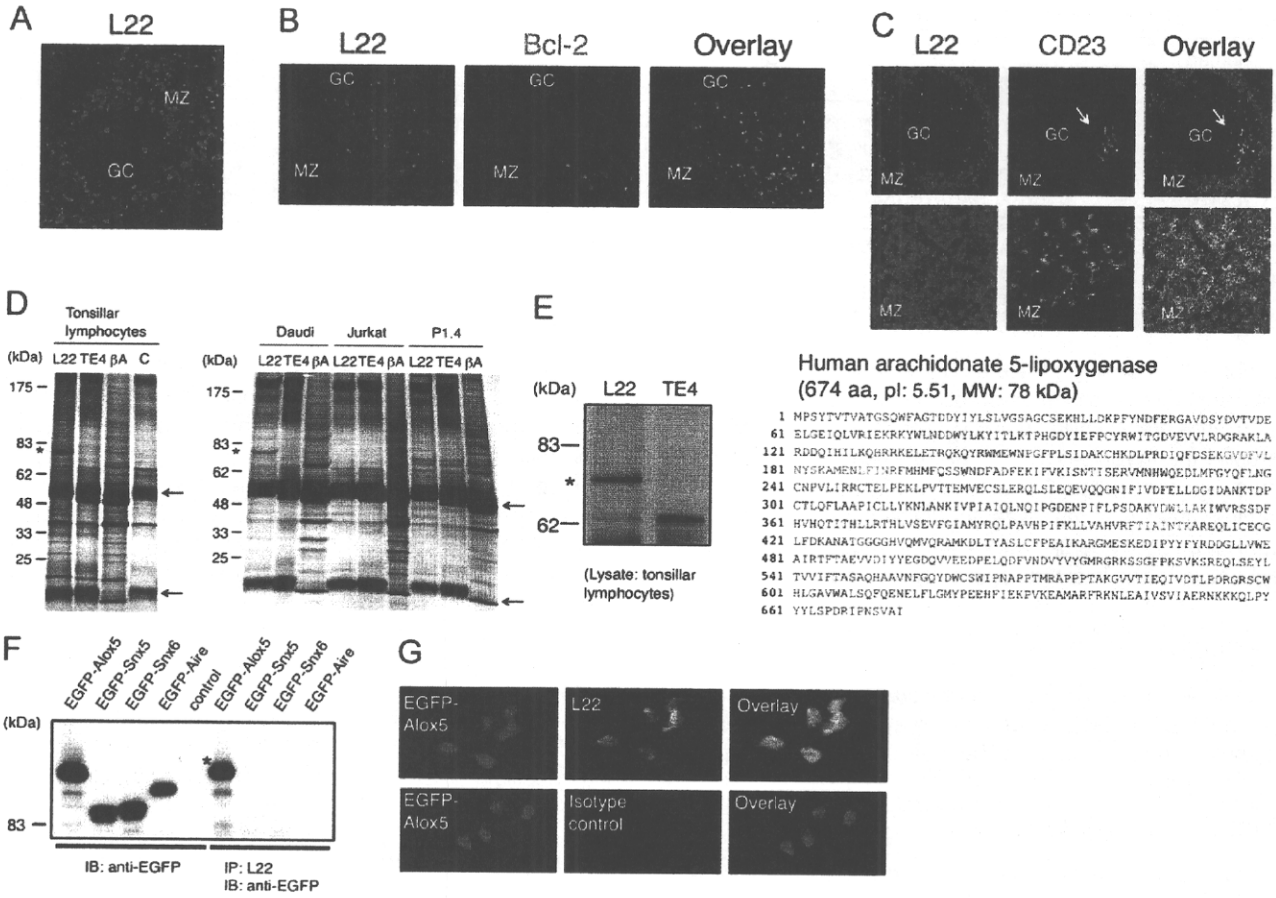

Figure 1. Mantle zone B cells of lymphoid tissues highly express Alox5. **A–C:** Immunohistochemical analysis of lymphoid follicles of tonsils with L22 mAbs. **A:** Mantle zone B cells around germinal centers express L22 Ag (green). Original magnification, ×200. **B:** Mantle zone B cells with L22 Ag (green) simultaneously express Bcl-2 (red). Original magnification, ×200. **C:** The mantle zone exhibits a mixed population of $L22^{+}CD23^{+}$ and $L22^{+}CD23^{-}$ B cells. **Upper panel** shows the lymphoid follicle containing follicular dendritic cells (**arrows**). **Lower panel** focuses on the mantle zone. Original magnification: ×200 (**upper panel**); ×400 (**lower panel**). In **A, B,** and **C,** the mantle zone and germinal center are represented as MZ and GC, respectively. The large L22-expressing cells within the GC are macrophages. **D:** Immunoprecipitation analysis of tonsillar lymphocytes and cell lines with L22 mAbs. After separation of immunoprecipitates, the proteins were visualized by silver staining. The **left** and **right panels** demonstrate bands that resulted from the lymphocytes of tonsils and cell lines, including Daudi B cells, Jurkat T cells, and P1.4 thymic epithelial cells. The band that specifically reacts to L22 mAbs is indicated by **asterisks** in each panel. L22, L22 mAbs; TE4, antithymic medullary epithelium mAbs; βA, anti–β-actin mAbs; C, isotype control. **Arrows** indicate light or heavy Ig chains bound to beads. **E:** Proteomics analysis of L22 Ags for identifying Alox5. Mass spectrometry of the band is indicated by an **asterisk** [**left panel**; same as (**D**)] revealed four different peptide sequences, including GVDFVLNYSK, AMENLFINR, YDWLLAK, and FTIAINTK. The protein sequence of Alox5 is shown in the **right panel**, where the four peptides are depicted in red, as directed by a Mascot search. **F:** Immunoprecipitation analysis of EGFP-tagged Alox5 and other human proteins with L22 mAb. HEK 293 cells were transiently transfected with a plasmid expressing EGFP-Alox5, EGFP–sorting nexin 5 (Snx5), EGFP–sorting nexin 6 (Snx6), or EGFP–autoimmune regulator (Aire), with expected molecular weights of 118, 86, 88, and 98 kDa, respectively. L22 mAbs bind to EGFP-Alox5 (**asterisk**) but not to other EGFP-tagged proteins. **G:** Immunohistochemical analysis of HEK 293 cells expressing EGFP-Alox5 with L22 mAb. L22 mAb (red) reacts to cells transiently expressing EGFP-Alox5 (green). Original magnification, ×400.

Alox5 Is Required for Specific Humoral Immune Responses

Next, we immunized *Alox5*$^{-/-}$ mice with various antigens and investigated their sera and immune cells. When SRBCs were administered as T-cell–dependent foreign antigens, these mice showed impaired production of SRBC-specific antibodies at the initial and recall phases (Figure 3, A and B). To further examine the role of Alox5 in B cells, we established bone marrow chimeras of *Rag1*$^{-/-}$ mice lacking B and T cells. $Lin^{-}Scal^{+}$ cells from the bone marrow of *Alox5*$^{+/+}$or *Alox5*$^{-/-}$ mice were transplanted into *Rag1*$^{-/-}$ mice (termed *Rag1*^{WT} or *Rag1*^{Alox5} mice, respectively). When immunized with SRBC, *Rag1*^{Alox5} could not fully produce SRBC-specific antibodies, whereas *Rag1*^{WT} mice could (Figure 3D). These results strongly suggested the requirement of Alox5 to produce specific antibodies from B cells.

Most Ig subclasses examined were at normal serum levels in unimmunized *Alox5*$^{-/-}$ mice, suggesting that Alox5 was not necessary to produce naturally occurring Igs (Figure 3C). To our surprise, however, IgG2a levels dependent on type 1 helper T cells were significantly reduced in *Alox5*$^{-/-}$ mice even after SRBC immunization (Figure 3, A and C). Similarly, *Rag1*^{Alox5} mice demonstrated low levels of IgG2a compared with *Rag1*^{WT} mice (data not shown). The inability of IgG2a production by *Alox5*$^{-/-}$ and *Rag1*^{Alox5} mice suggests the possible involvement of Alox5 in an unknown mechanism of class switching of the IgG2a heavy chain within B cells or of insufficient effects from neighboring cells such as helper T cells.[32,33]

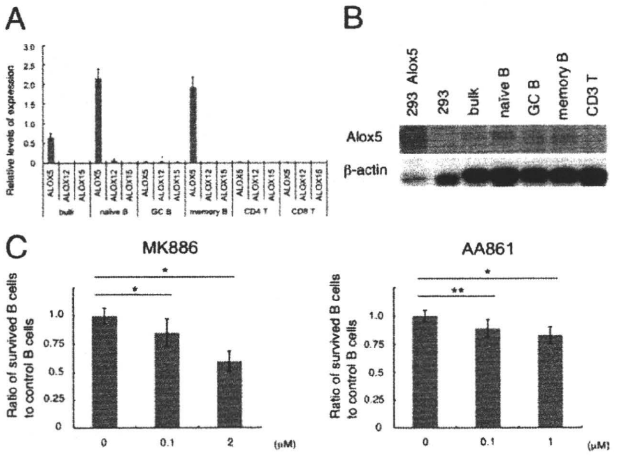

Figure 2. Expression and function of Alox5 in primary resting B cells. **A:** Quantitative PCR analysis of Alox5 in tonsillar lymphocytes. On the basis of the oxidation site of arachidonic acid, lipid oxidation enzymes are categorized into three types: Alox5, Alox12, and Alox15. Naive and memory B cells preferentially express transcripts of Alox5 but not Alox12 and Alox15. **B:** Immunoblotting analysis of Alox5 in tonsillar lymphocytes. Alox5 is abundantly expressed in naive and memory B cells. HEK 293 cells and their transient transformants expressing Alox5 were used as controls. **C:** B cells require Alox5 for their survival. 2×10^6 $CD19^+$ B cells from tonsils were cultured in serum-free AIM V medium (Invitrogen) in each well of a 24-well plate with or without MK886 or AA861 for 96 hours, and then flow cytometric analysis of B cells was performed. The data are demonstrated as the ratios of the $CD19^+$ population in the control media to these cells in the inhibitor-containing media, where the cells were counted as $CD19^+$ cells. Results are representative of three to four independent experiments. $^*P < 0.1$, $^{**}P < 0.05$.

Furthermore, we investigated the role of Alox5 in the regulation of memory-specific humoral responses. When the mice were administered NP36-CGG, although memory B cells with the $B220^+IgG1^+NP^+$ phenotype were indeed found in *Alox5*$^{-/-}$ mice, the number of memory B cells in *Alox5*$^{-/-}$ mice was almost half the number in *Alox5*$^{+/+}$ mice (Figure 3E). The serum titer of NP25-BSA or NP3-BSA specific IgG1 increased in *Alox5*$^{+/+}$ mice in response to the second challenge of NP36-CGG; however, this titer did not increase to the same extent in *Alox5*$^{-/-}$ mice, as observed in the SRBC immunization experiment (Figure 3F). Meanwhile, there were no significant differences in a ratio of the titer of NP25-BSA to that of NP3-BSA in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice at each date of investigation (Figure 3G). Therefore, Alox5 might regulate the number of memory B cells, whereas Alox5 would not be required for the processes of affinity maturation.[23]

Alox5 Regulates Survival and Function of Follicular B Cells

As previously reported, the spleen is relatively smaller in *Alox5*$^{-/-}$ mice than in wild-type mice (Figure 4A).[19] When immunized with SRBCs, the spleen of *Alox5*$^{+/+}$ mice gradually increased in weight up to approximately double the original weight. Immunization also increased the spleen weight in *Alox5*$^{+/-}$ and *Alox5*$^{-/-}$ mice; however, the spleens were smaller and their weights did not reach the weight attained by *Alox5*$^{+/+}$ mice. Inverted microscopy revealed that lymphoid nodules of the spleen did not develop well in *Alox5*$^{+/-}$ and *Alox5*$^{-/-}$ mice even after administration of SRBCs (Figure 4B). Indeed, immunohistochemical studies revealed fewer mature germinal centers in the lymphoid follicles in the spleen of SRBC-immunized *Alox5*$^{-/-}$ mice (Figure 4C). Furthermore, flow cytometry analysis indicated reduced numbers of germinal center cells ($B220^+PNA^+IgD^-$) in SRBC-immunized *Alox5*$^{-/-}$ mice (Figure 4D). These results suggest that the weak humoral responses to foreign antigens in *Alox5*$^{-/-}$ mice may be caused by inability to generate sufficient germinal centers.

Freshly isolated spleens of *Alox5*$^{-/-}$ mice had slightly fewer B cells ($B220^+$) than did those of *Alox5*$^{+/+}$ mice, even after SRBC immunization. Primary resting B cells are generally classified into 2 major subpopulations—follicular B cells ($B220^+CD21^{int}CD23^+$) and marginal zone B cells ($B220^+CD21^{hi}CD23^-$), each of which plays a unique role in the humoral immune response.[34,35] Follicular B cells play an important role in antibody production in a T-cell–dependent manner, and marginal zone B cells are considered to be innate-like cells. Interestingly, *Alox5*$^{-/-}$ mice demonstrated a significantly decreased number of follicular B cells, ie, approximately 60% of the number of cells in *Alox5*$^{+/+}$ mice (Figure 4E). We also observed the same tendency of follicular B cells in the spleen of *Rag1*^{Alox5} mice compared with those of *Rag1*^{WT} mice (Figure 4F). Note that Alox5 deficiency could lead to apoptosis of follicular B cells, as assessed by the cell surface expression of annexin V (Figure 4G).[36] These evidences imply that follicular B cells depend on Alox5 for maintaining the B-cell population; ie, the B-cell repertoires might be preserved by Alox5 to effectively produce specific antibodies.

We further investigated the features of follicular B cells of *Alox5*$^{-/-}$ mice. After examination of various molecules related to antibody production, the expression levels of the interleukin-21 (IL-21) receptor were profoundly reduced in follicular B cells of *Alox5*$^{-/-}$ mice (Figure 4H).[37,38] However, levels of IL-6, interferon γ receptors, and TLRs were not significantly altered in these cells. Similarly, there were no differences in the major histocompatibility complex class II expression of follicular B cells in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice, suggesting that Alox5 of follicular B cells might not affect the cellular presentation of foreign antigens to helper T cells (Figure 4I). Because Alox5 regulates the cell fate and IL-21–mediated responses of follicular B cells, Alox5-deficient mice might become incapable of producing antibodies specific to foreign antigens.

Alox5 Is Involved in the Generation of Tfh Cells

As noted, IgG2a is preferentially regulated by type 1 helper T cells; therefore, we considered the possible functional effect of Alox5 in the development of effector helper T cells. This might also be suggested by evidence that professional APCs other than primary B cells, such as macrophages or dendritic cells, possess Alox5.[11,39] Before this investigation, we analyzed the status of thymic selection in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice. Results revealed no significant differences between the populations of developing thymocytes in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$

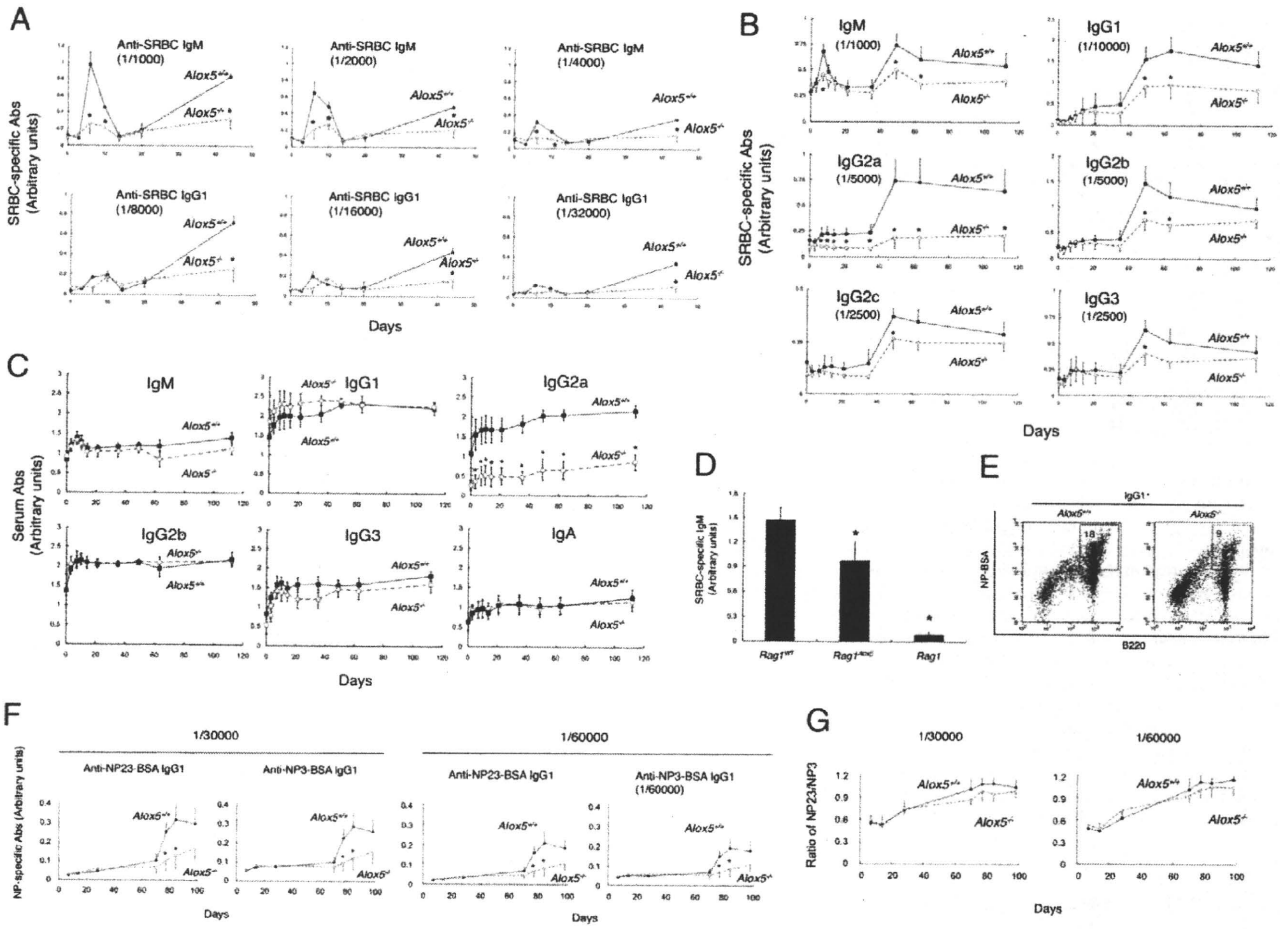

Figure 3. Functional defects of antibody responses specific to foreign antigens of Alox5-deficient mice. **A:** SRBC-specific serum titers of IgM and IgG1 of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice. Mice were immunized with SRBCs on days 0 and 28. SRBC specific IgM and IgG1 titers on consecutive days are shown in different dilutions. $n = 6$ to 8 mice per group. **B:** SRBC specific serum Ig titers of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice. The mice were immunized with SRBCs on days 0 and 42. Titers of SRBC specific IgM, IgG1, IgG2a, IgG2b, IgG2c, and IgG3 on consecutive days are shown in different dilutions. $n = 7$ to 10 mice per group. **C:** Impairment of IgG2a production in *Alox5*$^{-/-}$ mice. Serum levels of IgM, IgG1, IgG2a, IgG2b, IgG3, and IgA of the specimens studied in **B** are demonstrated. **D:** SRBC specific serum titers of IgM of *Rag1*^{WT} and *Rag1*^{Alox5} mice. Before immunization, *Rag1* chimera mice were examined for whether they produced Igs. Then mice were immunized with SRBCs on day 0, and the titers of SRBC specific IgM on day 7 are presented. Results from *Rag1* are also shown as a control. $n = 5$ to 6 mice per group. **E:** Low numbers of memory B cells (B220$^+$IgG1$^+$NP$^+$) in *Alox5*$^{-/-}$ mice. Mice were immunized with NP36-CGG on days 0 and 56. FACS analysis was used to assess B cells in the spleen on day 64. After gating IgG1$^+$ cells, B220$^+$NP$^+$ cells were found to compose 18% and 9% of IgG1$^+$ cells in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice, respectively. $n = 4$ to 6 mice in each group. **F:** Recall antibody responses of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice. The mice were immunized with NP36-CGG on days 0 and 63. Titers of NP23-BSA or NP3-BSA specific IgG1 at consecutive days are shown at different dilutions. $n = 6$ mice per group. **G:** Ratio of the titer of NP23 specific IgG1 to NP3 specific IgG1 obtained in **F**. No significant differences were observed between the ratios in *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice. Results of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice are depicted as solid and dashed lines, respectively. $n = 6$ to 10 mice per group. $^*P < .05$ compared with the wild-type control.

mice (Figure 5A). Previous studies have indicated the diversification of peripheral naive helper T cells into different types of helper T cells, among which Tfh cells (CD3$^+$CD4$^+$CD25$^-$CXCR5$^+$ICOS$^+$) have the distinguished property of driving specific antibody responses.[14,15] When a population of Tfh cells was studied, *Alox5*$^{-/-}$ mice unexpectedly lost their capacity to develop Tfh cells after SRBC immunization (Figure 5B). We next investigated the expression of receptors for Alox5-related lipid mediators in Tfh cells derived from human tonsils in two cases (Figure 5C). In these cases, Tfh cells rather than control helper T cells (CD3$^+$CD4$^+$) up-regulated the expression of v-maf musculoaponeurotic fibrosarcoma oncogene (MAF), Bcl-6, and IL-21, as reported in previous studies.[40–42] The control helper T cells expressed leukotriene receptors of BLT1 and BLT2 for leukotriene B4 and CysLTR1 or CysLTR2 for cysteinyl leukotrienes. In contrast, the levels of these receptors in Tfh cells seemed to be down-regulated or similar to those in control helper T cells. In fact, when human tonsillar T cells were examined by means of flow cytometry, CD4$^+$ T cells frequently presented BLT1 on the cell surface, but BLT1 was lost in Tfh cells (data not shown). These results imply that the signaling of Alox5-related lipid mediators would be required during the initiation of differentiation of naive helper T cells to Tfh cells, probably while in contact with professional APCs.

Alox5 Ensures Survival of Mice from Chronic Enterocolitis under Conventional Conditions

Our observations suggest the novel role of Alox5 in the regulation of adaptive humoral responses. To investigate

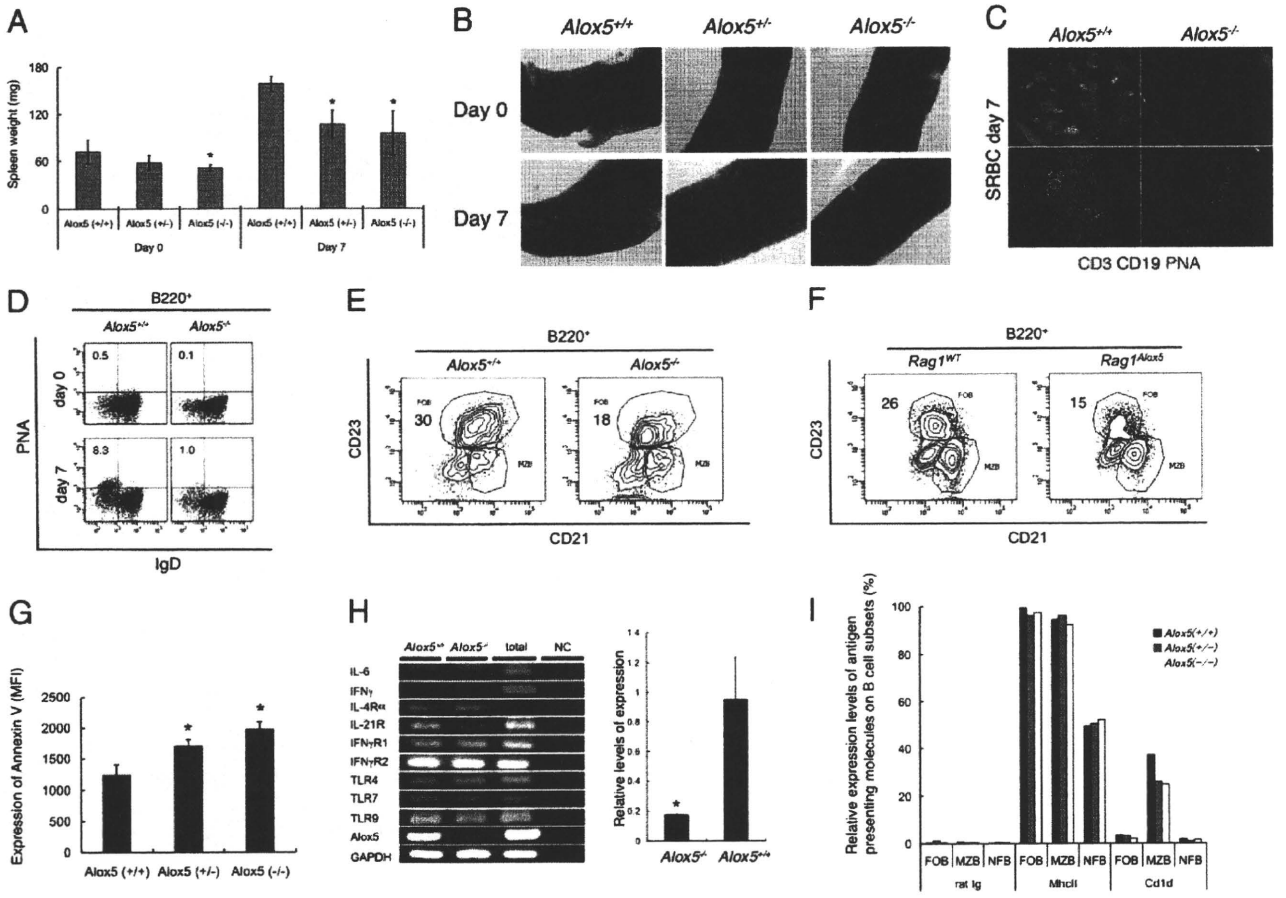

Figure 4. Analysis of antibody-producing cells in Alox5-deficient mice. **A:** Spleen weights of $Alox5^{+/+}$, $Alox5^{+/-}$, and $Alox5^{-/-}$ mice before (day 0) and after immunization with SRBCs (day 7). **B:** Features of the spleens of $Alox5^{+/+}$, $Alox5^{+/-}$, and $Alox5^{-/-}$ mice before (day 0) and after (day 7) immunization with SRBCs as examined using a stereomicroscope (SZX7, Olympus). Original magnification, ×15. **C:** Immunohistochemical analysis of the spleen of $Alox5^{+/+}$ and $Alox5^{-/-}$ mice after immunization with SRBCs (day 7). $CD3^+$ T cells (green), $CD19^+$ B cells (blue), and PNA^+ germinal center cells (red) are visualized. Original magnification: ×200 (upper panel) and ×400 (lower panel). **D:** FACS analysis of spleen cells of $Alox5^{+/+}$ and $Alox5^{-/-}$ mice before (day 0) and after (day 7) immunization with SRBCs. After immunization, the population of germinal center cells ($B220^+IgD^-PNA^+$) of $Alox5^{+/+}$ mice increased from 0.5% to 8.3% of the total $B220^+$ cells, whereas that of $Alox5^{-/-}$ mice increased from 0.1% to only 1.0%. **E, F:** A FACS analysis of follicular B cells ($B220^+CD21^{int}CD23^+$) of the spleen. **E:** Follicular B cells compose 30% and only 18% of the total $B220^+$ cell population in $Alox5^{+/+}$ and $Alox5^{-/-}$ mice, respectively. **F:** Follicular B cells compose 26% and 15% of the total $B220^+$ cell population in $Rag1^{WT}$ and $Rag1^{Alox5}$ mice, respectively. **G:** The expression levels of annexin V on follicular B cells of the spleen in $Alox5^{+/+}$, $Alox5^{+/-}$, and $Alox5^{-/-}$ mice after administration of SRBCs (day 7) as assessed by means of FACS analyzer. The mean fluorescence intensity (MFI) of annexin V on follicular B cells is shown. **H:** Expression levels of the transcripts of cytokines and receptors of follicular B cells sorted from the spleen of $Alox5^{+/+}$ and $Alox5^{-/-}$ mice as assessed by means of PCR. The **left panel** shows the RT-PCR results (25 cycles) investigating various molecules, including Alox5, and determines the down-regulation of the IL-21 receptor transcripts in the $Alox5^{-/-}$ cells. The **right panel** presents results from quantitative PCR of the IL-21 receptor of follicular B cells, indicating that the IL-21 receptor levels in these cells of $Alox5^{-/-}$ cells are approximately 20% of those in $Alox5^{+/+}$ cells. Total, total spleen cells; NC, no template control. **I:** The expression levels of major histocompatibility complex class II on spleen cells of $Alox5^{+/+}$, $Alox5^{+/-}$, and $Alox5^{-/-}$ mice. FOB, follicular B cells; MZB, marginal zone B cells ($B220^+CD21^{hi}CD23^-$); NFB, nonfollicular B cells ($B220^+CD21^{lo}CD23^-$). The results presented are representative of three to four independent experiments. In each experiment, 3 to 10 mice per group were used. $^*P < 0.05$ compared with the wild-type control.

whether humoral immunity regulated by Alox5 would work as a defense mechanism against microorganisms, an experimental colitis model was used in which DSS was orally administered to the mice in a conventional facility.[24] The results indicated that loss of Alox5 led to exaggerated enterocolitis (Figure 6A). Histologic examinations revealed severe erosion and an inflammatory reaction of the mucosa in $Alox5^{-/-}$ mice (Figure 6, B and C). Similar results were observed in $Rag1^{Alox5}$ mice, implying that the Alox5 of B cells plays a pivotal role in establishing humoral immunity against pathogens under conventional conditions.

Alox5 Enhances the Growth of MCLs

Finally, we investigated the expression profiles of Alox5 in B-cell lymphomas composed of small lymphoid cells, including MCL, small lymphocytic lymphoma (SLL), and marginal zone lymphoma. Currently, naive B cells of the mantle zone, antigen-experienced B cells, and postgerminal center B cells are considered to be the postulated cell origins of MCL, SLL, and marginal zone lymphoma, respectively.[43] Immunohistochemical studies using L22 mAbs on frozen sections revealed the high expression of Alox5 in MCL and SLL but not in marginal zone lymphoma (Figure 7, A–I). These results seem to be in agreement with our observations that Alox5 was preferentially presented in naive and memory B cells. When the MCL cell lines were examined, three cell lines, including G519, MINO, and REC1, possessed Alox5 (Figure 7J). Interestingly, AA861, an Alox5 inhibitor, possessed the capacity to reduce the growth of MINO cells (Figure 7K). Moreover, AA861 combined with vincristine, an antitumor re-

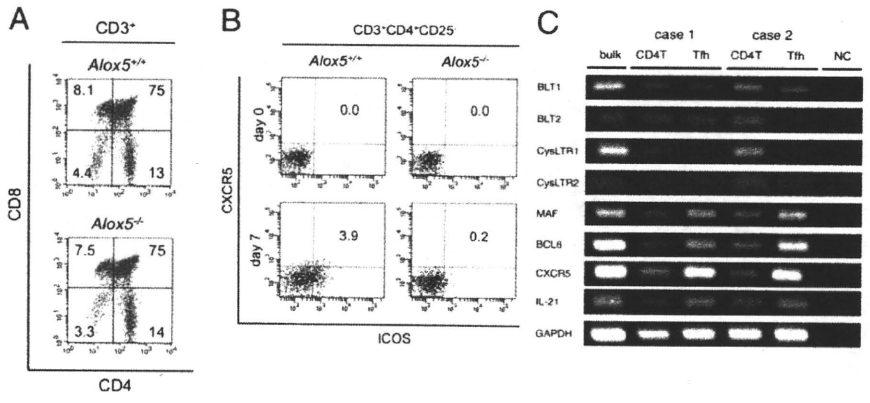

Figure 5. Developmental defects of Tfh cells in Alox5-deficient mice. **A:** FACS analysis of thymocytes in Alox5$^{+/+}$ and Alox5$^{-/-}$ mice. The population recognized by the expression of CD4 and CD8 are shown. **B:** FACS analysis of spleen cells of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice before (day 0) and after (day 7) immunization with SRBCs. After immunization, the population of Tfh cells (CD3$^+$CD4$^+$CD25$^-$ ICOS$^+$CXCR5$^+$) in *Alox5*$^{+/+}$ mice increased from 0.0% to 3.9% of the total CD3$^+$CD4$^+$ cells, and that of *Alox5*$^{-/-}$ mice increased from 0.0% to 0.2%. **C:** Expression levels of transcripts of leukotriene receptors in Tfh cells sorted from human tonsils. Results of two cases assessed by means of RT-PCR (28 cycles) are shown. Glyceraldehyde-3-phosphate dehydrogenase was used as the positive control. Bulk, total lymphocytes; NC, no template control. The results presented in (**A**) and (**B**) are representative of three to four independent experiments. In each experiment, three to six mice per group were tested.

agent for MCL, resulted in dramatic reduction of the growth activities of MINO cells. Therefore, Alox5 may be associated with the cellular integrities of not only primary B cells of the mantle zone, from which they originate, but also of MCL cells.

Discussion

In this study, we followed comprehensive immunoprecipitation and proteomics methods to identify an L22 Ag as Alox5. As implicated by the expression of Alox5 in mantle zone B cells (CD23$^+$ and CD23$^-$) around germinal centers, Alox5 plays a pivotal role in specific immunity as a regulator of cell fate and responsiveness to IL-21 of naive follicular B cells.[37,38,44] Alox5 defects also impinge on the generation of memory B cells, suggesting that it would completely support the preservation of B-cell repertories maintained by naive and memory B cells. The Alox5 pathway is essentially associated with a variety of inflammatory diseases, including asthma, atherosclerosis, rheumatoid arthritis, liver cirrhosis, and cancer, which are caused by an underlying anomaly of acquired immunity.[15,31] Therefore, primary B cells may act as a modulator in these pathologic situations. As a major source of leukotrienes in lymphoid tissues, primary B cells may contribute to establishment of the histologic features, such as lymphadenitis lesions.

Infection of mice with RNA or DNA viruses induces an antiviral antibody response, which is largely restricted to IgG2a. This also provides the functional importance of Alox5 as a coordinator of host defense. The regulation of IgG2a, which is an IL-4–independent Ig isotype, and the mechanism of interferon γ derived from type I helper T cells remain investigative priorities. Although we could not fully elucidate the precise mechanism of IgG2a production by Alox5, it has been previously reported that Alox5 deficiency may lead to an imbalance of type I and II helper T cells.[45] Thus far, we considered the possible involvement of Alox5 in the development of helper T-cell subpopulations. CD4$^+$ T cells possess leukotriene receptors, which can instructively work for the mobilization of effector T cells to inflammatory foci; however, little is known about the role of leukotrienes in the development of Tfh cells.[14,40–42,46] Leukotriene receptors encoded in germlines form a receptor spanning seven membranes coupled with G-proteins in the cytoplasm, similar to the chemokine receptors of Tfh cells, such as CXCR5, CCR6, and CXCR3. Leukotriene receptors seem to be down-regulated (or at least not up-regulated) in Tfh cells; therefore, such lipid mediators might act on the initial process of differentiation of naive helper T cells. The prerequisite for the differentiation of naive helper T cells is the interaction of the T-cell receptor on naive CD4$^+$ helper cells with major histocompatibility complex class II molecules on professional APCs. Therefore, professional APCs, which express Alox5, profoundly affect the initial steps of the differentiation of naive helper T cells. *Rag1*^{Alox5} mice exhibited defects of specific antibody responses to foreign antigens, although Tfh cells could be recognized in response to the administration of foreign antigens in these

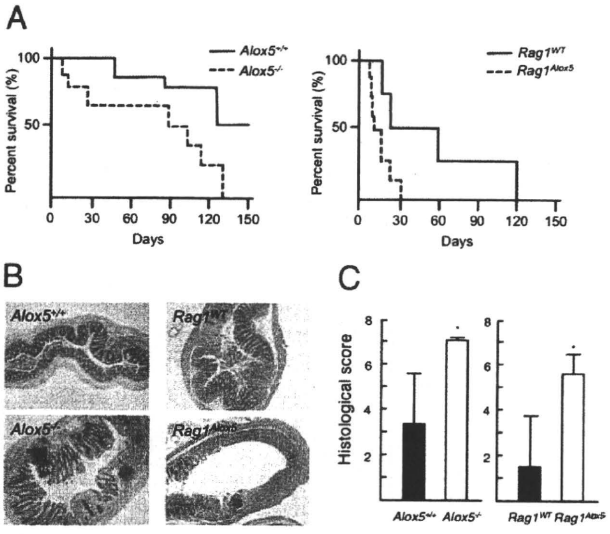

Figure 6. Alox5 defects attenuate intestinal tissue damage, with high mortality in experimentally induced chronic enterocolitis models. **A:** Survival curves of DSS-induced colitis models. Mice were maintained in a conventional facility and were allowed to freely drink water containing 1.5% DSS. The **left** and **right panels** illustrate the percentage survivals of *Alox5*$^{+/+}$ and *Alox5*$^{-/-}$ mice and *Rag1*^{WT} and *Rag1*^{Alox5} mice, respectively. $n = 7$ to 8 per group. **B:** Histologic findings of mice intestines after DSS administration on day 7. The **left** and **right panels** demonstrate the intestines of *Alox5*$^{+/+}$ or *Alox5*$^{-/-}$ mice and *Rag1*^{WT} or *Rag1*^{Alox5} mice, respectively. The intestines of *Alox5*$^{-/-}$ and *Rag1*^{Alox5} mice manifested severe enterocolitis. Original magnification, ×100. **C:** The total histologic score is presented as mean with SEM ($n = 3$ to 4 mice per group). $^*P < 0.05$ compared with the wild-type control.

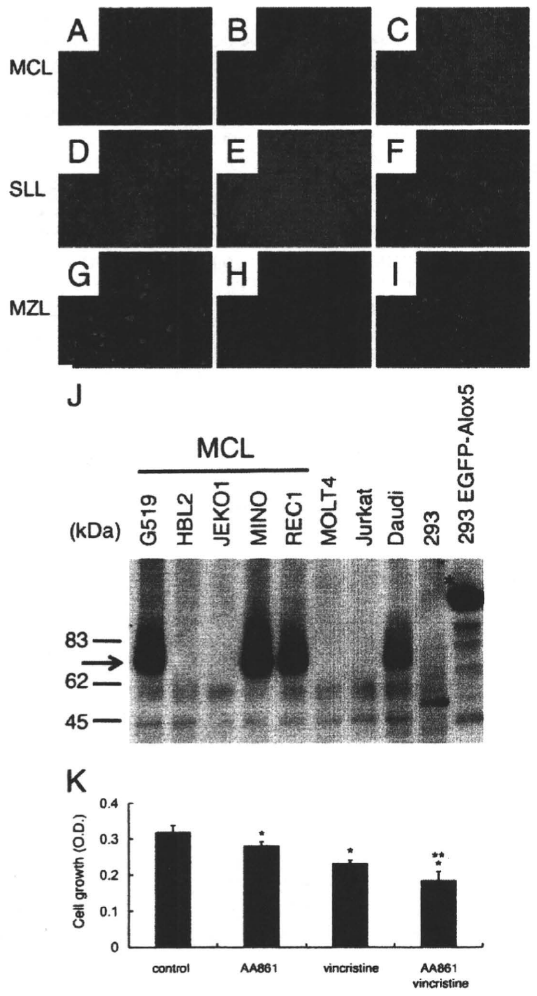

Figure 7. Alox5 defines cell growth of mantle cell–derived B-cell malignancies. **A–I:** Alox5 expression in B-cell lymphomas composed of small lymphoid cells. Immunohistochemical analysis using L22 mAbs was performed on frozen sections of independent tumor tissues: MCL (**A–C**), SLL (**D–F**), and marginal zone lymphoma (MZL) (**G–I**). Original magnification, ×200. **J:** Alox5 expression in cells derived from MCL at the protein level (**arrow**). Immunoblot analysis with anti-Alox5 pAbs was performed on human cell lines. HEK 293 cells with or without EGFP-Alox5 (**asterisk**) were used as the control. **K:** Cell growth of MINO under 10 μmol/L AA861 or 50 μg/ml of vincristine assessed by using WST1. A 96-well plate containing 1×10^5 cells per well was incubated with reagents for 24 hours. Results are representative of four independent experiments. $^*P < 0.01$ compared with the control; $^{**}P < 0.05$ compared with vincristine alone.

mice (data not shown). Thus, the lipid mediators derived from macrophages or dendritic cells but not from B cells would affect the process of differentiation of Tfh cells.

In this study, we could not fully elucidate a mechanism of the production of leukotrienes from primary B cells. Macrophages or dendritic cells produce Alox5-related leukotrienes by activating cytosolic phospholipase A2. It may be supposed that B-cell receptor signaling leads to the mobilization of calcium ions through inositol phosphate, leading to the activation of cytosolic phospholipase A2 and the subsequent liberation of arachidonate from membrane glycerolipids as a substrate of Alox5. We observed that *Alox5*$^{-/-}$ mice can produce B1 B cells ($B220^+CD5^+IgM^+$), related to producing natural antibodies, in the spleen and peritoneal cavity (data not shown). In this context, leukotrienes of primary B2 B cells may regulate the adaptive humoral immune response.[47] Indeed, we found transcripts of Alox5 in mouse follicular B cells, and we are trying to detect Alox5 in these cells at protein levels.

Maintenance of the B-cell repertoire is one of the most important elements in achieving adaptive humoral responses and protecting the host from pathogens. Perhaps primary naive and memory B cells express Alox5 and preserve their integrity to maintain the B-cell repertoire. According to this story, it was of interest to note that MCL, one of the most refractory tumors against conventional therapies, might depend at least in part on the function of Alox5 in terms of cellular growth. The evidence that primary B cells and MCL, which originates from primary B cells, rely on Alox5 for their cellular integrity can provide insights for understanding the unique tumor biology of MCLs. Further investigations should elucidate the mechanism of the additive effects of Alox5 inhibitors as chemotherapeutic reagents to treat MCL.[48,49]

In summary, we demonstrated the fundamental role of Alox5 in establishing specific antibody responses. Alox5 regulates not only primary resting B cells of the naive and memory B-cell phenotypes but also Tfh cell generation, thereby preserving specific antibody production. It has not yet been determined whether Alox5-related lipid mediators take part in the plasticity of the generation of helper T cells. However, studies of the expression profiles of leukotriene receptors on helper T-cell species would enable us to recognize the biological significance of lipid mediators in the differentiation of helper T cells. Lipid metabolism of arachidonic acid, affected by amounts from the oral intake or function of related enzymes, may affect the primary B-cell response by altering IL-21– and Tfh-mediated stimulation.

Acknowledgments

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