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CD19 Expression in B Cells Regulates Atopic Dermatitis in a Mouse Model

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Atopic dermatitis is an inflammatory cutaneous disorder characterized by dry skin and relapsing eczematous skin lesions. Besides antibody production, the contribution of B cells to the pathogenesis of atopic dermatitis is unclear. In mice, repeated epicutaneous sensitization with ovalbumin induces inflamed skin lesions resembling human atopic dermatitis and therefore serves as an experimental model for this condition. To investigate the role of B cells in a murine model of atopic dermatitis, ovalbumin-sensitized allergic skin inflammation was assessed in mice lacking CD19. In ovalbumin-sensitized skin from CD19-deficient mice, the number of eosinophils and $CD4^+$ T cells was reduced, and both epidermal and dermal thickening were decreased. Following *in vitro* stimulation with ovalbumin, CD19 deficiency significantly reduced the proliferation of $CD4^+$, but not $CD8^+$, T cells from spleen and draining lymph nodes. Furthermore, splenocytes and draining lymph node cells from ovalbumin-sensitized CD19-deficient mice secreted significantly less IL-4, IL-13, and IL-17 than ovalbumin-sensitized wild-type mice. These results suggest that CD19 expression in B cells plays a critical role in antigen-specific $CD4^+$ T-cell proliferation and T helper 2 and 17 responses in a murine model of atopic dermatitis. Furthermore, the present findings may have implications for B-cell–targeted therapies for the treatment of atopic dermatitis. *(Am J Pathol 2013, 182: 2214–2222; http://dx.doi.org/10.1016/j.ajpath.2013.02.042)*

Atopic dermatitis (AD) is one of the most common inflammatory cutaneous disorders, characterized by dry, itchy skin and relapsing eczematous skin lesions, which affects approximately 15% to 30% of children and 2% to 10% of adults.[1] Histologically, AD is characterized by epidermal and dermal thickening with marked infiltration of activated T cells, eosinophils, and monocytes/macrophages within the dermis.[1] Approximately 60% to 90% of patients with AD show increased serum total IgE against environmental and/or food allergens.[2–4] In addition, the expression of T helper (Th) 2 cytokines, such as IL-4, IL-5, and IL-13, is increased in the acute skin lesions of AD,[5,6] suggesting that Th2 cells play critical roles in disease development.

Skin barrier dysfunction is a critical feature of AD. Recent studies have shown that more than 10% of patients with AD have mutations in the filaggrin gene, which is important for skin barrier function.[7,8] It has been hypothesized that a disrupted skin barrier facilitates antigen penetration and epicutaneous sensitization, leading to allergic skin inflammation in patients with AD.[9] Moreover, IL-4 and IL-13 reduce filaggrin gene and protein expression in keratinocytes.[10] Thus, a genetic and/or acquired defect in filaggrin is likely to play an important role in the development of AD. In mice, repeated epicutaneous sensitization of tape-stripped skin with ovalbumin (OVA), mimicking epicutaneous allergen exposure to epidermal barrier dysfunction, was found to induce the appearance of inflamed pruritic skin lesions at the application site, as well as local and systemic Th2 responses. Because of the resemblance of these lesions to human AD,[11,12] this experimental method can serve as a convenient experimental model.

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Historically, B cells have been considered to mediate humoral immune responses by differentiating into antibody (Ab)-secreting plasma cells.[13] However, recent studies have revealed that B cells also serve as antigen-presenting cells,[14] secrete a variety of cytokines,[15] provide costimulatory signals, and promote T-cell activation.[15,16] Moreover, IL-10–producing B cell subsets can inhibit innate and adaptive immune responses, inflammation, and autoimmunity, demonstrating the existence of regulatory B cells.[13,17–19] Thus, in addition to Ab production, B cells have multiple diverse immune functions.

The fate and function of B cells are controlled by signal transduction through B-cell receptors, which are further modified by other cell-surface molecules, including CD19, CD21, CD22, CD40, CD72, and Fcγ receptor IIb.[20] CD19 is a general rheostat that defines signaling thresholds critical for humoral immune responses and autoimmunity.[21] CD19 is a B-cell–specific cell-surface molecule of the Ig superfamily expressed by early pre-B cells in humans and mice until plasma cell differentiation.[22,23] Human CD19 and mouse CD19 are functionally equivalent *in vivo*.[22] B cells from CD19-deficient ($CD19^{-/-}$) mice are hyporesponsive to a variety of transmembrane signals, including B-cell receptor ligation.[22] CD20, a B-cell–specific cell-surface molecule involved in the regulation of B-cell activation and Ca^{2+} transport, is initially expressed by pre-B cells in humans and mice with continued expression until plasma cell differentiation.[24,25] Although the role of B cells, besides Ab production, in the pathogenesis of AD remains unclear, B-cell depletion in humans with the chimeric human anti-CD20 monoclonal antibody (mAb) rituximab results in an improvement of AD,[26,27] suggesting that B cells play important roles in the development of this condition. Therefore, in the present study, we examined the importance of B cells in an OVA-sensitized allergic skin inflammation model using $CD19^{-/-}$ and wild-type (WT) mice.

Materials and Methods

Mice

WT C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). $CD19^{-/-}$ (C57BL/6 × 129) mice were generated as described previously[28] and backcrossed for 7 to 12 generations onto the C57BL/6 background before use in this study. Lack of cell-surface CD19 expression was verified by two-color immunofluorescence staining with flow cytometric analysis. All mice were bred in a specific pathogen–free barrier facility and used at 8 to 12 weeks of age. All studies were approved by the Committee on Animal Experimentation (University of Tokyo, Japan).

Epicutaneous Sensitization

Epicutaneous sensitization of mice was performed as described previously.[12] Briefly, the dorsal skin of anesthetized mice was shaved and tape-stripped six times. Next, 100 μg of OVA (Grade V; Sigma-Aldrich, St. Louis, MO) in 100 μL of PBS or 100 μL of PBS alone was placed on a patch of 1 × 1-cm sterile gauze, which was secured to the dorsal skin with a transparent bio-occlusive dressing (Tegaderm; 3M Health Care, St. Paul, MN). Each mouse had a total of three 1-week exposures to the patch separated from each other by 2-week intervals.

Histological Analysis

Mice were sacrificed 1 day after removal of the patch after the third sensitization (day 50). Skin samples were removed, and segments were fixed in 10% buffered formalin. After paraffin embedding, sections (5 μm thick) were cut and stained with H&E for eosinophil counting and with toluidine blue for mast cell counting. For immunohistochemistry, paraffin-embedded tissues were cut into 6-μm-thick sections, deparaffinized in xylene, and then dehydrated in PBS. Deparaffinized sections were treated with endogenous peroxidase blocking reagent (Dako, Glostrup, Denmark) and proteinase K (Dako) for 6 minutes at room temperature. Sections were then incubated with rat mAb specific to mouse CD4 (#2H9; ReliaTech, Wolfenbüttel, Germany), CD8 (D-9; Santa Cruz Biotechnology, Santa Cruz, CA), and B220 (RA3-6B2; BD Biosciences, San Jose, CA). Rat IgG (Southern Biotechnology Associates, Birmingham, AL) was used as a control for nonspecific staining. Sections were then incubated sequentially (20 minutes at 37°C) with a biotinylated rabbit anti-rat IgG secondary Ab followed by a horseradish peroxidase–conjugated avidin–biotin complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were developed with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide, and counterstained with methyl green. Stained cells were counted in 10 random grids under high magnification (×400) using a light microscope. Each section was examined independently by two investigators (K.Y. and M.K.) in a blinded manner.

Serum Ab Determination

Mice were bled and serum samples were collected on day 50 (1 day after the end of epicutaneous sensitization). All serum samples were stored at −70°C until use. Serum levels of OVA-specific IgG1, IgG2a, and IgE Abs were measured with a specific enzyme-linked immunosorbent assay (ELISA) kit (Alpha Diagnostic International, San Antonio, TX), according to the manufacturer's protocol. Each sample was tested in duplicate.

Abs and Immunofluorescence Analysis

Anti-mouse mAbs against B220 (RA3-6B2), CD19 (1D3), CD5 (53-7.3), CD1d (1B1), CD4 (H129.19), CD8 (53-6.7), and CD25 (PC61) were obtained from BD Biosciences. For intracellular staining, mAbs against FoxP3 (FJK-16s; eBiosciences, San Diego, CA) and the Cytofix/Cytoperm kit (BD Biosciences) were used. Single-cell suspensions of the spleen and draining lymph nodes (pooled bilateral axial and

inguinal lymph nodes) were prepared by gentle dissection. Peritoneal cavity leukocytes were isolated with 10 mL of cold (4°C) PBS injected into the peritoneum of sacrificed mice followed by gentle massage of the abdomen. Viable cells were counted using a hemocytometer, with relative lymphocyte percentages determined by flow cytometry. Single-cell leukocyte suspensions were stained on ice using predetermined optimal concentrations of each Ab for 20 to 60 minutes and fixed as previously described.[23] Cells with the light scatter properties of lymphocytes were analyzed by immunofluorescence staining and a FACSVerse flow cytometer (BD Biosciences). Background staining was determined using unreactive isotype-matched control mAbs (Caltag Laboratories, San Francisco, CA) with gates positioned to exclude ≥98% of unreactive cells.

Lymphocyte Subset Isolation

Magnetic-activated cell sorting technology (Miltenyi Biotec, Bergisch Gladbach, Germany) was used to purify lymphocyte populations according to the manufacturer's instructions. B220 mAb-coated microbeads and $CD4^+$ and $CD8^+$ T-cell isolation kits (Miltenyi Biotec) were used to purify B cells, $CD4^+$ T cells, and $CD8^+$ T cells, respectively. When necessary, the cells were enriched a second time using a fresh magnetic-activated cell sorting column to obtain purities >95%.

In Vitro T-Cell Proliferation Assays

On day 50, 3×10^5 purified $CD4^+$ or $CD8^+$ T cells harvested from the spleen or draining lymph nodes were cultured in 96-well plates in 200 μL of complete medium (RPMI 1640 containing 10% fetal calf serum, 200 μg/mL penicillin, 200 U/mL streptomycin, 4 mmol/L L-glutamine, and 5×10^{-5} mol/L 2-mercaptoethanol; all from Invitrogen, Carlsbad, CA) with 1.5×10^5 mitomycin C (Sigma-Aldrich)-treated splenic B cells and 200 μg/mL OVA. 5-Bromo-2′-deoxyuridine (BrdU; cell proliferation BrdU ELISA; Roche, Indianapolis, IN) was added during the final 2 hours of 4-day cultures. BrdU incorporation was then assessed by measuring absorbance at 450 nm.

Analysis of *in Vitro* Cytokine Synthesis

Single-cell suspensions of the spleen and draining lymph nodes were prepared in complete medium. Cells were cultured in complete medium at 2×10^6/mL in 24-well plates in the presence of 200 μg/mL OVA. Supernatant was collected after 96 hours of culture. The levels of IL-4, IL-10, IL-13, IL-17, and interferon (IFN)-γ in the supernatants were determined by ELISA according to the manufacturer instructions (R&D Systems, Minneapolis, MN).

Adoptive Transfer of B Cells

Splenic B cells were purified using CD19 mAb-coated microbeads (Miltenyi Biotech). The cells were enriched a second time using a fresh magnetic-activated cell sorting column to obtain purities >95%. Then, 2×10^7 $CD19^+$ B cells from naive WT mice were transferred intravenously into *CD19*$^{-/-}$ mice. Two days later, the recipient mice were epicutaneously sensitized with OVA to induce allergic skin inflammation.

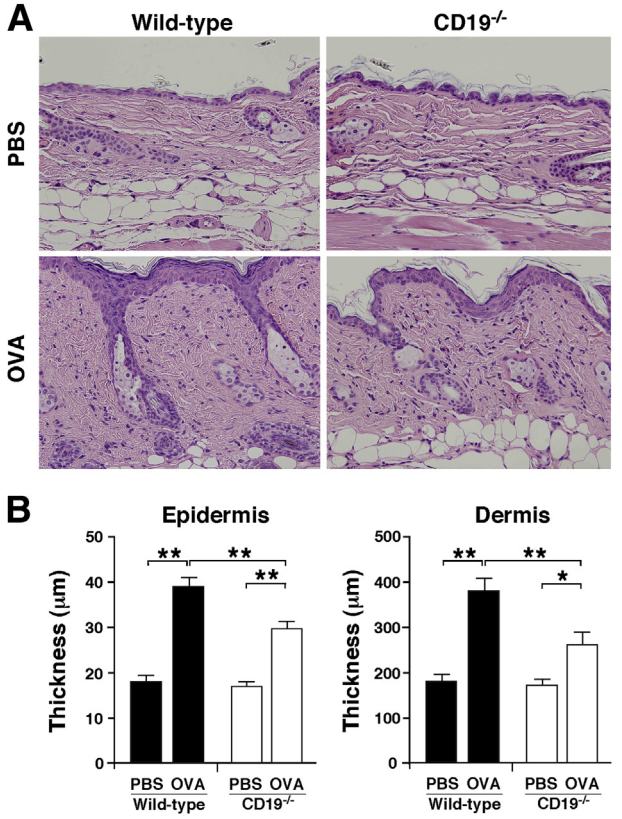

Figure 1 Decreased allergic skin inflammation in *CD19*$^{-/-}$ mice. The shaved back skin of WT and *CD19*$^{-/-}$ mice was epicutaneously sensitized with PBS or OVA. **A:** Representative skin sections from WT and *CD19*$^{-/-}$ mice stained with H&E. Original magnification, ×200. **B:** Epidermal and dermal thickness in WT and *CD19*$^{-/-}$ mice. Values represent means ± SEM from $n \geq 5$ mice per group. Significant differences between sample means are indicated as $^*P < 0.05$, $^{**}P < 0.01$. Similar results were obtained in at least two independent experiments.

Statistical Analysis

All data are expressed as means ± SEM. The *U*-test was used for determining the level of significance of differences in sample means, and the Bonferroni test was used for multiple comparisons.

Results

Decreased Severity of OVA-Sensitized Allergic Skin Inflammation in *CD19*$^{-/-}$ Mice

To assess whether CD19 expression played a role in the pathogenesis of OVA-sensitized allergic skin inflammation, we sensitized *CD19*$^{-/-}$ and WT mice with OVA epicutaneously

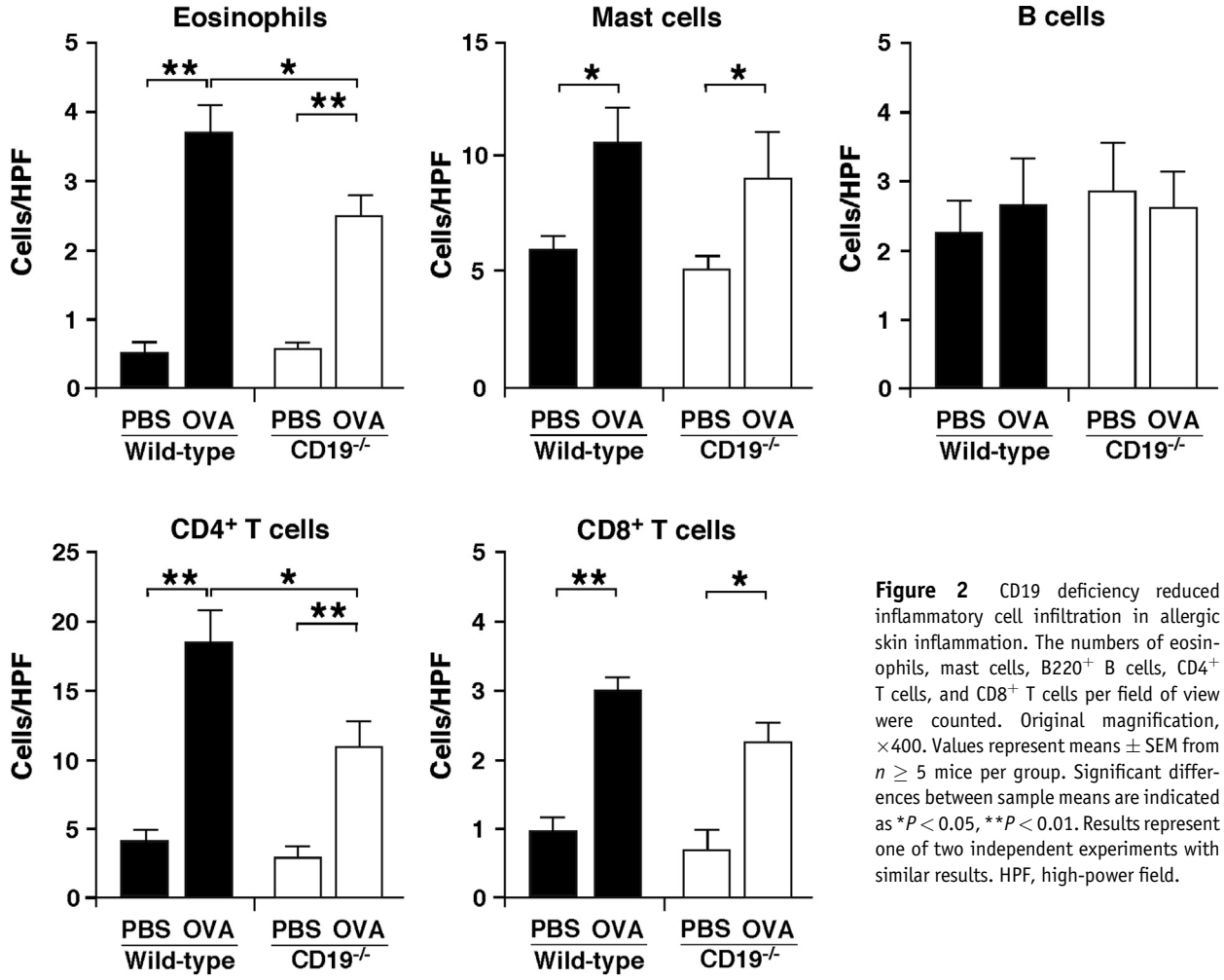

Figure 2 CD19 deficiency reduced inflammatory cell infiltration in allergic skin inflammation. The numbers of eosinophils, mast cells, $B220^+$ B cells, $CD4^+$ T cells, and $CD8^+$ T cells per field of view were counted. Original magnification, ×400. Values represent means ± SEM from $n \geq 5$ mice per group. Significant differences between sample means are indicated as $^*P < 0.05$, $^{**}P < 0.01$. Results represent one of two independent experiments with similar results. HPF, high-power field.

over 7 weeks, and the site of repeated sensitization was histopathologically assessed. Epicutaneous sensitization with OVA induced thickening of the epidermis and dermis in both WT and *CD19*$^{-/-}$ mice, but to a lesser degree in *CD19*$^{-/-}$ mice (Figure 1). Furthermore, OVA sensitization significantly increased the numbers of eosinophils, mast cells, and $CD4^+$ and $CD8^+$ T cells in both WT and *CD19*$^{-/-}$ mice, but the numbers of eosinophils and $CD4^+$ T cells were significantly lower in *CD19*$^{-/-}$ than in WT mice after sensitization with OVA (Figure 2). There were no significant differences in the numbers of mast cells, B cells, and $CD8^+$ T cells between WT and *CD19*$^{-/-}$ mice. These results show that allergic skin inflammation was suppressed in *CD19*$^{-/-}$ mice compared with WT mice.

CD19 Deficiency Inhibits OVA-Specific Ab Production

The effect of CD19 deficiency on serum Ab responses was also assessed after repeated OVA sensitization. OVA-sensitized WT mice were able to mount OVA-specific IgG1, IgG2a, and IgE Ab responses following sensitization, whereas no OVA-specific Abs were detected in the serum of PBS-sensitized mice (Figure 3). By contrast, the serum levels of IgG1, IgG2a, and IgE anti-OVA Abs remained significantly lower in *CD19*$^{-/-}$ mice sensitized with OVA; levels in these animals were not significantly higher than those in WT mice sensitized with PBS. Thus, CD19 deficiency significantly attenuated OVA-specific Ab production in OVA-sensitized allergic skin inflammation.

The Effects of Repeated OVA Sensitization on the Numbers of Regulatory B Cells and T Cells

The effects of CD19 deficiency on the numbers of $CD4^+CD25^+FoxP3^+$ regulatory T cells in the spleen and draining lymph nodes, peritoneal $CD5^+B220^+$ B1-a cells, and splenic $CD1d^{hi}CD5^+$ regulatory B cells (B10 cells)[19] were also assessed by flow cytometry after repeated OVA sensitization. Without OVA sensitization, *CD19*$^{-/-}$ mice had significantly reduced $B220^+$ B cells in the spleen and peritoneal cavity compared with WT mice ($P < 0.01$), whereas $B220^+$ B-cell numbers in the draining lymph nodes were comparable between WT and *CD19*$^{-/-}$ mice (Table 1). The numbers of $B220^+$ B cells in the spleen, draining lymph nodes, and peritoneal cavity increased during OVA-sensitized allergic skin inflammation in both WT and *CD19*$^{-/-}$ mice,

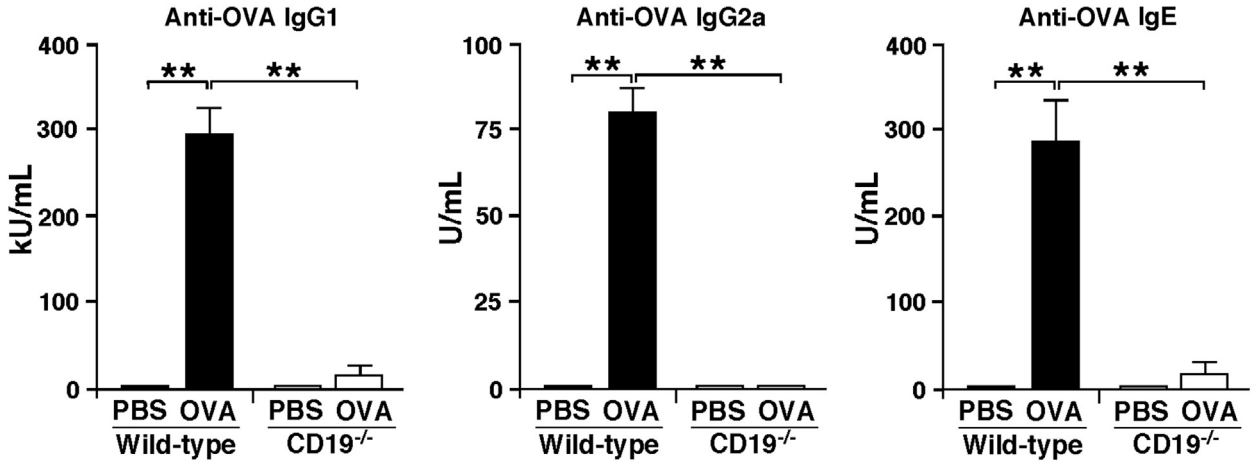

Figure 3 Impaired antigen-specific Ab production in *CD19*$^{-/-}$ mice. Serum OVA-specific IgG1, IgG2a, and IgE concentrations in OVA-sensitized WT and *CD19*$^{-/-}$ mice. Values represent means ± SEM from $n \geq 5$ mice per group. Significant differences between sample means are indicated as $^{**}P < 0.01$. Similar results were obtained in at least two independent experiments.

although these changes were not significant. The numbers of regulatory T cells in the spleen and draining lymph nodes were significantly increased during OVA-sensitized allergic skin inflammation in both WT and *CD19*$^{-/-}$ mice ($P < 0.05$ and $P < 0.01$, respectively). The numbers of regulatory T cells in the spleen and draining lymph nodes were comparable in OVA-sensitized WT and *CD19*$^{-/-}$ mice. The numbers of peritoneal B-1a cells were significantly decreased in *CD19*$^{-/-}$ mice compared with WT mice, as previously described,[23] but there was no effect on these peritoneal B-1a cell numbers after OVA sensitization of either WT or *CD19*$^{-/-}$ mice. Moreover, OVA sensitization did not affect splenic regulatory B-cell numbers in WT mice, whereas no significant numbers of regulatory B cells were observed in *CD19*$^{-/-}$ mice, regardless of OVA sensitization as described.[19] Thus, repeated epicutaneous OVA sensitization affected the numbers of regulatory T cells, which did not correlate with the observed decreased disease severity in *CD19*$^{-/-}$ mice.

CD19 Deficiency Attenuates OVA-Specific CD4$^+$ T-Cell Proliferation

The effects of CD19 deficiency on OVA-specific CD4$^+$ and CD8$^+$ T cell responses was evaluated after repeated OVA sensitization. Spleen and draining lymph node CD4$^+$ and CD8$^+$ T cells were purified after OVA sensitization, and OVA-specific T-cell proliferation was quantified *in vitro* using purified mitomycin C–treated B cells from WT mice sensitized with OVA as antigen-presenting cells in the presence of PBS or OVA. Spleen and draining lymph node CD4$^+$ T-cell recall responses to OVA in OVA-sensitized *CD19*$^{-/-}$ mice were reduced by 40% ($P < 0.01$) and 66% ($P < 0.01$) compared to OVA-sensitized WT mice, respectively (Figure 4A). By contrast, OVA-specific CD8$^+$ T-cell proliferation was equivalent in WT and *CD19*$^{-/-}$ mice (Figure 4B). Thus, CD19 deficiency impaired the expansion of antigen-specific CD4$^+$ T cells, but not CD8$^+$ T cells, following OVA sensitization.

Th2 and Th17 Cytokine Production Is Decreased in *CD19*$^{-/-}$ Mice

Because CD19 regulates OVA-specific T-cell proliferation, it is possible that CD19 also affects cytokine production in response to OVA stimulation. Therefore, we stimulated OVA-sensitized splenocytes and draining lymph node cells with PBS or OVA *in vitro* and, using ELISA, examined whether the loss of CD19 affected cytokine secretion.

Table 1 Cell Numbers in Wild-Type and *CD19*$^{-/-}$ Mice

		Cell number ($\times 10^{-5}$)			
Tissue	Subset	Wild-type PBS	*CD19*$^{-/-}$ PBS	Wild-type OVA	*CD19*$^{-/-}$ OVA
Spleen	B220$^+$	499 ± 45	235 ± 32**	532 ± 58	262 ± 39**
	CD1d ^{hi}CD5$^+$B220$^+$	16 ± 3	0.2 ± 0.1**	20 ± 4	0.3 ± 0.1**
	CD4$^+$CD25$^+$FoxP3$^+$	15 ± 2	14 ± 3	21 ± 3*	20 ± 4*
Draining lymph node	B220$^+$	11 ± 2	12 ± 3	14 ± 3	14 ± 2
	CD4$^+$CD25$^+$FoxP3$^+$	2.5 ± 0.4	2.3 ± 0.6	4.4 ± 0.8**	4.9 ± 1.0**
Peritoneal cavity	B220$^+$	12 ± 1.8	2.5 ± 0.4**	11 ± 2.2	2.3 ± 0.1**
	CD5$^+$B220$^+$	1.5 ± 0.4	0.09 ± 0.02**	1.6 ± 0.3	0.08 ± 0.03**

Data are expressed as means ± SEM of at least four mice.
$^*P < 0.05$, $^{**}P < 0.01$ versus PBS-sensitized wild-type mice.

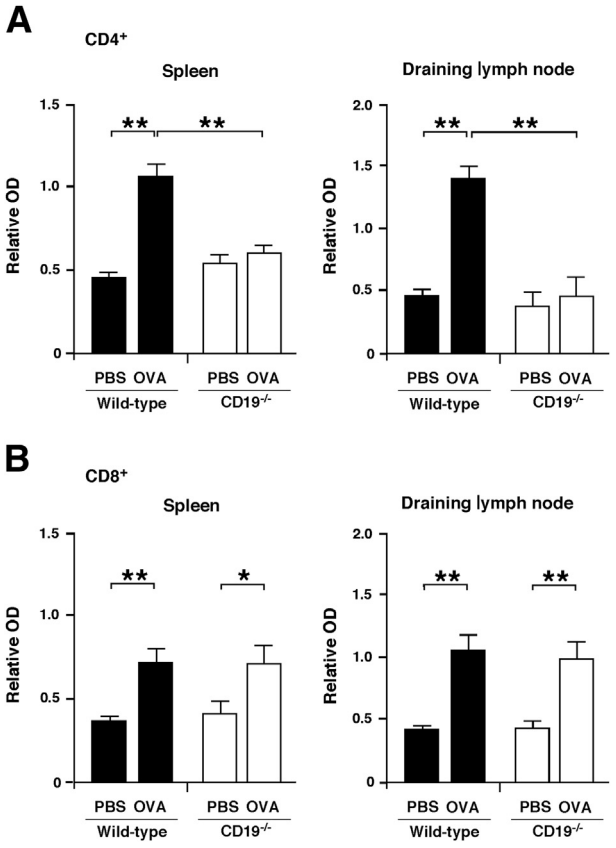

Figure 4 CD19 deficiency inhibits OVA-specific $CD4^+$ T-cell proliferation. $CD4^+$ (**A**) or $CD8^+$ (**B**) T cells were purified from spleen or draining lymph nodes and cultured with mitomycin C-treated B cells from OVA-sensitized WT mice in the presence of PBS or OVA. Values represent means ± SEM BrdU incorporation from triplicate cultures. All data are representative of two independent experiments; $n \geq 5$ mice per group. Significant differences between sample means are indicated as $^*P < 0.05$, $^{**}P < 0.01$. OD, optical density.

OVA-sensitized WT splenocytes secreted more IL-4, IL-13, IL-17, and IL-10 than PBS-sensitized WT splenocytes (Figure 5A). OVA-sensitized *CD19*$^{-/-}$ splenocytes exhibited reduced secretion of IL-4, IL-13, IL-17, and IL-10 compared to OVA-sensitized WT splenocytes. Similarly, WT draining lymph node cells treated with OVA showed increased IL-4, IL-13, IL-17, and IL-10 production compared to cells treated with PBS, whereas draining lymph node cells from OVA-sensitized *CD19*$^{-/-}$ mice secreted significantly less IL-4, IL-13, IL-17, and IL-10 relative to those from OVA-sensitized WT mice (Figure 5B). Thus, CD19 deficiency decreased Th2 and Th17 cytokine secretion in an OVA-sensitized model of allergic skin inflammation.

Adoptive Transfer of $CD19^+$ B Cells Restored the Severity of Allergic Skin Inflammation in *CD19*$^{-/-}$ Mice

Genetic deficiency of CD19 may affect normal T-cell development and impair allergic skin inflammation. Therefore, we next assessed whether CD19 expression in B cells

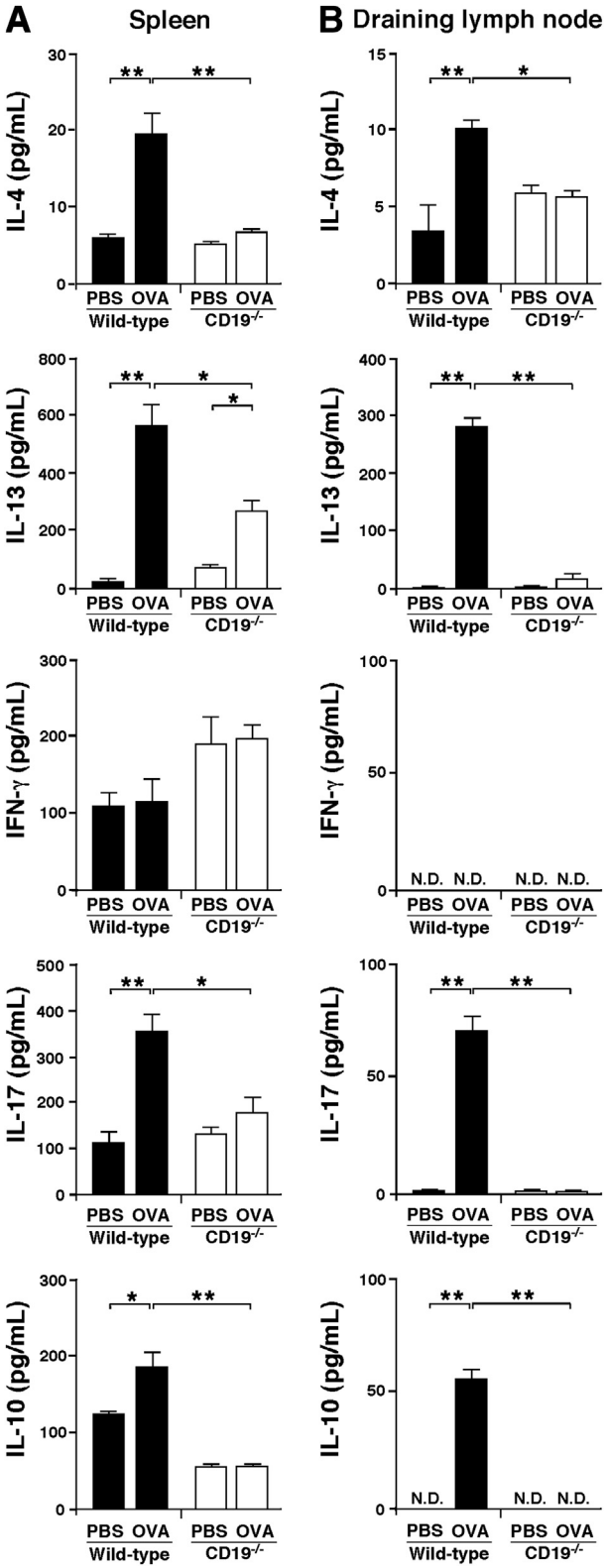

Figure 5 CD19 deficiency suppresses Th2 and Th17 cytokine production. IL-4, IL-13, IFN-γ, IL-17, and IL-10 secretion by splenocytes (**A**) and draining lymph node cells (**B**) from OVA-sensitized WT and *CD19*$^{-/-}$ mice after *in vitro* PBS or OVA stimulation. Values represent means ± SEM from $n \geq 5$ mice per group. Significant differences between sample means are indicated as $^*P < 0.05$, $^{**}P < 0.01$. Similar results were obtained in at least two independent experiments. N.D., not detected.

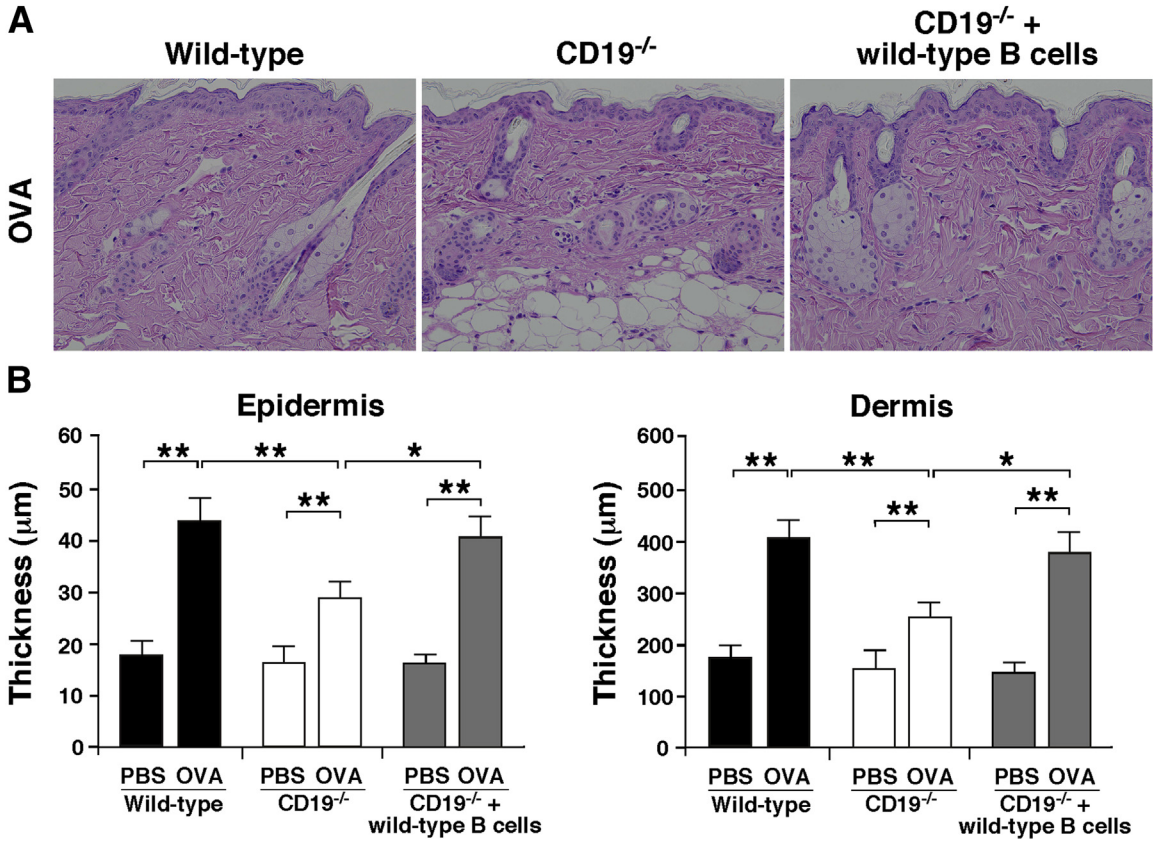

Figure 6 Adoptive transfer of WT B cells in allergic skin inflammation. WT splenic B cells were purified from naive WT mice and transferred into *CD19*$^{-/-}$ mice. Two days later, the recipient mice were epicutaneously sensitized with OVA to induce allergic skin inflammation. **A:** Representative skin sections stained with H&E from WT, *CD19*$^{-/-}$, and WT B-cell—transferred *CD19*$^{-/-}$ mice. Original magnification, ×200. **B:** Epidermal and dermal thickness in WT, *CD19*$^{-/-}$, and WT B-cell—transferred *CD19*$^{-/-}$ mice. Values represent means ± SEM from $n \geq 4$ mice per group. Significant differences between sample means are indicated as $^{*}P < 0.05$, $^{**}P < 0.01$.

was responsible *in vivo* for allergic skin inflammation. B cells were purified from the spleen of naive WT mice and transferred to *CD19*$^{-/-}$ mice before OVA sensitization (2×10^7 cells, >95% CD19$^+$). Transferring WT B cells into *CD19*$^{-/-}$ mice significantly enhanced thickening of the epidermis and dermis ($P < 0.05$ for both) (Figure 6). The *CD19*$^{-/-}$ mice that received WT B cells developed allergic skin inflammation of the same severity as that in WT mice. Therefore, reduced allergic skin inflammation of *CD19*$^{-/-}$ mice is enhanced to normal levels when spleen B cells from WT mice are transferred, indicating the pathogenic role of CD19 expression in B cells.

Discussion

Previous studies have shown that B-cell depletion with CD20 mAb reduces CD4$^+$ T-cell activation during immune responses to low-dose, but not high-dose, antigens.[29] B-cell depletion also suppresses both arthritogenic collagen-specific CD4$^+$ T-cell proliferation in murine collagen-induced arthritis and B-cell—specific CD4$^+$ T-cell proliferation in pancreatic lymph nodes of NOD mice.[29,30] Consistently, the results of the present study showed that CD19 expression in B cells is required for antigen-specific CD4$^+$ T-cell activation following epicutaneous sensitization. Therefore, it is most likely that B cells contribute to antigen-specific CD4$^+$ T-cell activation and expansion during allergic skin inflammation. Moreover, antigen-specific B cells are considered to be essential for Th2 cell development.[31] B cells control parasite infection by promoting Th2 cell development in mice.[32,33] Of note, the results of the present study show that CD19 expression in B cells promoted, not only IL-4 and IL-13, but also IL-17 production. It has been reported that the numbers of Th17 cells are increased in patients with asthma and acute AD.[34,35] In addition, B-cell—depletion therapy with CD20 mAb inhibits Th17 responses in patients with rheumatoid arthritis,[36] suggesting the potential role of B cells in Th17 responses. Thus, B cells are likely to enhance both Th2 and Th17 responses, thereby regulating allergic inflammatory responses. By contrast, IL-17 expression in patients with AD is significantly decreased compared with those with psoriasis, a representative Th17-dominant disease, although it is slightly increased compared with healthy individuals.[37] Furthermore, we have examined IL-17 mRNA expression using real-time PCR and found that expression in the inflamed skin was not affected by OVA sensitization in

either WT or *CD19*$^{-/-}$ mice (data not shown). Therefore, the role of Th17 cells in allergic diseases remains largely unclear. Additional studies will therefore be required to clarify the role of Th17 responses in AD.

B cells play both positive effector and negative regulatory roles during immune responses.[13] CD19 deficiency may either decrease or increase inflammation depending on the disease model. It has previously been demonstrated that CD19 deficiency is beneficial in mouse models of systemic sclerosis,[38] rheumatoid arthritis,[39] and pulmonary fibrosis,[40] whereas CD19 deficiency enhances inflammation in the contact hypersensitivity response,[19] an experimental autoimmune encephalomyelitis model of human multiple sclerosis,[41] and a dextran sulfate sodium–induced colitis model of human ulcerative colitis.[18] Because *CD19*$^{-/-}$ mice have few, if any, CD1d^{hi}CD5^{+} regulatory B10 cells,[19] CD19 deficiency may lead to worsening of the inflammatory response in diseases that have a predominance of regulatory B cells relative to effector B cells. Our experimental findings revealed that the splenic B10 cell subset did not have a critical role in the inhibition of OVA-sensitized allergic skin inflammation. Consistently, rituximab-induced B-cell depletion in humans led to the improvement of AD,[26,27] suggesting the predominance of effector B cells relative to regulatory B cells in this condition. However, it is also possible that the balance between opposing positive and negative regulatory B-cell functions changes during the course of disease in AD. In mice, B-cell depletion before experimental autoimmune encephalomyelitis induction causes a deterioration in disease symptoms, whereas B-cell depletion after the development of experimental autoimmune encephalomyelitis symptoms inhibits pathogenic T-cell expansion and decreases disease severity.[42] Further studies are needed to determine the contribution of each B-cell subset to the pathogenesis of AD.

The absence of CD19 from birth affects normal B-cell development, and the number of B220^{+} B cells was found to be significantly reduced in *CD19*$^{-/-}$ mice.[22,43] The proliferative capacity of *CD19*$^{-/-}$ B cells to mitogens is also lower than WT B cells.[22] Therefore, suppressed allergic skin inflammation in *CD19*$^{-/-}$ mice is likely due to the decreased B-cell numbers and/or impaired proliferation capacity of B cells, resulting in impaired allergic skin inflammation. Moreover, it was reported that peritoneal B-1a cells are also decreased in *CD19*$^{-/-}$ mice compared with WT mice,[23] although it has also been shown that peritoneal B-1a cells promote Th17 cell differentiation in mice.[44] Thus, reduced peritoneal B-1a cells may decrease IL-17 secretion in *CD19*$^{-/-}$ mice. Future studies may determine the precise mechanisms by which B-cell CD19 expression is involved in allergic skin inflammation.

In conclusion, the current findings demonstrate that CD19 expression in B cells plays a pathogenic role in OVA-sensitized skin inflammation by enhancing antigen-specific CD4^{+} T-cell expansion and Th2 and Th17 responses. Currently, patients with AD are treated with corticosteroids and immunosuppressive drugs, but these therapies can cause severe side effects such as infection and may fail in some severe cases. The therapeutic use of B-cell–targeted therapies that effectively eliminate B cells or simply reduce B-cell hyperactivity may hold promise for the treatment of allergic skin diseases such as AD. Examining the effects of B-cell depletion in a murine model of OVA-sensitized skin inflammation with an intact immune system using mouse anti-mouse CD20 mAb can provide a preclinical test for B-cell–based immunotherapy for AD in humans.

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