CD19 Expression in B Cells Is Important for Suppression of Contact Hypersensitivity

Rei Watanabe,^{*,†} Manabu Fujimoto,^{*,‡}
Nobuko Ishiura,^{*} Yoshihiro Kuwano,^{*}
Hiroko Nakashima,^{*} Norihito Yazawa,^{*}
Hitoshi Okochi,[†] Shinichi Sato,[§]
Thomas F. Tedder,[¶] and Kunihiko Tamaki^{*}

From the Department of Dermatology,^{*} Faculty of Medicine, University of Tokyo, Tokyo, Japan; the Department of Regenerative Medicine,[†] Research Institute, International Medical Center of Japan, Tokyo, Japan; the Department of Dermatology,[‡] Kanazawa University Graduate School of Medical Science, Kanazawa, Japan; the Department of Dermatology,[§] Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; and the Department of Immunology,[¶] Duke University Medical Center, Durham, North Carolina

Contact hypersensitivity (CHS) is a cutaneous immune reaction mediated mainly by antigen-specific effector T cells and is regarded as a model for Th1/Tc1-mediated inflammation. However, recent reports have suggested pivotal roles of B cells in CHS. CD19 serves as a positive B-cell response regulator that defines signaling thresholds critical for B-cell responses. In the current study, we assessed the role of the B-cell-specific surface molecule CD19 on the development of CHS by examining CD19-deficient mice. Although CD19-deficient mice are hyposensitive to a variety of transmembrane signals, CD19 loss resulted in increased and prolonged reaction of CHS, suggesting an inhibitory role of CD19 expression in CHS. Sensitized lymph nodes and elicited ear lesions from CD19-deficient mice exhibited Th1/Tc1-shifted cytokine profile with increased interferon- γ expression and decreased interleukin-10 expression. Adoptive transfer experiments revealed that CD19 expression in recipient mice was required for optimal suppression of CHS response, indicating its role in the elicitation phase. Furthermore, spleen B cells, especially marginal zone B cells, from wild-type mice were able to normalize exaggerated CHS reactions in CD19-deficient mice. Thus, CD19 expression in B cells is critical for termination of CHS responses, possibly through the function of regulatory B cells. (*Am J Pathol* 2007, 171:560–570; DOI: 10.2353/ajpath.2007.061279)

Delayed-type hypersensitivity and related contact hypersensitivity (CHS) are cutaneous immune reactions mediated by antigen-specific effector T cells. The CHS response develops in two distinct phases: sensitization and elicitation. In mice, the CHS sensitization phase is developed by primary skin painting immunization on the body with reactive hapten antigen. Then CHS effector T cells are activated by binding to complexes of antigen peptides and major histocompatibility complex molecules on antigen-presenting cells. Cytokines produced by Tc1 cells [interferon (IFN)- γ], Th1 cells [interleukin (IL)-2, IFN- γ , and tumor necrosis factor- α], Th2 cells (IL-4 and IL-10), and Langerhans cells (IL-12 and IL-18) are essential for the optimal initiation of sensitization phase.^{1–3} Subsequently, CHS elicitation phase is induced by re-exposure via painting the same reactive hapten at a separate skin site. Soon after the elicitation of the local secondary response by antigen challenge, very small numbers of circulating sensitized antigen-specific T cells are recruited into the extravascular space at the skin challenge site from the circulation and then interact again with antigen/peptide-major histocompatibility complexes on antigen-presenting cells. Activated T cells release proinflammatory cytokines such as IFN- γ , which induce local tissue cells to produce chemokines that recruit and activate an infiltrate of bone marrow-derived leukocytes, leading to characteristic late 24- to 48-hour effector responses.^{4–8} It is known that in the elicitation phase the main effector cells are IFN- γ -producing CD8⁺ Tc1 cells.^{9–11} Thus, CHS is a model for type 1-mediated inflammation.^{5,6}

Recent studies on B cells have demonstrated that B cells regulate immune responses via various ways that had not been previously appreciated. In addition to Ig secretion, B cells have crucial roles such as antigen presentation, cytokine production, and the regulation of

Supported by the Ministry of Education, Science, and Culture of Japan (grant-in-aid to M.F.) and the National Institutes of Health (grants CA105001, CA96547, and AI56363 to T.F.T.).

Accepted for publication April 27, 2007.

Address reprint requests to Manabu Fujimoto, M.D., Department of Dermatology, Kanazawa University Graduate School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan. E-mail: fujimoto-m@umin.ac.jp.

lymphoid organogenesis, effector T-cell differentiation, and dendritic cell (DC) function. Accordingly, B cells have been demonstrated to play significant roles in the circumstances in which B cells had not been considered to participate primarily. Indeed, recent reports suggest important roles of B cells in CHS. For example, mice exposed to contact allergen showed an increase in the percentage of antigen-specific B-1 cells in the draining lymph nodes (LNs).^{6,12} In the early phase of elicitation, likewise, IgM/IgG isotype antibodies (Abs) produced by B cells are critical for mast cell and platelet activation, resulting in an increased vascular permeability via release of serotonin and tumor necrosis factor- α .^{13–15} Because B-cell fate and function are tightly regulated by signal transduction through B-cell receptor (BCR) and functionally interrelated cell-surface receptors, such as CD19, CD21, CD22, CD40, CD72, and Fc γ RIIb,¹⁶ modulation of these receptors may be a potential strategy for regulating CHS and other disorders mediated by type 1 response. Among them, CD19 is generally considered a positive B-cell response regulator that governs intrinsic and stimulant-dependent signaling thresholds in B cells.¹⁷ CD19 is a member of the Ig superfamily expressed only on B cells and follicular DCs.¹⁸ CD19 functions as a specialized adapter protein regulating Src family protein tyrosine kinases, phosphatidylinositol 3-kinase, and Vav and thus serves as a key molecule for multiple signaling pathways crucial for modulating basal and BCR-induced signals.^{19–23}

In the current study, we assessed the roles of CD19 in CHS. Surprisingly, although B cells from CD19-deficient (CD19^{-/-}) mice are hyposensitive to a variety of transmembrane signals,^{24–26} CD19 loss resulted in increased and prolonged reactivity of CHS, suggesting an inhibitory role of CD19 expression in the etiology of CHS.

Materials and Methods

Mice

C57BL/6 and BALB/c wild-type mice were purchased from Clea Japan Inc. (Tokyo, Japan). CD19^{-/-} (C57BL/6 $\times$ 129) mice were generated as described²⁴ and backcrossed onto C57BL/6 strain 12 times. All mice used were 8 to 12 weeks of age and were housed in a specific pathogen-free barrier facility. All studies and procedures were approved by the Animal Committee of International Medical Center of Japan.

Sensitization and Elicitation of CHS

Mice were sensitized with 25 μ l of 0.5% 2,4-dinitrofluorobenzene (DNFB; Sigma-Aldrich, St. Louis, MO), in acetone and olive oil (4:1), on shaved abdominal skin for 2 consecutive days. Five days later, CHS was elicited by applying 20 μ l of 0.25% DNFB on the right ear. The left ear was treated with acetone/olive oil alone. Ear thickness was measured in a blinded manner with a micrometer (Mitutoyo, Kawasaki, Japan) before and after the chal-

lenge for 240 hours, every 24 hours. The degree of CHS was determined as swelling of the hapten-challenged ear compared with that of the vehicle-treated ear and was expressed in mm $\times$ 10⁻² (mean $\pm$ SEM). Mice that were ear challenged without previous sensitization also served as negative controls. As for sensitization with fluorescein isothiocyanate (FITC), 400 μ l of 0.5% FITC (Sigma-Aldrich) in acetone and dibutylphthalate (1:1) was applied on shaved abdominal skin of each mouse, followed by loading 20 μ l of 0.5% FITC on the right ear and acetone/dibutylphthalate alone on the left ear (control) 5 days later. Ear thickness was evaluated as was in that of DNFB-elicited mice.

Histology

Ear samples were taken before and 24 and 120 hours after DNFB challenge and fixed in 4% formalin for routine histology with hematoxylin and eosin (H&E) staining. Alternatively, for immunohistological staining, samples were frozen in OCT compound (Sakura Finetek, Torrance, CA). Cryostat-cut tissue sections were fixed in acetone for 5 minutes and incubated with 10% normal rabbit serum in phosphate-buffered saline (PBS) (10 minutes, 37°C) to block nonspecific staining. Sections were then incubated with rat monoclonal Abs specific for mouse macrophages (F4/80; BD PharMingen, San Diego, CA), CD4 (RM4-5; BD PharMingen), CD8 (53-6.7; BD PharMingen), and B220 (RA3-6B2; BD PharMingen) at predetermined optimal concentration. Rat IgG (Southern Biotechnology, Birmingham, AL) was used as a control for nonspecific staining. Sections were incubated sequentially (20 minutes, 37°C) with a biotinylated rabbit anti-rat IgG and then horseradish peroxidase-conjugated avidin-biotin complexes (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were developed with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide and then counterstained with methyl green. The sections were examined with $\times$ 20 objective. Infiltrated cells were counted per one field of view. Five fields of view were selected at random in one section, and three samples were examined per each staining.

Adoptive Cell Transfer of Immune Response

Donor mice were treated with DNFB as above. Inguinal LN cells were harvested 5 days later from the same number of donors as recipients, and a mixture of 5 $\times$ 10⁶ cells was adoptively transferred intravenously, in 0.25 ml of PBS. B cells (2 $\times$ 10⁶ cells) or T cells (2 $\times$ 10⁶ cells) were alternately isolated from inguinal LN cell suspensions by autoMACS (Miltenyi Biotec, Auburn, CA) using anti-B220-coupled microbeads or anti-Thy-1.2-coupled microbeads, respectively (Miltenyi Biotec), and also transferred intravenously. To collect peritoneal B-1 cells, 5 ml of PBS was injected into the abdominal cavity of sensitized mice 5 days after sensitization and then reclaimed, followed by B220⁺CD5⁺ B-1 cell isolation by flow cytometry sorting using an Epics Altra flow cytometer

(Beckman Coulter, Miami, FL). Splenocytes were also harvested from DNFB-treated mice 5 days after sensitization or nonimmunized mice. B cells and T cells were further isolated from single cell suspensions by autoMACS (Miltenyi Biotec) using anti-B220-coupled microbeads and anti-Thy-1.2-coupled microbeads, respectively. In addition, splenic marginal zone (MZ) B cells were purified by sorting CD21^{high}CD23^{low} cells from splenocytes using an Epics Altra flow cytometer (Beckman Coulter). CD19⁺ DCs were purified by sorting CD11c⁺CD19⁺ cells by a flow cytometer from splenic cell suspensions that were prepared after a 1-hour incubation of spleen with collagenase D (Roche Diagnostics, Basel, Switzerland). Purity of each isolated population was always 95 to 98%. Then, splenocytes (2×10^7 cells), peritoneal B-1 cells (2×10^6 cells), splenic B-2 cells (10^7 cells), splenic T cells (10^7 cells), splenic MZ B cells (2×10^6 cells), or splenic CD19⁺ DCs (1×10^5 cells) were transferred intravenously to the recipients indicated. Ear thickness of the recipients, at least five mice per group, was measured with a micrometer before the transfers. Twenty-four hours later, the recipients were challenged on the right ear with 20 μ l of 0.25% DNFB as above and on the left ear with acetone/olive oil alone. The subsequent increases in ear thickness were determined at 24, 120, and 240 hours after challenge. The thickness of the control ears was subtracted from experimental responses to yield net ear swelling.

Immunofluorescence Analysis

Inguinal LNs were harvested from wild-type and CD19^{-/-} mice 5 days after the sensitization with DNFB. LNs from nontreated mice were used as control. Single cell suspensions were stained for two- and/or three-color immunofluorescence analysis at 4°C using FITC-conjugated anti-CD8, FITC-conjugated anti-CD25, phycoerythrin-conjugated anti-CD4, and phycoerythrin-Cy5-conjugated anti-Thy1.2 mAbs (BD PharMingen) at predetermined optimal concentrations for 20 minutes as described.²⁷ Erythrocytes were lysed after staining using FACS lysing solution (Becton Dickinson, San Jose, CA). Labeled cells were analyzed on an Epics Altra flow cytometer (Beckman Coulter) with fluorescence intensity shown on a 4-decade log scale. Positive and negative populations of cells were determined using unreactive isotype-matched Abs (Southern Biotechnology) as controls for background staining.

Mixed Leukocyte Reaction

Spleen CD4⁺ T cells from C57BL/6 wild-type mice, C57BL/6 CD19^{-/-} mice, and BALB/c wild-type mice were isolated from spleen cell suspensions by autoMACS using anti-CD4-coupled microbeads (Miltenyi Biotec). Splenic DCs were isolated using anti-CD11c-coupled microbeads (Miltenyi Biotec) after a 1-hour incubation of spleen with collagenase D (Roche Diagnostics) at 37°C. The isolated CD4⁺ T cells were stained with 2 μ mol/L

carboxyfluorescein diacetate succinimidyl ester (CFSE) using a Vybrant CFDA SE cell tracer kit (Molecular Probes, Eugene, OR). CD4⁺ T cells from C57BL/6 wild-type and CD19^{-/-} mice and DCs from BALB/c mice, or CD4⁺ T cells from BALB/c mice and DCs from C57BL/6 wild-type and CD19^{-/-} mice, were mixed at the indicated concentrations in RPMI 1640 culture medium including 10% bovine serum albumin, 10 mmol/L HEPES, and 55 μ mol/L 2-mercaptoethanol and then incubated in 96-well round-bottomed culture plates at 37°C under 5% CO₂ concentration. Five days later, CFSE fluorescence intensity of the surviving CD4⁺ T cells was analyzed using an Epics Altra flow cytometer (Beckman Coulter). Cells were stained by 5 μ g/ml propidium iodide (Sigma-Aldrich), and proliferation potency of CD4⁺ T cells and antigen-presenting capability of DCs were measured via the size of cells (forward scatter), viability (propidium iodide uptake), and degree of cell division (CFSE fluorescence intensity).

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Inguinal LNs taken 2 days after DNFB sensitization and ear specimens taken before and 24 and 120 hours after the DNFB challenge were homogenized in Isogen S (Wako, Tokyo, Japan), and total RNA was isolated following the instructions of the user's manual. RNA concentration was determined using NanoDrop (NanoDrop Technologies, Wilmington, DE) by A260 value of the samples. Total RNA was reverse transcribed to cDNA using a reverse transcription system with random hexamers (Promega, Madison, WI). Quantitative RT-PCR was performed using the TaqMan system (Applied Biosystems, Foster City, CA) on an ABI Prism 7000 sequence detector (Applied Biosystems) according to the manufacturer's instructions. TaqMan probes and primers for IFN- γ , IL-2, IL-4, IL-10, IL-12 p40, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems. Relative expression of real-time PCR products was determined using the $\Delta\Delta C_T$ technique. Nontreated inguinal LNs from wild-type mice were used as the calibrator. Briefly, each set of samples was normalized using the difference in threshold cycle (C_T) between the target gene and housekeeping gene (GAPDH): $\Delta C_T = (C_{T \text{ target gene}} - C_{T \text{ GAPDH}})$. Relative mRNA levels were calculated by the expression $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = \Delta C_{T \text{ sample}} - \Delta C_{T \text{ calibrator}}$. Each reaction was performed at least in triplicate.

Statistical Analysis

All data are shown as mean values $\pm$ SEM. Mann-Whitney *U*-test was used for determining the level of significance of differences between sample means. A *P* value less than 0.05 was considered statistically significant.

Figure 1. Augmented CHS reaction in CD19^{-/-} mice. **A:** Wild-type and CD19^{-/-} mice were sensitized by epicutaneous application of 0.5% DNFB, and thickness of ear lobes challenged with 0.25% DNFB or carrier (control) 5 days later was measured at indicated time points. **B:** Wild-type and CD19^{-/-} mice were sensitized and challenged by application of 0.5% FITC or carrier (control), and thickness of ear lobes were measured. Each experiment included five to seven mice and was repeated at least three times. **P* < 0.05, ***P* < 0.01.

Results

CD19^{-/-} Mice Exhibit Exaggerated CHS Reactions

To assess whether CD19 expression has a role in CHS, CD19^{-/-} and wild-type mice were challenged with DNFB after sensitization, and ear swelling was measured before and every 24 hours after challenge. DNFB-induced increase of ear thickness was observed as early as 12 hours after challenge and was significantly higher in CD19^{-/-} mice than in wild-type mice at 24 hours (*P* < 0.05; Figure 1A). In wild-type mice, ear swelling reached peak at 24 hours after challenge, then decreased gradually, and returned to normal approximately at 72 hours (Figure 1A). By contrast, CD19^{-/-} mice significantly exhibited prolonged and enhanced swelling of the challenged ear, which remained for more than 240 hours (Figure 1A). Thus, CD19^{-/-} mice demonstrated augmented and prolonged CHS reactions to DNFB. This increased response was not limited to CHS against DNFB, because CD19^{-/-} mice exhibited similarly augmented response to FITC application (Figure 1B), although somewhat less remarkable than DNFB application. Thus, CD19^{-/-} mice are more susceptible to CHS. The degree of CHS reaction was also assessed histopathologically. Cellular infiltrate and edema were ob-

served in the DNFB-painted ears of both CD19^{-/-} and wild-type mice 24 hours after challenge (Figure 2). The majority of the infiltrating cells were polymorphonuclear cells and lymphoid cells in both mice, although the number of infiltrating cells was markedly higher by threefold in CD19^{-/-} mice. The degree of edema was similarly enhanced in CD19^{-/-} mice. As for the ear specimens taken 120 hours after challenge, cell infiltration and edema almost disappeared in wild-type mice, whereas the ears of CD19^{-/-} mice still contained many infiltrating cells, accompanied with hyperkeratosis and acanthosis (Figure 2). Therefore, CD19^{-/-} mice exhibit more severe CHS response, both clinically and pathologically, than wild-type mice.

CD8⁺ T-Cell Infiltration Is Increased in the Inflamed Ear of CD19^{-/-} Mice with CHS Reaction

The profile of infiltrating cells in the ear lesion was further examined immunohistologically. At 24 hours, there was more infiltration of macrophages, polymorphonuclear cells, and lymphocytes in the inflamed ear of CD19^{-/-} mice than that of wild-type mice (Figure 3). The frequency of CD8⁺ T cells was 1.6-fold higher in CD19^{-/-} mice than in wild-type mice (*P* < 0.01), and polymorphonuclear cells also infiltrated in high rate (200% of wild type, *P* < 0.01). Consequently, CD4/CD8 ratio of the infiltrating T cells was significantly lower in the inflamed ear of CD19^{-/-} mice than that of wild-type mice (2.6 in wild-type and 1.2 in CD19^{-/-} mice at 24 hours, *P* < 0.01; Figure 3). This profile of infiltrating cells at 24 hours was similar to that at 120 hours except for polymorphonuclear cells, which were no longer observed at 120 hours (Figure 3). B-cell infiltration was not observed in wild-type or CD19^{-/-} mice (data not shown). There was no difference in numbers of CD4⁺ or CD8⁺ T cells or CD4/CD8 T-cell ratios in blood, LNs, or spleens between naive wild-type and CD19^{-/-} mice (Figure 4 and data not shown). In addition, there was no significant difference of CD4⁺CD25⁺ regulatory T-cell numbers in inguinal LNs between wild-type and CD19^{-/-} mice both before and after sensitization (Figure 4). However, the ratio of CD8⁺ T cells both in inguinal LNs and spleens increased after sensitization with DNFB in CD19^{-/-} mice, compared with wild-type mice [21.5 ± 1.4% in wild-type LN (*n* = 5) versus 31.4 ± 1.9% in CD19^{-/-} LN (*n* = 5), *P* < 0.01; Figure 4 and data not shown]. Therefore, CD8⁺ T-cell activation appeared relatively exaggerated in the CHS response in CD19^{-/-} mice compared with wild-type mice.

Increased IFN-γ and Decreased IL-10 Expression in CHS Reactions of CD19^{-/-} Mice

Figure 5 shows cytokine mRNA expression in DNFB-sensitized inguinal LNs. Inguinal LNs were harvested from wild-type and CD19^{-/-} mice 2 days after sensitization, and cytokine expression was analyzed by quantita-

Figure 2. Histopathology of CHS-elicited ear pinnae in wild-type and CD19^{-/-} mice. Wild-type and CD19^{-/-} mice were sensitized and challenged by DNFB as in Figure 1A. Representative sections before sensitization with DNFB (A), 24 hours after elicitation (B), and 120 hours after elicitation (C) of ear lobes from wild-type mice (top) and CD19^{-/-} mice (bottom). The sections were stained with H&E. Original magnifications, $\times 200$.

tive RT-PCR. IFN- γ mRNA expression was slightly but not significantly elevated in LNs from CD19^{-/-} mice compared with those from wild-type mice (Figure 5A). Expression levels other Th1/Tc1 cytokines including IL-2 and IL-12 p40 were equivalent between wild-type and CD19^{-/-} mice. By contrast, Th2 cytokine expressions, such as IL-4 and IL-10, were decreased in LNs from CD19^{-/-} mice compared with those from wild-type mice (IL-4, 57% of wild type; IL-10, 33% of wild type; Figure 5A). This tendency was similar in DNFB-painted ears. CHS-elicited ears of wild-type and CD19^{-/-} mice were resected 24 hours after paint, and mRNA expression of each ear extract was assessed. mRNA analysis of DNFB-painted ears of CD19^{-/-} mice demonstrated significantly increased expressions of IFN- γ and IL-2 $\times 10$ -fold and 5-fold, respectively, whereas IL-10 expression was significantly decreased to 38% of wild-type mice (Figure 5B). These data suggest that cytokine profiles are shifted to Th1/Tc1 in the CHS reaction of CD19^{-/-} mice.

CD19 Expression in Recipient Mice Influenced the Duration of CHS Reactions

To determine the phase of CHS responses that CD19 expression regulates, inguinal LN cells from wild-type mice were adoptively transferred to unsensitized CD19^{-/-} mice or unsensitized wild-type mice 5 days

after sensitization. Then, 24 hours after cell transfer, 20 μ l of 0.25% DNFB was loaded onto the ear of recipient CD19^{-/-} mice or wild-type mice. As in Figure 6A, ear swelling at 24 and 48 hours was comparable between recipient CD19^{-/-} mice and wild-type mice. By contrast, ear swelling remained significantly augmented in CD19^{-/-} mice at 120 and 240 hours, when CHS reaction already ceased in wild-type mice (Figure 6A). Likewise, when inguinal LN cells of sensitized CD19^{-/-} mice were adoptively transferred to naive CD19^{-/-} mice or naive wild-type mice, there was no significant difference in ear swelling between recipient CD19^{-/-} mice or wild-type mice at 24 and 48 hours, whereas recipient CD19^{-/-} mice showed significantly increased response than recipient wild-type mice at 120 and 240 hours (Figure 6A). When the donor cells were from CD19^{-/-} mice, the magnitude of the responses tended to be slightly exaggerated at 24 and 48 hours compared with the case in which sensitized wild-type LN cells were used (120% at 24 hours and 140% at 48 hours), although the differences were not significant. Therefore, although CHS is fully transferable to naive mice with sensitized LN cells from the donor wild-type or CD19^{-/-} mice, CD19 deficiency in the recipient mice prolongs the duration of the responses once CHS is induced. Collectively, these results suggest that CD19 expression in recipient mice is required for the optimal late-phase suppression of the CHS reactions.

Figure 3. Profile of infiltrating cells in CHS lesion in wild-type and CD19^{-/-} mice. The numbers of total infiltrating cells (A), CD4⁺ T cells and CD8⁺ T cells (B), macrophages (D), and polymorphonuclear cells (E) per one field of view (×200) in the DNFB-challenged ear lobes were counted. In C the rate of CD4⁺ T cells and CD8⁺ T cells is shown based on the numbers in B. **P* < 0.05, ***P* < 0.01.

B Cells from Sensitized LNs Suppress Augmented CHS Reaction in Recipient CD19^{-/-} Mice

Next, T cells or B cells were purified from inguinal LNs from sensitized mice and were transferred to wild-type or CD19^{-/-} mice. T cells were able to induce initial CHS reactions at the magnitude similar with those observed in whole cell transfer (Figure 6, A and B). CHS did not occur when B cells alone were transferred to naive mice (Figure 6C). Although acute CHS responses at 24 hours were

Figure 4. T lymphocytes present in LNs from CD19^{-/-} mice before or after sensitization. Inguinal LNs were harvested before or after sensitization with DNFB in wild-type and CD19^{-/-} mice. In A, cells were stained with anti-CD4 and anti-CD8 Abs and analyzed by two-color flow cytometric analysis. Numeric characters (%) indicate the rates of CD4⁺ cells and CD8⁺ cells in total lymphoid cells. In B, Thy1.2⁺ cells were gated and examined for CD4 and CD25 expression by three-color flow cytometric analysis. Numeric characters (%) in B indicate the rate of CD25⁺ cells in CD4⁺ cells in wild-type mice and CD19^{-/-} mice before and after sensitization. Results represent those obtained with five mice.

similar between whole cell transfer and T-cell transfer, augmented CHS responses in recipient CD19^{-/-} mice were more obvious and enhanced compared with recipient wild-type mice at 48 to 240 hours when T cells alone were transferred from wild-type or CD19^{-/-} mice (Figure 6, A and B). Recipient wild-type mice showed normal course and intensity of CHS responses regardless of the presence or absence of B cells from sensitized donor mice or genotype of donor mice (Figure 6, A and B). By contrast, whereas recipient CD19^{-/-} mice mounted similar responses between whole inguinal LN cell transfer from wild-type mice and from CD19^{-/-} mice (Figure 6A), T-cell transfer from CD19^{-/-} mice induced a more enhanced reaction in recipient CD19^{-/-} mice at 120 and 240 hours than T-cell transfer from wild-type mice (2.3- and 2.2-fold increase at 120 hours and 240 hours, re-

Figure 5. Cytokine expression in LNs and ear lobes of wild-type and CD19^{-/-} mice. Inguinal LNs 2 days after sensitization (A) and ear lobes 24 hours after elicitation (B) were collected from wild-type and CD19^{-/-} mice (five per group), and RNA was extracted from the specimens. The mRNA levels of IFN-γ, IL-2, IL-12 p40, IL-4, and IL-10 were analyzed by quantitative RT-PCR and normalized with internal control GAPDH. Data are shown as mean ± SEM from five mice. **P* < 0.05, ***P* < 0.01.

Figure 6. Adoptive cell transfer of LN cells in CHS reaction. Whole cells (A), T cells (B), and B cells (C) were prepared from inguinal LNs of sensitized wild-type or CD19^{-/-} mice and transferred to unsensitized mice as indicated. CHS was elicited 24 hours after transfer using DNFB. Ear thickness was measured as in Figure 1, and the ear swelling values indicate the difference between the ear thickness before and after each time point. Each experiment included three mice and was repeated at least three times. **P* < 0.05, ***P* < 0.01.

spectively; Figure 6B). Especially, comparison between whole cell transfer and T-cell transfer from CD19^{-/-} mice into recipient CD19^{-/-} mice demonstrated that T-cell transfer induced significantly enhanced ear swelling than whole cell transfer at 120 hours by 1.6-fold (Figure 6, A and B). Therefore, although weaker than wild-type B cells, even CD19^{-/-} B cells from sensitized LNs are also likely to have an inhibitory effect, which was obvious when recipient mice were deficient with CD19 expression. In addition, when inguinal LN T cells from sensitized CD19^{-/-} mice and inguinal LN B cells from sensitized wild-type mice were simultaneously transferred into CD19^{-/-} mice, wild-type B cells also showed an inhibitory effect on CHS response in CD19^{-/-} mice (data not shown). Collectively, whereas late-phase B cell-mediated suppression was dominantly dependent on CD19 expression in recipient mice, sensitized B cells from donor mice also had an additive inhibitory effect onto CHS reaction when wild-type B cells were absent in recipient mice.

Proliferation Potency of T Cells and Antigen-Presenting Capacity of DCs Are Comparable between Wild-Type and CD19^{-/-} Mice

The above data suggest that CD19 expression in B cells of nonimmune recipient mice suppresses CHS reaction and also that sensitized LN B cells are likely to have an inhibitory role especially when recipient mice lack CD19 expression. However, B-cell infiltration was not observed in the challenged ear of wild-type mice or CD19^{-/-} B cells (data not shown). Therefore, to test the possibility that intrinsic defect in T cells or DCs have an influence on

Figure 7. *In vitro* T-cell and DC function in CD19^{-/-} mice. **A:** Proliferation of splenic CD4⁺ T cells from CD19^{-/-} and wild-type mice in response to anti-TCR Ab. CFSE fluorescence intensity of CD4⁺ T cells 5 days after incubation. The rate of cells with reduced fluorescence intensity, ie, T cells with cell division, were regarded as the index reflecting cell proliferation and used in **B** and **C**. **B:** Proliferative response of splenic CD4⁺ T cells from CD19^{-/-} and wild-type mice by allogeneic DCs in mixed leukocyte reaction assays. Splenic CD4⁺ T cells of C57BL/6 wild-type or CD19^{-/-} mice were stained with CFSE, mixed with splenic DCs of BALB/c mice, and incubated for 5 days. The rate of the cells with reduced CFSE intensity was measured. **C:** Capacity of splenic DCs from CD19^{-/-} and wild-type mice to stimulate allogeneic CD4⁺ T cells in mixed leukocyte reaction assays. Splenic CD4⁺ T cells of BALB/c wild-type mice, stained with CFSE, were mixed with splenic DCs of C57BL/6 wild-type or CD19^{-/-} mice and incubated for 5 days. The rate of the T cells with reduced CFSE intensity was measured as in **B**.

augmented CHS reaction in CD19^{-/-} mice, mixed leukocyte reaction was performed. First, CFSE-stained CD4⁺ T cells from C57BL/6 wild-type or CD19^{-/-} mice were mixed with splenic DCs from BALB/c wild-type mice in the indicated concentrations. After a 5-day incubation, the rate of proliferated T cells was analyzed by flow cytometric analysis. As shown in Figure 7B, there was found no significant difference in the rate of proliferated T cells through the stimulation of BALB/c DCs between wild-type and CD19^{-/-} mice. Next, CFSE-stained CD4⁺ T cells from BALB/c mice were mixed with splenic DCs from C57BL/6 wild-type or CD19^{-/-} mice. DCs from wild-type and CD19^{-/-} mice similarly stimulated BALB/c CD4⁺ T cells (Figure 7C). Consequently, proliferation potency of T cells and antigen-presenting capacity of DCs *in vitro* are considered comparable between wild-type and CD19^{-/-} mice.

Spleen Cells from Sensitized Mice Have an Inhibitory Effect on CHS

In Figure 8, spleens were harvested from DNFB-sensitized wild-type or CD19^{-/-} mice and used for adoptive

Figure 8. Adoptive cell transfer of spleen cells in CHS reaction. Whole splenocytes (A), splenic T cells (B), and splenic B cells (C) were isolated from spleen of sensitized wild-type or CD19^{-/-} mice, and transferred to unsensitized mice as indicated. CHS was elicited 24 hours after transfer. Ear swelling was assessed as described in Figure 6. Each experiment included three mice and was repeated at least three times. **P* < 0.05, ***P* < 0.01.

cell transfer analysis. CHS was elicited in unsensitized recipient mice to which splenic cells were transferred. It is of note that CHS reaction in naive CD19^{-/-} mice ceased normally when spleen cells from wild-type mice were transferred, which contrasted with inguinal LN cell transfer (Figures 6A and 8A). Nonetheless, when spleen cells from sensitized CD19^{-/-} mice were transferred to naive wild-type or CD19^{-/-} mice, CHS response was augmented and prolonged as was observed in inguinal LN cell transfer (Figures 6A and 8A): ear swelling was comparable between recipient wild-type and CD19^{-/-} mice at 24 hours, whereas ear swelling remained significantly higher in recipient CD19^{-/-} mice at 48, 120, and 240 hours compared with recipient wild-type mice (Figure 8A). Therefore, augmented CHS reaction of CD19^{-/-} mice is suppressed to normal when spleen cells from sensitized wild-type mice were transferred, indicating an inhibitory role of spleen cells from wild-type mice, but not from CD19^{-/-} mice.

Spleen B Cells from Wild-Type Mice Suppress Augmented CHS Reaction in CD19^{-/-} Mice

To investigate further the inhibitory feature of spleen cells, sensitized spleen cells were isolated to B cells or T cells and transferred to naive wild-type or CD19^{-/-} mice (Figure 8, B and C). Sensitized spleen T cells were capable of eliciting the CHS reaction (Figure 8B), and CHS was not induced by B cells alone (Figure 8C). Importantly, sensitized spleen T cells from wild-type mice did not amend the prolonged ear swelling in CD19^{-/-} mice at 120 and 240 hours (Figure 8B), whereas the CHS reaction normally ceased when whole spleen cells from sensitized wild-type mice were transferred to CD19^{-/-} mice (Figure 8A). Furthermore, when spleen B cells from sen-

Figure 9. Adoptive transfer of sera and B-cell subsets in CHS reaction. Sera, peritoneal B-1 cells, splenic B-2 cells, and splenic MZ B cells from wild-type mice 5 days after sensitization (A) or before sensitization (B) were transferred to CD19^{-/-} mice. CHS was elicited 24 hours after transfer. Ear swelling was assessed as described in Figure 6. Each experiment included three mice and was repeated at least three times. **P* < 0.05, ***P* < 0.01.

sitized wild-type mice were transferred to CD19^{-/-} mice, ear swelling was suppressed to the levels in wild-type mice (Figure 9A). Consequently, splenic B cells from wild-type mice are suggested to have some inhibitory role in amending the prolonged CHS reactions in CD19^{-/-} mice.

Splenic MZ B Cells of Wild-Type Mice Have an Inhibitory Role in CHS

Next, to examine the inhibitory mechanisms of B cells, sera, peritoneal B-1 cells, and spleen MZ B cells as well as whole spleen B cells were harvested from sensitized or nonimmune wild-type mice and then transferred to CD19^{-/-} mice that were sensitized 5 days before. Recipient CD19^{-/-} mice were elicited for CHS 24 hours after transfer. By contrast to splenic B cells, neither sera nor peritoneal B-1 cells from sensitized wild-type mice affected ear swelling in CD19^{-/-} mice (Figure 9A). Notably, however, the CHS reaction of CD19^{-/-} mice normally ceased by CD21^{high}CD23^{low} MZ B-cell transfer from sensitized wild-type mice (Figure 9A). Furthermore, this suppression was equally observed when whole spleen B cells or MZ B cells was retained from naive wild-type mice that had not been sensitized with DNFB

Figure 10. Adoptive transfer of splenic CD19⁺ DCs in CHS reaction. Splenic CD19⁺ DCs from wild-type mice 5 days after sensitization were sorted and then transferred to CD19^{-/-} mice that were also sensitized 5 days before. PBS-injected wild-type or CD19^{-/-} mice served as controls. CHS was elicited 24 hours after transfer. Ear swelling was assessed as described in Figure 6. Each experiment included three mice and was repeated at least three times. **P* < 0.05, ***P* < 0.01.

(Figure 9B). Therefore, spleen MZ B cells of wild-type mice have an intrinsic inhibitory role in CHS.

Recently a novel population of murine splenic DCs expressing CD19 has been described.^{28,29} CD19⁺ DCs have been demonstrated to play a suppressive role in T-cell-mediated immunity.³⁰ To assess the possibilities that the inhibition of CHS responses by transferring wild-type splenic B cells resulted from the contamination of CD19⁺ splenic DCs and/or that the augmented CHS responses observed in CD19^{-/-} mice was attributable to the lack of CD19⁺ splenic DCs, we performed transfer experiments of splenic CD19⁺ DCs from wild-type mice in CHS (Figure 10). Splenic CD19⁺ DCs from sensitized wild-type mice were isolated and injected into sensitized CD19^{-/-} mice, followed by elicitation with DNFB. However, the exaggerated CHS reaction in CD19^{-/-} mice was not altered by CD19⁺ DCs from wild-type mice.

Discussion

CD19 is a B-cell-specific transmembrane molecule that positively regulates B-cell response to a range of extracellular signals.^{31,32} B cells from CD19^{-/-} mice are hyporesponsive to various stimuli,^{24–26} and consequently CD19^{-/-} mice generally exhibit an immunodeficient phenotype. However, the current study has demonstrated that CHS was more intense and prolonged in CD19^{-/-} mice than in wild-type mice (Figure 1). This was consistent with cytokine profiles of the skin and LNs, which were shifted to Th1/Tc1, in CD19^{-/-} mice (Figure 5). CD19 expression primarily influences elicitation phase rather than sensitization phase (Figure 6), and the duration of CHS reactions depends on CD19 expression in recipient mice in the transfer experiments. Nonetheless, because T cells from sensitized CD19^{-/-} mice can induce stronger CHS reactions in unsensitized CD19^{-/-} mice than those from sensitized wild-type mice (Figure 6, A and B), sensitized LN B cells also seem to have an inhibitory function. Considering that B cells were absent in the lesion and that no functional defects in T cells and DCs from CD19^{-/-} mice have been identified so far (Figure 7), the lack of CD19 expression in LN or spleen B cells seemed to influence effector T-cell activation and/or differentiation. Furthermore, transfer of LN or spleen B cells from sensitized wild-type mice can rescue the exaggerated response in CD19^{-/-} mice (Figures 6, 8, and 9), directly suggesting the presence of B cells that have an inhibitory

function in CHS. Importantly, MZ B cells from wild-type mice can resolve augmented CHS response in CD19^{-/-} mice (Figure 9). In addition, MZ B cells from naive wild-type mice can also achieve the same suppression. Collectively, CD19 expression in B cells is important for the suppression of CHS reaction.

Although it is generally considered that CHS is mediated mainly by CHS effector T cells, recent studies have suggested that B cells also have some important roles in eliciting CHS reactions. For instance, the local recruitment of effector T cells at the secondary challenge depends on a cascade involving antigen-specific IgM.³³ Furthermore, pan-B cell-deficient JH^{-/-} mice,³³ B-cell-deficient μ MT mice,³⁴ and Btk-defective Xid mice with B-1 cell deficiency and partial B-2 cell deficiency³⁵ have defective CHS responses, suggesting that CHS-initiating IgM Abs are produced by the B-1 cells in the thoracic and peritoneal cavity.³³ The current study has demonstrated that altered B-cell function can also enhance or prolong CHS responses. Intriguingly, CD19^{-/-} mice show significantly reduced number of B-1 cells in the peritoneal cavity.^{24,25} Serum Ig levels, especially IgM and IgG levels, are saliently decreased in CD19^{-/-} mice.²⁴ Both CD19 and Btk are critical signaling molecules that regulate B-cell development, activation, and survival.^{32,36} Although CD19 and Btk partially function along common signaling pathways,³⁷ CD19^{-/-} mice and Xid or Btk^{-/-} mice exhibit resembling but different phenotypes. For instance, Xid or Btk^{-/-} mice lack B-1a cells, whereas B-1a population is decreased but present in CD19^{-/-} mice. In addition, Xid mice have a decreased number of B-1b cells,³⁸ whereas CD19^{-/-} mice have normal number of B-1b cells.³⁹ Consequently, Xid mice are hyporesponsive to T-cell-independent type 2 antigen whereas CD19^{-/-} mice exhibit hyperresponsiveness.²⁶ Besides, Btk^{-/-} mice show augmented expressions of both Th1 and Th2 cytokines in immune responses,⁴⁰ whereas CD19^{-/-} mice show Th1-shifted cytokine balance.⁴⁰ In the current study, serum transfer or peritoneal B-1 cell transfer did not alter CHS reaction (Figure 9), suggesting that augmented CHS responses in CD19^{-/-} mice were not caused by B-1 cell functions or CHS-initiating IgM. CD19^{-/-} mice have a normal number of B-1b cells, which may be sufficient for CHS induction. Alternatively, since another recent study has demonstrated augmented CHS response in Btk^{-/-} mice,⁴⁰ mice strain difference for the dependency on IgM and/or B-1 cells may exist in CHS.

Recent studies have emphasized B cells as an important source of IFN- γ ,^{41–43} whereas B cells down-regulate Th1 response via the release of IL-10.^{44,45} In our study, increased IFN- γ and decreased IL-10 mRNA expressions in CD19^{-/-} mice were demonstrated, compared with wild-type mice, both in sensitized inguinal LNs and in CHS-elicited lesions (Figure 6). These data suggest that cytokine balance is shifted to Th1/Tc1 in CD19^{-/-} mice during CHS responses. In addition, we have previously demonstrated that CD19 expression influences IL-10 and IFN- γ productions from B cells positively and negatively, respectively.⁴⁶ Recently, effector B cells have been proposed to be

classified into effector B-1 (Be1) cells and effector B-2 (Be2) cells.⁴⁷ Furthermore, a line of studies have indicated that IL-10-producing regulatory B cells exist and play important roles in suppression of various diseases in mice and humans, including inflammatory bowel disease, experimental autoimmune encephalomyelitis (EAE), arthritis, and systemic lupus erythematosus.^{44,45,48–52} The augmented Th1 response in the absence of CD19 expression was also observed in EAE,⁴⁶ in which CD19^{−/−} mice exhibited higher clinical and pathological severity scores than wild-type mice and demonstrated specific increase of CD8⁺ T cells in spinal cord. Although the results in EAE and CHS have similarity, transferred wild-type B cells rescued augmented response of CHS but not EAE in CD19^{−/−} mice.⁴⁶ Instead, transferred CD19^{−/−} B cells exacerbated EAE induced in wild-type mice. Thus, it can be postulated that Be1 cells play a major role in exacerbating EAE in CD19^{−/−} mice and that the absence of regulatory B cells is responsible for augmented CHS reaction in CD19^{−/−} mice. It remains unknown how CD19 regulates cytokine production. However, because CD19 regulates BCR-induced phosphorylation of STAT1,⁵³ which has a critical role in IFN-induced response, alterations in STAT1 function by CD19 loss may affect the signaling pathway leading to lopsided cytokine manifestation. In addition, phosphatidylinositol 3-kinase deficiency results in skewed cytokine profile to Th1 in DCs.⁵⁴ Because CD19 regulates phosphatidylinositol 3-kinase activation,^{23,37,55} similar signaling mechanisms may also exist in B cells. Collectively, the loss of CD19 expression in B cells leads to the augmented Th1 cytokine production from B cells, which may exaggerate Th1/Tc1 responses mediated by T cells probably through the interaction in LNs and spleen. B cell-T cell interactions with B-cell antigen presentation favors Th2, rather than Th1, differentiation.^{56,57} Therefore, impaired ability of B-cell antigen presentation in CD19^{−/−} mice can lead to Th1-directed T-cell balance, resulting in increased IFN- γ and decreased IL-10 expression.

Several conclusions can be obtained from adoptive transfer experiments. First, CD19 expression in recipient mice (naive wild-type B cells) is required for normal termination of CHS. Second, sensitized LN B cells either from wild-type or CD19^{−/−} mice also have some inhibitory function when they were implanted to CD19^{−/−} mice. By contrast, splenic CD19⁺ DCs, which have been recently demonstrated to suppress T-cell responses, did not modify the prolonged CHS reaction in CD19^{−/−} mice. Third, transfer of splenic B cells, specifically MZ B cells, from naive wild-type mice can suppress augmented CHS in CD19^{−/−} mice. Intriguingly, CD19^{−/−} mice are known to lack MZ B cells.⁵⁸ Therefore, CD19 affects the development of MZ B cells, which may contain the fraction of regulatory B cells. Because MZ B cells share phenotypic and functional features with recently proposed regulatory B cells,^{48,59–62} CD19 may be critical for the development and function of regulatory B cells.

References

1. Fujisawa H, Kondo S, Wang B, Shivji GM, Sauder DN: The role of CD4 molecules in the induction phase of contact hypersensitivity cytokine profiles in the skin and lymph nodes. *Immunology* 1996, 89:250–255
2. Gorbachev AV, Fairchild RL: Induction and regulation of T-cell priming for contact hypersensitivity. *Crit Rev Immunol* 2001, 21:451–472
3. Wang B, Feliciani C, Howell BG, Freed I, Cai Q, Watanabe H, Sauder DN: Contribution of Langerhans cell-derived IL-18 to contact hypersensitivity. *J Immunol* 2002, 168:3303–3308
4. Marchal G, Seman M, Milon G, Truffa-Bachi P, Zilberfarb V: Local adoptive transfer of skin delayed-type hypersensitivity initiated by a single T lymphocyte. *J Immunol* 1982, 129:954–958
5. Kalish RS, Askenase PW: Molecular mechanisms of CD8⁺ T cell-mediated delayed hypersensitivity: implications for allergies, asthma, and autoimmunity. *J Allergy Clin Immunol* 1999, 103:192–199
6. Askenase PW: Allergy. Principles and Practice, ed 5. Edited by E Middleton, EF Ellis, JW Yunginger, CE Reed, NF Adkinson, WW Busse. St. Louis, Mosby Co., 1998, pp 323–341
7. Szczepanik M, Akahira-Azuma M, Bryniarski K, Tsuji RF, Kawikova I, Ptak W, Kiener C, Campos RA, Askenase PW: B-1 B cells mediate required early T cell recruitment to elicit protein-induced delayed-type hypersensitivity. *J Immunol* 2003, 171:6225–6235
8. Fong TA, Mosmann TR: The role of IFN- γ in delayed-type hypersensitivity mediated by Th1 clones. *J Immunol* 1989, 143:2887–2893
9. Enk AH: Allergic contact dermatitis: understanding the immune response and potential for targeted therapy using cytokines. *Mol Med Today* 1997, 3:423–428
10. Akiba H, Kehren J, Ducluzeau MT, Krasteva M, Horand F, Kaiserlian D, Kaneko F, Nicolas JF: Skin inflammation during contact hypersensitivity is mediated by early recruitment of CD8⁺ T cytotoxic 1 cells inducing keratinocyte apoptosis. *J Immunol* 2002, 168:3079–3087
11. Martín A, Gallino N, Gagliardi J, Ortiz S, Lascano AR, Diller A, Daraio MC, Kahn A, Mariani AL, Serra HM: Early inflammatory markers in elicitation of allergic contact dermatitis. *BMC Dermatol* 2002, 2:9–16
12. Gerberick GF, Cruse LW, Miller CM, Ridder GM: Selective modulation of B-cell activation markers CD86 and I-Ak on murine draining lymph node cells following allergen or irritant treatment. *Toxicol Appl Pharmacol* 1999, 159:142–151
13. Askenase PW, Bursztajn S, Gershon MD, Gershon RK: T cell-dependent mast cell degranulation and release of serotonin in murine delayed-type hypersensitivity. *J Exp Med* 1980, 152:1358–1374
14. van Loveren H, Meade R, Askenase PW: An early component of delayed-type hypersensitivity mediated by T cells and mast cells. *J Exp Med* 1983, 157:1604–1617
15. Askenase PW, Geba GP, Levin J, Ratzlaff RE, Anderson GM, Ushio H, Ptak W, Matsuda H: A role for platelet release of serotonin in the initiation of contact sensitivity. *Int Arch Allergy Immunol* 1995, 107:145–147
16. Tsubata T: Co-receptors on B lymphocytes. *Curr Opin Immunol* 1999, 11:249–255
17. Fujimoto M, Sato S: B cell signaling and autoimmune diseases: CD19/CD22 loop as a B cell signaling device to regulate the balance of autoimmunity. *J Dermatol Sci* 2007, 46:1–9
18. Tedder TF, Isaacs CM: Isolation of cDNAs encoding the CD19 antigen of human and mouse B lymphocytes. A new member of the immunoglobulin superfamily. *J Immunol* 1989, 143:712–717
19. Fearon DT, Carroll MC: Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu Rev Immunol* 2000, 18:393–422
20. Fujimoto M, Poe JC, Inaoki M, Tedder TF: CD19 regulates B lymphocyte responses to transmembrane signals. *Semin Immunol* 1998, 10:267–277
21. Tooze RM, Doody GM, Fearon DT: Counterregulation by the coreceptors CD19 and CD22 of MAP kinase activation by membrane immunoglobulin. *Immunity* 1997, 7:59–67
22. Fujimoto M, Fujimoto Y, Poe JC, Jansen PJ, Lowell CA, DeFranco AL, Tedder TF: CD19 regulates Src family protein tyrosine kinase activation in B lymphocytes through processive amplification. *Immunity* 2000, 13:47–57
23. Buhl AM, Pleiman CM, Rickert RC, Cambier JC: Qualitative regulation of B cell antigen receptor signaling by CD19: selective requirement

- for PI3-kinase activation, inositol-1,4,5-trisphosphate production and Ca^{2+} mobilization. *J Exp Med* 1997, 186:1897–1910
24. Engel P, Zhou LJ, Ord DC, Sato S, Koller B, Tedder TF: Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. *Immunity* 1995, 3:39–50
25. Rickert RC, Rajewsky K, Roes J: Impairment of T-cell-dependent B-cell responses and B-1 cell development in CD19-deficient mice. *Nature* 1995, 376:352–355
26. Sato S, Steeber DA, Tedder TF: The CD19 signal transduction molecule is a response regulator of B-lymphocyte differentiation. *Proc Natl Acad Sci USA* 1995, 92:11558–11562
27. Fujimoto M, Bradney AP, Poe JC, Steeber DA, Tedder TF: Modulation of B lymphocyte antigen receptor signal transduction by a CD19/CD22 regulatory loop. *Immunity* 1999, 11:191–200
28. Mellor AL, Baban B, Chandler PR, Manlapat A, Kahler DJ, Munn DH: Cutting edge: CpG oligonucleotides induce splenic CD19⁺ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN type 1 signaling. *J Immunol* 2005, 175:5601–5605
29. Baban B, Hansen AM, Chandler PR, Manlapat A, Bingaman A, Kahler DJ, Munn DH, Mellor AL: A minor population of splenic dendritic cells expressing CD19 mediates IDO-dependent T cell suppression via type I IFN signaling following B7 ligation. *Int Immunol* 2005, 17:909–919
30. Mellor AL, Munn DH: IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol* 2004, 4:762–774
31. Tedder TF, Poe JC, Fujimoto M, Haas KM, Sato S: The CD19-CD21 signal transduction complex of B lymphocytes regulates the balance between health and autoimmune disease: systemic sclerosis as a model system. *Curr Dir Autoimmun* 2005, 8:55–90
32. Fujimoto M, Poe JC, Hasegawa M, Tedder TF: CD19 regulates intrinsic B lymphocyte signal transduction and activation through a novel mechanism of processive amplification. *Immunol Res* 2000, 22:281–298
33. Tsuji RF, Szczepanik M, Kawikova I, Paliwal V, Campos RA, Itakura A, Akahira-Azuma M, Baumgarth N, Herzenberg LA, Askenase PW: B cell-dependent T cell responses: IgM antibodies are required to elicit contact sensitivity. *J Exp Med* 2002, 196:1277–1290
34. Macpherson AJ, Lamarre A, McCoy K, Harriman GR, Odermatt B, Dougan G, Hengartner H, Zinkernagel RM: IgA production without μ or δ chain expression in developing B cells. *Nat Immunol* 2001, 2:625–631
35. Hardy RR, Hayakawa K: CD5 B cells, a fetal B cell lineage. *Adv Immunol* 1994, 55:297–339
36. Satterthwaite AB, Witte ON: The role of Bruton's tyrosine kinase in B-cell development and function: a genetic perspective. *Immunol Rev* 2000, 175:120–127
37. Fujimoto M, Poe JC, Satterthwaite AB, Wahl MI, Witte ON, Tedder TF: Complementary roles for CD19 and Bruton's tyrosine kinase in B lymphocyte signal transduction. *J Immunol* 2002, 168:5465–5476
38. Riggs J, Howell K, Matechin B, Matlack R, Pennello A, Chiasson R: X-chromosome-linked immune-deficient mice have B-1b cells. *Immunology* 2003, 108:440–451
39. Haas KM, Poe JC, Steeber DA, Tedder TF: B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to *S. pneumoniae*. *Immunity* 2005, 23:7–18
40. Kawakami Y, Inagaki N, Salek-Ardakani S, Kitaura J, Tanaka H, Nagao K, Kawakami Y, Xiao W, Nagai H, Croft M, Kawakami T: Regulation of dendritic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3. *Proc Natl Acad Sci USA* 2006, 103:153–158
41. Pang Y, Norihisa Y, Benjamin D, Kantor RR, Young HA: Interferon-gamma gene expression in human B-cell lines: induction by interleukin-2, protein kinase C activators, and possible effect of hypomethylation on gene regulation. *Blood* 1992, 80:724–732
42. Dayton MA, Knobloch TJ, Benjamin D: Human B cell lines express the interferon gamma gene. *Cytokine* 1992, 4:454–460
43. Metzger DW, Vogel LA, Van Cleave VH, Lester TL, Buchanan JM: The effects of IL12 on B-cell subset function. *Res Immunol* 1995, 146:499–505
44. Fillatreau S, Sweeney CH, McGeachy MJ, Gray D, Anderton SM: B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002, 3:944–950
45. Mauri C, Gray D, Mushtaq N, Londei M: Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med* 2003, 197:489–501
46. Matsushita T, Fujimoto M, Hasegawa M, Komura K, Takehara K, Tedder TF, Sato S: Inhibitory role of CD19 in the progression of experimental autoimmune encephalomyelitis by regulating cytokine response. *Am J Pathol* 2006, 168:812–821
47. Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, Johnson LL, Swain SL, Lund FE: Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol* 2000, 1:475–482
48. Mizoguchi A, Bhan AK: A case for regulatory B cells. *J Immunol* 2006, 176:705–710
49. Korganow AS, Ji H, Mangialaio S, Duchatelle V, Pelanda R, Martin T, Degott C, Kikutani H, Rajewsky K, Pasquali JL, Benoist C, Mathis D: From systemic T cell self-reactivity to organ-specific autoimmune disease via immunoglobulins. *Immunity* 1999, 10:451–461
50. Yan J, Mamula MJ: B and T cell tolerance and autoimmunity in autoantibody transgenic mice. *Int Immunol* 2002, 14:963–971
51. Brummel R, Lenert P: Activation of marginal zone B cells from lupus mice with type A(D) CpG-oligodeoxynucleotides. *J Immunol* 2005, 174:2429–2434
52. Lenert P, Brummel R, Field EH, Ashman RF: TLR-9 activation of marginal zone B cells in lupus mice regulates immunity through increased IL-10 production. *J Clin Immunol* 2005, 25:29–40
53. Su L, Rickert RC, David M: Rapid STAT phosphorylation via the B cell receptor. Modulatory role of CD19. *J Biol Chem* 1999, 274:31770–31774
54. Fukao T, Tanabe M, Terauchi Y, Ota T, Matsuda S, Asano T, Kadowaki T, Takeuchi T, Koyasu S: PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat Immunol* 2002, 3:875–881
55. Carter RH, Fearon DT: CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science* 1992, 256:105–107
56. Stockinger B, Zal T, Zal A, Gray D: B cells solicit their own help from T cells. *J Exp Med* 1996, 183:891–899
57. Macaulay AE, DeKruyff RH, Goodnow CC, Umetsu DT: Antigen-specific B cells preferentially induce CD4⁺ T cells to produce IL-4. *J Immunol* 1997, 158:4171–4179
58. Wang Y, Brooks SR, Li X, Anzelon AN, Rickert RC, Carter RH: The physiologic role of CD19 cytoplasmic tyrosines. *Immunity* 2002, 17:501–514
59. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS, Bhan AK: Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. *Immunity* 2002, 16:219–230
60. Su TT, Guo B, Wei B, Braun J, Rawlings DJ: Signaling in transitional type 2 B cells is critical for peripheral B-cell development. *Immunol Rev* 2004, 197:161–178
61. Wei B, Velazquez P, Turovskaya O, Spricher K, Aranda R, Kronenberg M, Birnbaumer L, Braun J: Mesenteric B cells centrally inhibit CD4⁺ T cell colitis through interaction with regulatory T cell subsets. *Proc Natl Acad Sci USA* 2005, 102:2010–2015
62. Dalwadi H, Wei B, Schrage M, Spicher K, Su TT, Birnbaumer L, Rawlings DJ, Braun J: B cell developmental requirement for the $\text{G}\alpha\text{i}2$ gene. *J Immunol* 2003, 170:1707–1715