[Frontiers in Bioscience 12, 1790-1803, January 1, 2007]

Aberrant B1 cell trafficking in a murine model for lupus

Sho Ishikawa and Kouji Matsushima

Department of Molecular Preventive Medicine, School of Medicine, The University of Tokyo, Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033

TABLE OF CONTENTS

1. ABSTRACT

B lymphocyte chemoattractant (BLC/CXCL13) is ectopically and highly expressed in the target organs such as the thymus and kidney in aged (NZB x NZW)F1 (BWF1) mice, a murine model for SLE. Ectopic expression of BLC/CXCL13 was attributed to mature myeloid DCs infiltrating in the target organs. DCs were also increased in the peripheral blood in aged BWF1 mice and differentiated into BLC/CXCL13- producing DCs in the presence of TNF-α or IL-1β, but not IFN-α or IFN-γ. BLC/CXCL13 expression in mature myeloid DCs was confirmed in bone marrow derived-DCs generated *in vitro* in the presence of GM-CSF and TNF-α. B1 cells expressed higher level of CXCR5 and migrated towards BLC/CXCL13 much better than B2 cells. B1 cells failed to home to the peritoneal cavity and preferentially recruited to the target organs in aged BWF1 mice developing lupus nephritis. B1, but not B2 cells possessed a potent antigen presenting activity in allo-MLR and activated autologous thymic CD4 T cells in the presence of IL-2. $CXCR5^+$ CD4 T cells were also increased in aged BWF1 mice and they enhanced IgG production by B1 cells *in vitro*. These results suggest a possible involvement of aberrant B1 cell trafficking in activation of autoreactive CD4 T cells and autoantibody production in the target organs during the development of lupus, providing a new insight for the pathogenesis of B1 cells in lupus.

2. INTRODUCTION

B1 cells are a specialized cell population that is distinguished from conventional B cells (B2 cells) by their origin, antigen specificity, cell surface phenotype, and unique tissue distribution (1-3). B1 cells were first described as Ly-1 $(CD5)^+$ B cells and considered to be involved in the development of autoimmune diseases because increased frequency of B1 cells was observed in particular autoimmune-prone mice including (NZB x NZW)F1 (BWF1) mice. In consistence with this idea, it was demonstrated that elimination of B1 cells from the peritoneal cavity by injection of distilled water decreased anti-dsDNA Ab production and pathogenic changes in the kidney in BWF1 mice (4). It was also described that down-regulation/elimination of B1 cells by administration of anti-IL-10 antibody (5, 6) delays the onset and disease severity in BWF1 mice.

B1 cells produce IgM antibodies (Ab) with broad specificity including for self antigens (7) and they are able to undergo isotype switching and somatic hypermutation (8, 9), suggesting that B1 cell can contribute to produce pathogenic high-affinity IgG autoantibodies. On the other hand, B1 cells possess a potent antigen presenting activity comparable to dendritic cells (10, 11). Lanzavecchia *et al.* (12) reported that B cells could efficiently uptake antigens through their surface immunoglobulin receptors, process, and then present to specific T cells in a MHC-restricted manner. These findings may indicate that B1 cells are involved in the pathogenesis of murine lupus as APCs to activate auto-reactive T cells.

In human, elevated levels of $CD5^+$ B cells have been documented in patients with autoimmune disorders including Sjogren's syndrome, rheumatoid arthritis and SLE (13-15). However, several evidences also suggest minor role of $CD5^+$ B cells in the development of SLE (16, 17). Due to different frequency and localization of $CD5^+$ B cells among the species (18), and also to possible conversion of CD5 expression during B cell activation (19), the pathological significance for $CD5^+$ B cells in the development of SLE still remains controversial. In this article, we highlight possible roles of aberrant B1 cell trafficking in the pathogenesis of murine lupus.

3. ABERRANT HIGH EXPRESSION OF BLC/CXCL13 BY MYELOID DCS IN THE TARGET ORGANS IN AGED BWF1 MICE DEVELOPING LUPUS NEPHRITIS

BWF1 mice spontaneously develop systemic autoimmune disorders characterized by production of a variety of IgG autoantibodies and massive deposition of immune complexes in glomeruli in the kidney (20, 21). A marked mononuclear cell infiltration in the target organs including the kidney and lung is another characteristic in aged BWF1 mice. More than 95 % of the BWF1 female mice die from renal failure before 12 months of age. RT-PCR analysis revealed that BLC/CXCL13 expression was markedly increased in the target organs including the kidney and the thymus in aged BWF1 mice developing lupus nephritis (Figure 1A) (22). Quantitative PCR analysis revealed that BLC/CXCL13 gene expression in the thymus and kidney of aged BWF1 mice is 1500 and 500 times higher, respectively compared to that in young mice. BLC protein was also highly expressed in the cellular infiltrates in these organs with a reticular pattern of staining (Figure 1B). Some, but not all blood vessels in the thymic medulla were heavily stained with anti-BLC mAb. BLC expression in the thymus of similarly-aged NZB and NZW mice was much lower than that in aged BWF1 mice (data not shown). It is generally accepted that BLC/CXCL13 is expressed by follicular dendritic cells (FDCs) in B cell follicles of the secondary lymphoid tissues (23, 24). However, aberrant BLC/CXCL13 expression in the target organs in aged BWF1 mice was attributed to mature myeloid DCs. $CD11b^+CD11c^+$ cells were markedly increased in the spleen and thymus and purified $CD11b^+CD11c^+$ cells preferentially expressed BLC/CXCL13 mRNA (Figure 1C). It was also demonstrated that $CD11b^+CD11c^+$ cells were increased in the peripheral blood in aged BWF1 mice and differentiated into BLC/CXCL13 expressing-mature DCs in the presence of TNF-α (Figure 1D, E) (25). Both TNF-α and IL-1β, but neither IFN-α nor IFN-γ could induce BLC gene expression in bone marrow-derived immature DCs generated in the presence of GM-CSF (Figure 1F). Since TNF-α and IL-1β expression is in fact enhanced in the target organs in aged BWF1 mice (26, our unpublished data), it is likely that peripheral blood DCs differentiate into mature DCs expressing BLC/CXCL13 in the target organs during the development of lupus.

4. BLC/CXCL13 CHEMO-ATTRACTS MORE B1 CELLS THAN B2 CELLS

BLC/CXCL13 chemo-attracted more B1 cells than B2 cells presumably due to higher expression of CXCR5 on B1 cells (Figure 2A, B) (22). Preferential chemotaxis of B1 cells towards BLC was also observed in other mouse strains including NZB, NZW, BALB/c, and C57BL/6 (data not shown). Although SLC/CCL21 and SDF-1/CXCL12 also have weak chemotactic activity for B1 cells, there was no difference in chemotaxis by these chemokines between B1 and B2 cells (unpublished data).

5. DEFECTIVE B1 CELL HOMING TO THE PERITONEAL CAVITY AND PREFERENTIAL LOCALIZATION IN THE TARGET ORGANS

It has been recently reported that BLC is essential for B1 cell homing to the peritoneal cavity via omentum milky spots (27). In CXCL13 deficient mice, few B1 cells were present in the peritoneal and pleural cavity, but present in the spleen. CFSE labeled-B1 cells failed to migrate to the peritoneal cavity in CXCL13 deficient mice when injected i.v. Interestingly, B1 cells failed to home to the peritoneal cavity in aged BWF1 mice developing lupus nephritis while B1 cell homing to the peritoneal cavity was intact in young BWF1 mice (28). When CFSE-labeled B1 cells were i.v. injected into young BWF1 mice, a number of labeled cells accumulated in the omentum milky-spots while only few cells were observed in aged BWF1 mice (Figure 3A). B1 cell accumulation in the omentum milky-

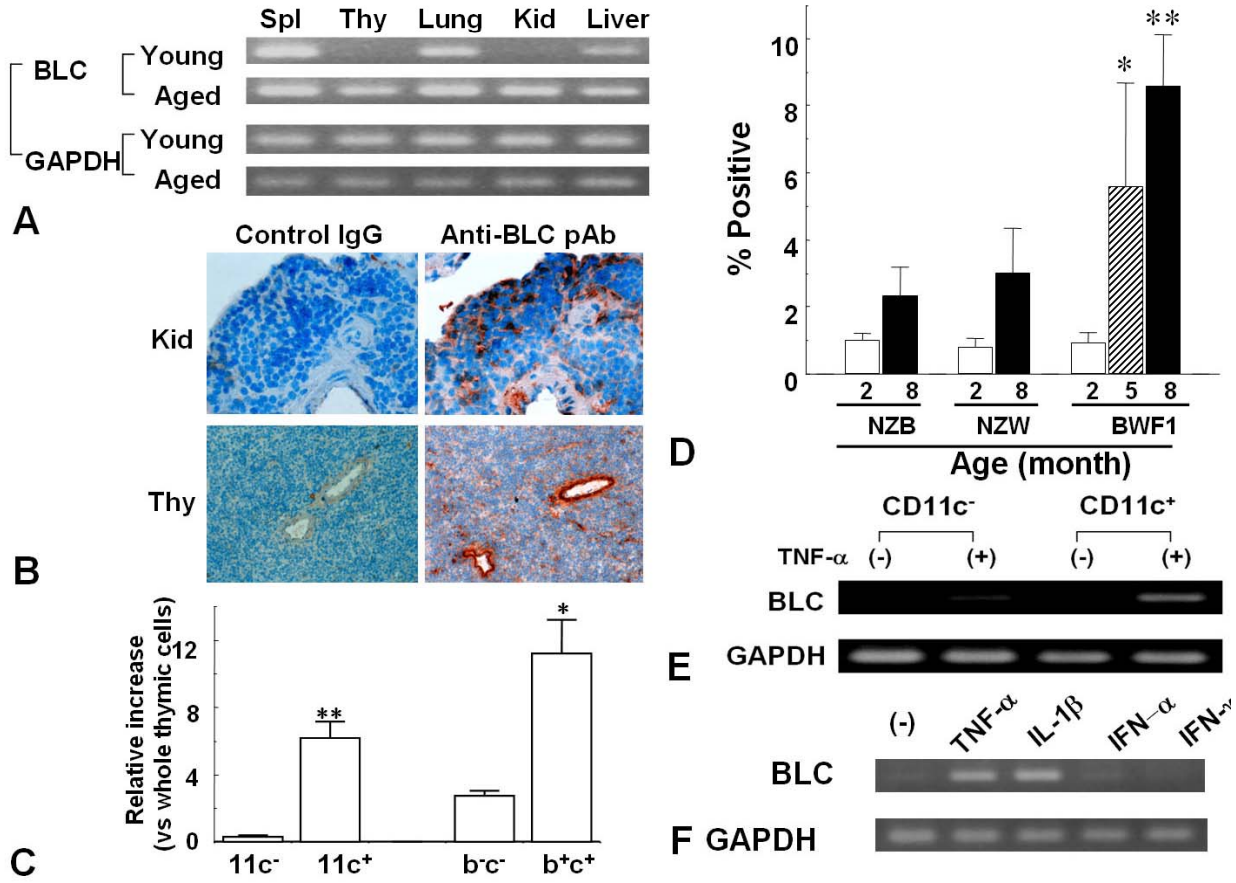

Figure 1. Ectopic high BLC expression by DCs in aged BWF1 mice. A. RT-PCR analysis was performed on total cellular RNAs obtained from the spleen, thymus, lung, kidney, and liver of young and aged BWF1 mice. B. Cryosections from kidney and thymus of aged BWF1 mice were stained with goat anti-BLC pAbs followed by biotinylated rabbit anti-goat pAb and HRP-labeled streptavidin. Representative pictures from one of three aged BWF1 mice are presented. (C) Quantitative RT-PCR analysis for BLC expression on $CD11c^+$ cells purified MACS® magnetic beads coupled with anti-CD11c mAb and FCM sorted-$CD11b^+CD11c^+$ cells obtained from aged BWF1 thymus. BLC quantity in each purified cell population was expressed relative to normalized BLC quantity in whole thymic cells. D. Increased DCs in the peripheral blood. PBLs from young and aged NZB, NZW, and BWF1 mice were stained with FITC-labeled anti-CD11b and PE-labeled anti-CD11c Abs and analyzed on EPICS flow cytometer. The mean ± SD for $CD11b^+CD11c^+$ cells were presented (n=3 for NZB and NZW mice; n=7 for 2 and 8 month-old BWF1 mice, n=5 for 5 month old BWF1 mice). Statistical analysis was performed by Student's t test. * $p<0.03$, ** $p<0.0002$ as compared to the 2 month old value. E. TNF-α induces BLC expression in DCs. $CD11c^+$ and $CD11c^-$ cells obtained from PBLs of aged BWF1 mice were cultured in the presence or absence of TNF-α for 3 days and RT-PCR analysis for BLC expression was performed. F. Induction of BLC expression in bone marrow derived-DCs by TNF-α and IL-1β, but not by IFN-α and IFN-γ. Bone marrow cells were cultured in the presence of GM-CSF for 6 days with removal of floating cells at day 3. TNF-α (10 ng/ml), IL-1β (10 ng/ml), IFN-α (100U/ml), or IFN-γ (100U/ml) for 3 days was added at day 6 and cultured for additional 3 days. RT-PCR analysis for BLC expression was performed.

spots was intact in similarly aged BALB/c mice, suggesting that the defective B1 cell homing to the peritoneal cavity in aged BWF1 mice was not merely an age-related phenomenon. It was also demonstrated that the number of peritoneal macrophages in the peritoneal cavity, a major cell source for BLC/CXCL13, was markedly decreased in aged BWF1 mice compared to those in young BWF1 mice (28).

Instead of homing to the peritoneal cavity, B1 cells injected i.v. preferentially migrated to the target organs including the kidney, thymus, and lung in aged BWF1 mice. When the mixture of CFSE-labeled B1 cells and CMTMR-labeled B2 cells were injected i.v. into the same aged BWF1 mice, more B1 cells were recruited in the cellular infiltrates in the target organs than B2 cells (Figure 3B). In consistence with these results, the frequency of B1 cells in total B cells in the target organs in aged BWF1 mice was significantly higher than that in secondary lymphoid tissues (28). These results suggest that aberrant high expression of BLC/CXCL13 in the target organs of aged BWF1 mice and decreased number of BLC producing

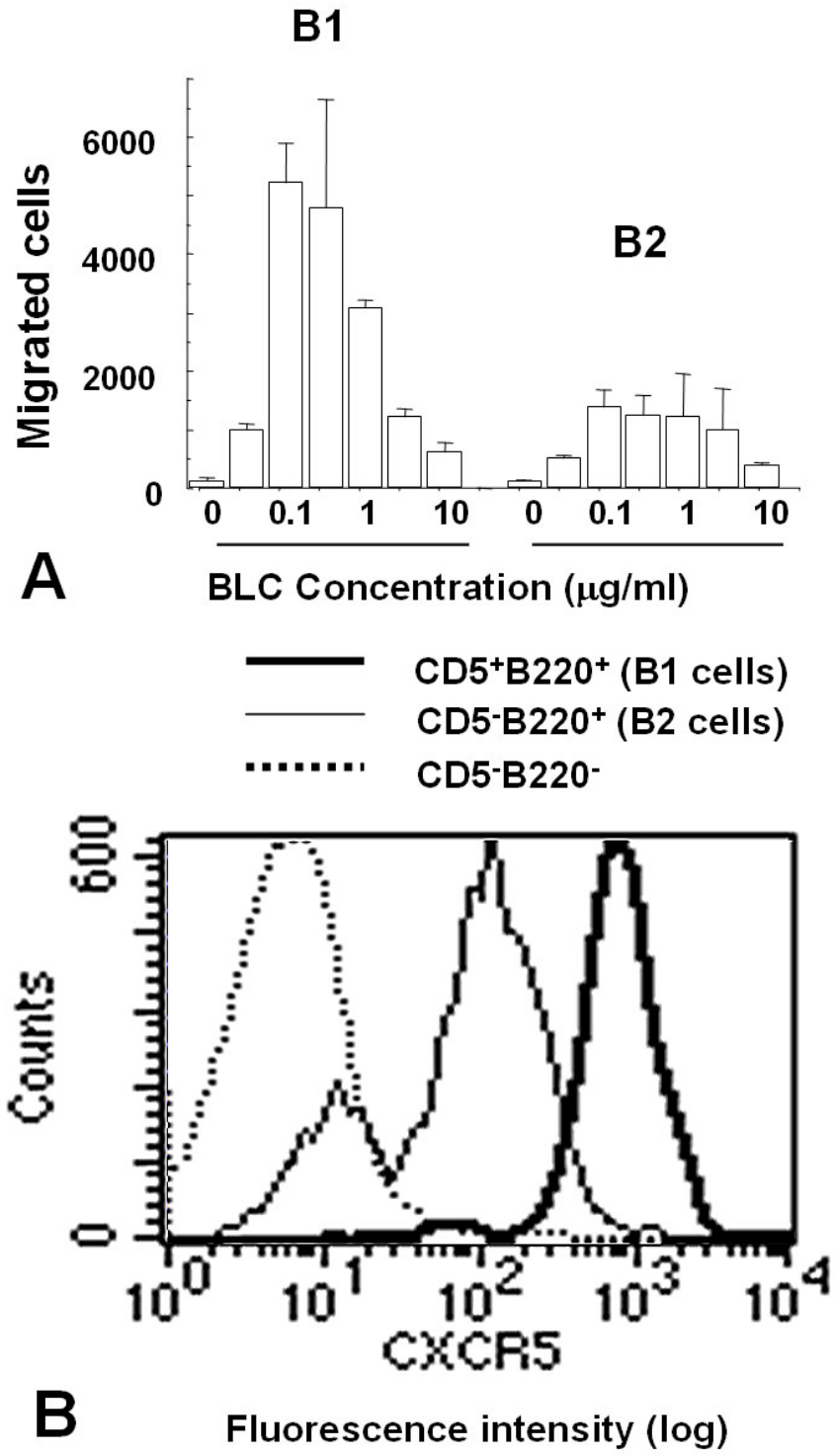

Figure 2. Preferential chemotaxis of B1 cells towards BLC. A. Mixture of spleen and peritoneal cells from young BWF1 mice were stained for FITC-labeled CD5 and PE-B220 and sorted into B1 and B2 cells on an EPICS ELITE® cell sorter and then subjected to BLC chemotaxis assay. Migrated cells were counted on a flow cytometer under the constant flow rate. B. Higher CXCR5 expression on B1 than B2 cells. Mixture of spleen and peritoneal cells from young BWF1 mice were stained with anti-CXCR5, anti-CD5, and anti-B220 mAbs and then analyzed for CXCR5 expression on gated B1 or B2 population. Mean fluorescence channels for B1 and B2 cells were 796 ± 18 (n=3) and 113 ± 9.0 (n=3), respectively ($p<0.001$). Staining on $CD5^-$ $B220^-$ cells was presented as a negative control.

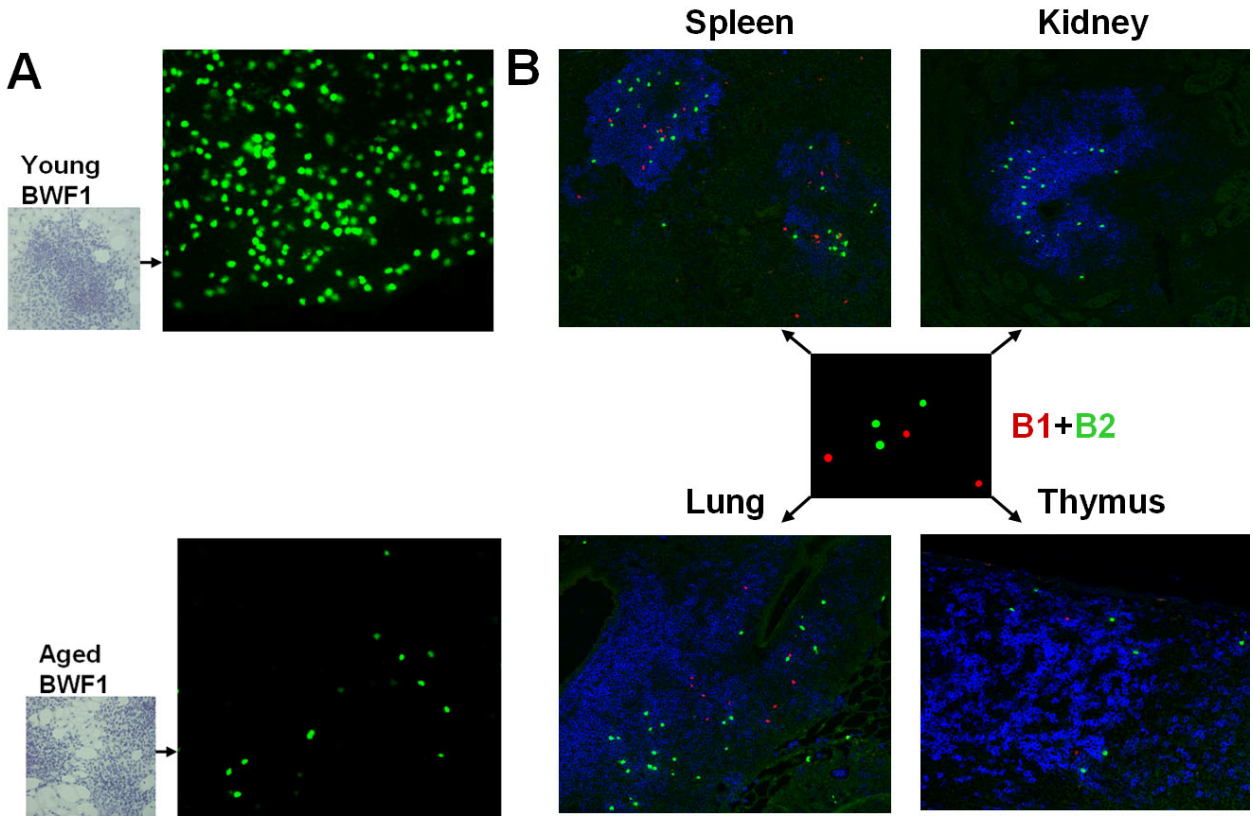

Figure 3. Failure of B1 cells homing to the peritoneal cavity and migration to the target organs in aged BWF1 mice. A. Decreased B1 cell accumulation to the omentum milky-spots in aged BWF1 mice. Four million CFSE labeled-peritoneal B1 cells were injected i.v. into young and aged BWF1 mice. Omentum milky-spots were analyzed 24 hr after injection under confocal laser scanning microscope (x100). B. Preferential B1 cell recruitment to non-lymphoid target organs including the kidney and lung. B1 and B2 cells were labeled with CFSE (green) and CMTMR (red), respectively and the 1:1 mixture of B1 and B2 cells was injected i.v. into the same aged BWF1 mice. Mice were sacrificed 24 hr after injection and analyzed under confocal laser scanning microscope (x200). Cellular infiltrates were highlighted using biotin-conjugated B220 mAbs (blue).

peritoneal macrophages result in defective B1 cell homing to the peritoneal cavity and preferential B1 cell trafficking to the target organs.

6. POTENT ANTIGEN PRESENTING ABILITY OF B1 CELLS

Mohan *et al.* (10) reported splenic B1a cells possessed potent antigen-presenting capability in NZM2410 lupus-prone mice. Peritoneal B1 cells obtained from BWF1 as well as BALB/c mice also induced a good alloreactive response in MLR when used as antigen presenting cells while B2 cells showed poor antigen presenting ability (Figure 4A) (11). Antigen presenting ability of B1 cells was as potent as that of splenic DCs and is not restricted to B1 cells derived from autoimmune mice. Peritoneal B1 cells expressed significantly higher levels of costimulatory molecules including CD80, CD86, and ICAM-1 than splenic B2 cells while the level of MHC class II expression was similar between B1 and B2 cells (Figure 4B).

7. B1 CELL MIGRATION TO THE THYMUS MAY RESULT IN AUTOREACTIVE CD4 T CELL ACTIVATION

Thymic post-capillary venules (PVS) were markedly enlarged in aged BWF1 mice and a number of B cells were infiltrated in the enlarged PVS (Figure 5A) (11). Approximately 15 % of infiltrated $B220^{+}$ cells are CD5 positive. The boundary between thymic PVS and parenchyma became unclear and some B cells were readily detected in the thymic medulla. BLC protein and PNAd were co-localized on luminal surfaces of HEV-like blood vessels in enlarged PVS. Recruitment of B1 cells to the enlarged thymic PVS and transmigration across the basement membranes to the thymic medulla was observed when CFSE labeled-B1 cells were injected i.v. into aged BWF1 mice (Figure 5B).

It is of great interest what would happen to tolerance induction when B1 cells which have a potent antigen presenting activity aberrantly migrate to the thymus. When thymocytes labeled with CFSE were cultured with B1 or B2 cells in the presence of IL-2, small,

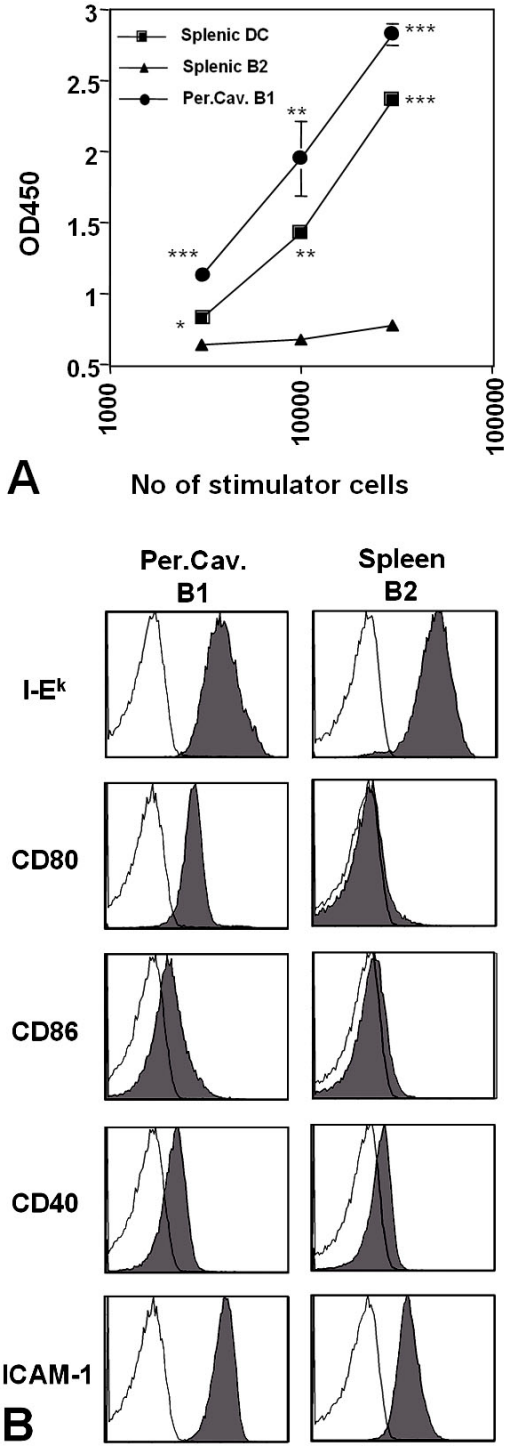

Figure 4. B1 cells as potent APCs. A. Allogeneic mixed lymphocyte reaction (MLR). 3 x 10^5 splenic $CD4^+$ T cells from C57BL/6 mice were co-cultured with either one of mitomycin C treated peritoneal B1 cells, splenic B2 cells or splenic DCs isolated from 2 month-old BWF1 mice. T cell proliferation was assessed by WST-1 assay 96 hr after culture. *p<0.05, **p<0.01, ***p<0.001 as compared to splenic B2 cells. B.Expression of co-stimulatory molecules on B1 and B2 cells. Whole peritoneal cells or splenocytes were stained with B220, CD5 and either one of MHC class II, CD80, CD86, CD40 and ICAM-1. Expression levels of each molecule on gated peritoneal B1 ($CD5^+B220^{lo}$) and splenic B2 ($CD5^-B220^{hi}$) cells are shown (gray histograms). Background staining is also represented as white histograms.

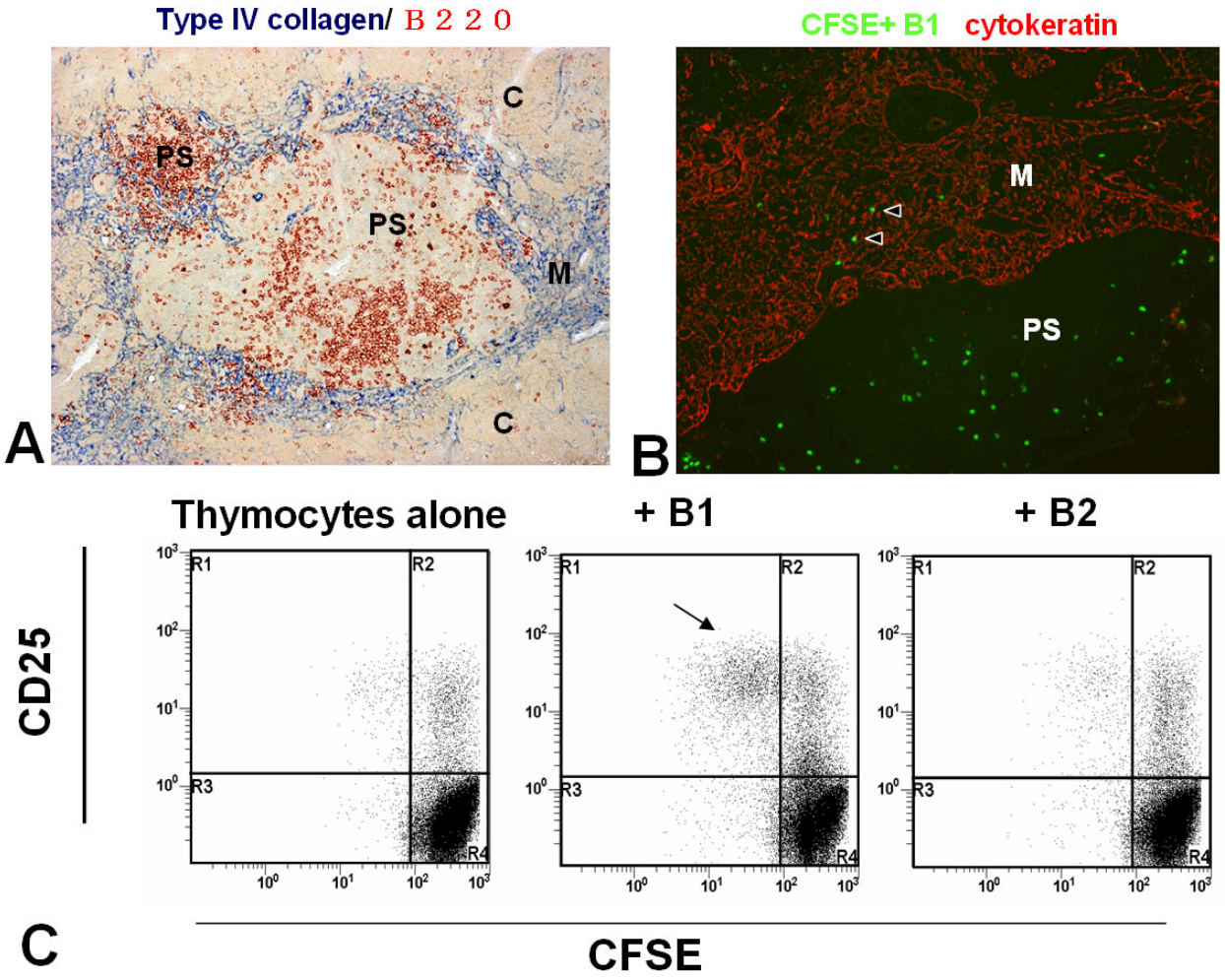

Figure 5. B1 cell migration to enlarged thymic perivascular spaces (PS) in aged BWF1. A. Enlarged thymic PS of 8 month-old BWF1 mice. Frozen sections of thymus from 8 month-old BWF1 mice were doubly stained with antibodies to B220 (red) and either one of cytokeratin (blue) (x200). M:medulla, C:cortex. B. Recruitment of B1 cells into thymic PS and the medulla. Ten million of CFSE-labeled peritoneal B1 cells were transferred intravenously into aged BWF1 mice. Three days later, thymic sections were stained with antibodies to cytokerarin (red) (x200). (C) Activation of self-reactive T cells in the adult thymus by B1 cells. Half million CFSE-labeled adult thymocytes were cultured with 5 x 10^4 of either peritoneal B1 cells or splenic B2 cells in the presence of 5 U/ml of recombinant human IL-2. After 96 hr after culture, cells were stained with FITC anti-CD4 and PE anti-CD25 mAb and analyzed on FCM. Dividing cells are indicated by arrows (gated for CD4 T cells).

but significant number of $CD4^+$ T cells in adult thymus were activated by syngeneic B1, but not B2 cells as evidenced by diluted intensity of CFSE and by CD25 expression (Figure 5C). B1 cells obtained from NZB, NZW, BALB/c and C57BL/6 mice also stimulated proliferation and activation of syngeneic T cells, demonstrating a generalized ability of B1 cells to activate CD4 T cells, some of them being potentially autoreactive. Activated $CD4^+$ T cells showed no TCR Vβ skewing after B1 cell stimulation (unpublished data). Furthermore, B1 cells obtained from MHC class II deficient mice failed to activate thymic CD4 T cells (unpublished data). These data suggest that CD4 T cells were not activated by retroviral superantigens encoded by MMTV, but by self-peptides associated with MHC class II molecules on B1 cells. Possible roles of B1 cell-activated CD4 T cells in the pathogenesis of lupus in BWF1 mice remain to be elucidated.

8. $CXCR5^+CD4$ T CELLS WITH SIMILAR PHENOTYPE TO FOLLICULAR HELPER T CELLS (T_{FH}) MAY BE INVOLVED IN IGG AUTOANTIBODY PRODUCTION BY B1 CELLS IN BWF1 MICE

Recent studies have revealed the existence of the third cellular subset of helper T cells designated as follicular helper T cells (T_{FH}) (29, 30). T_{FH} cells show distinct cytokine profiles from those of Th1 or Th2 cells, and they are able to enhance IgG and IgA antibody production by tonsillar B cells in the absence of APCs. It was also demonstrated that T_{FH} cells express CXCR5, ICOS, CD69 and MHC class II molecules and migrated

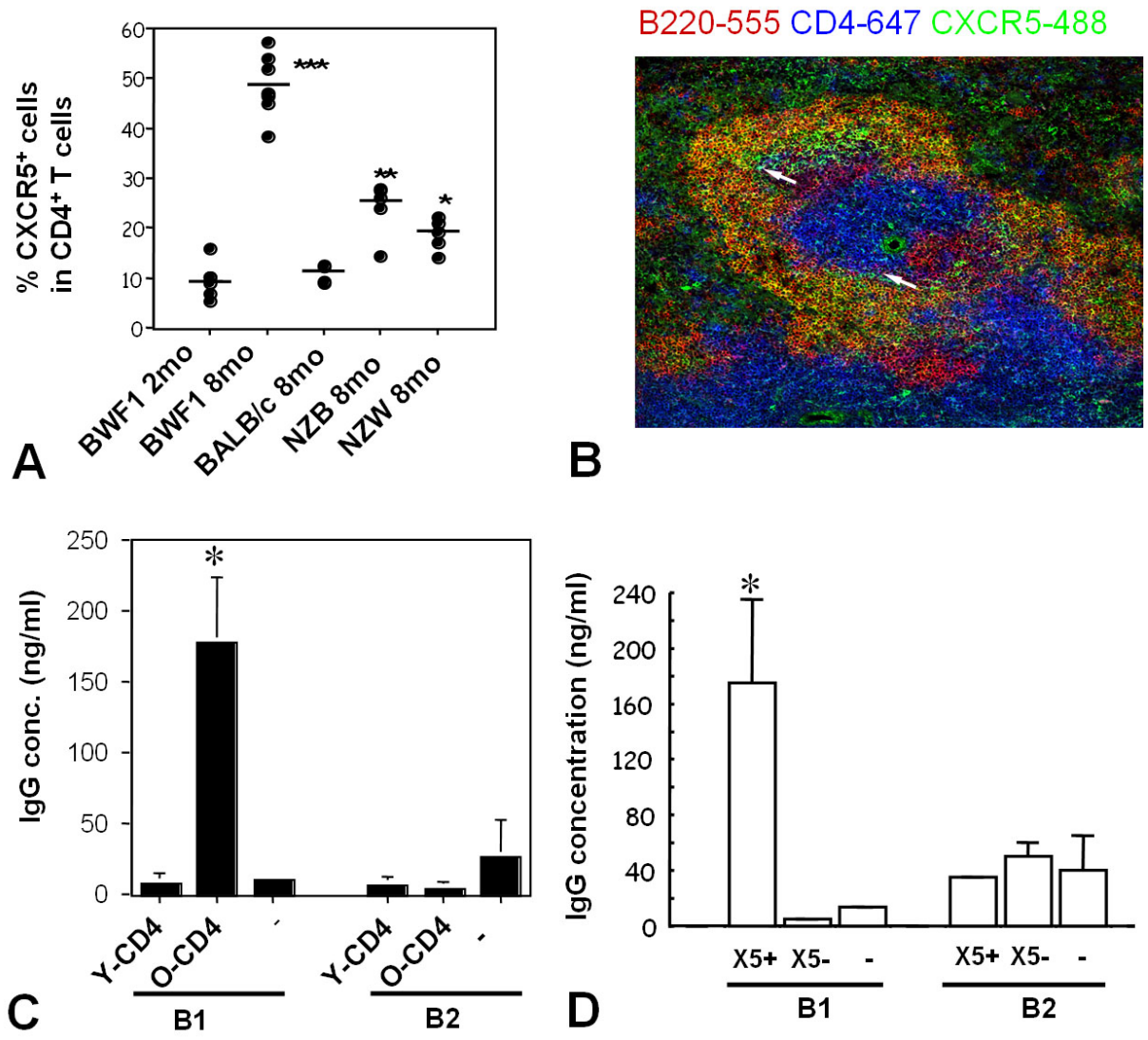

Figure 6. CXCR5$^+$CD4 T cells enhance IgG Ab production by B1 cells. A. Increased frequency of CXCR5$^+$ CD4 T cells in aged BWF1 mice. Spleen cells from young BWF1, aged BWF1, NZB, NZW, BALB/c mice were stained with FITC-anti-CD4 and PE-anti-CXCR5 mAb and analyzed on EPICS flow cytometer. The mean percentage of CXCR5$^+$ CD4 T cells among CD4 T cells were presented. *p<0.02, **p<0.004, *** p<0.000003 as compared to the 2 mo old value. B. Cryostat section of spleen obtained 8 mo-old BWF1 mice stained with Alexa488 labeled-anti-CXCR5 (green), Alexa555 labeled-anti-B220 (red), and Alexa647 labeled-anti-CD4 mAbs and were observed under confocal laser microscopy (x200). CXCR5+ CD4 T cells were localized in T cell area and B cell follicles as indicated by arrows. C. Enhanced IgG antibody production by B1 cells in the presence of CD4 T cells obtained from aged BWF1 mice. CD4 splenic T cells from young or aged BWF1 mice were purified by using anti-CD4 mAb conjugated-MACS beads. Each CD4 T cell population was cultured with peritoneal B (B1 enriched) or splenic B (B2 enriched) cells purified with anti-B220 conjugated-MACS beads. Ten days later, culture supernatants were collected and IgG concentrations were determined by ELISA. * p<0.004 as compared to the group without T cells. D. Enhanced IgG antibody production by B1 cells in the presence of CXCR5$^+$ CD4 T cells. Spleen cells were stained with FITC-anti-CD4 and PE-anti-CXCR5 mAbs and CXCR5$^+$ and CXCR5$^-$ CD4 T cells were sorted on EPICS flow cytometer. Each T cell population was cultured with peritoneal B (B1 enriched) or splenic B (B2 enriched) cells. Ten days later, culture supernatants were collected and IgG concentration was determined by ELISA. * p<0.05.

towards BLC/CXCL13. It has not been examined, however, whether or not T_{FH} cells promote IgG autoantibody production by B1 cells. In aged BWF1 mice, the percentage of CXCR5$^+$ CD4 T cells were markedly increased while similarly aged NZB and NZW mice also showed a much lower percentage of CXCR5$^+$ CD4 T cells (Figure 6A). CXCR5$^+$CD4 T cells in aged BWF1 mice expressed CD69, ICOS, and MHC class II molecules、showing a similar cell

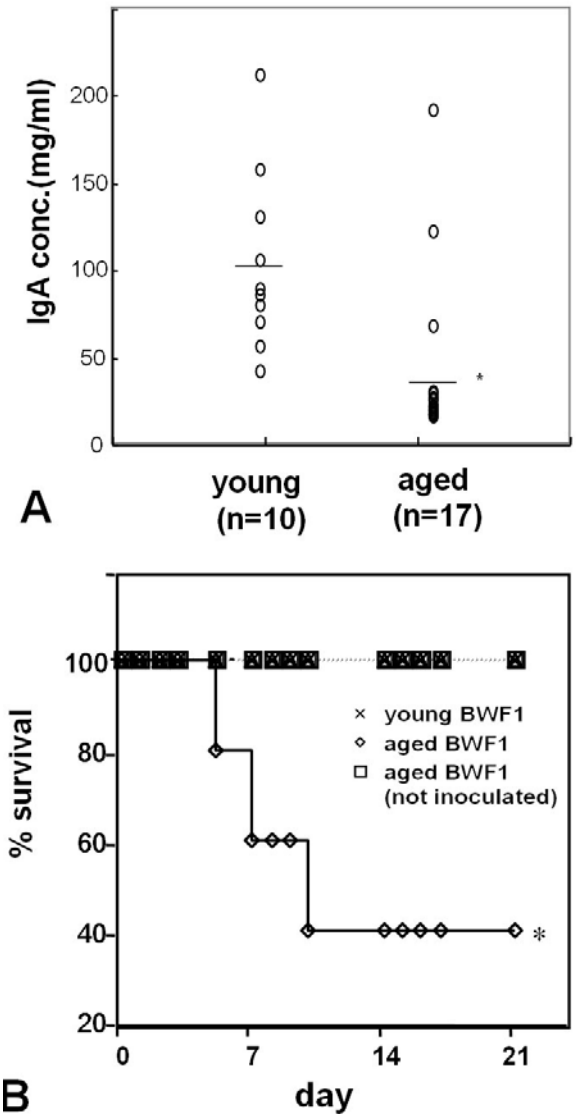

Figure 7. Impaired mucosal immunity in the gut of aged BWF1 mice. A. IgA concentrations in the feces of young and aged BWF1 mice (n=10 and 17, respectively) were determined by ELISA. Each circle represents the IgA level of an individual mouse and bars represent median values. *, $P < 0.03$. B. Increased susceptibility to bacterial infection in aged BWF1 mice. Mice were infected with *E. coli* (10^9 CFU B41 strain) on day 1, 2, 3 and mortality was monitored daily. Each group consisted of five mice: × = young BWF1; ◇ = aged BWF1 infected with *E. coli*; □ = aged BWF1 without infection. *$p < 0.05$.

surface phenotype to human tonsillar T_{FH} cells. $CXCR5^+CD4$ T cells were localized in the B cell follicle as well as in the T cell area in the spleen (Figure 6B). CD4 T cells obtained from aged, but not young BWF1 mice enhanced IgG antibody production by B1 cells while they did not enhance IgM production while they had no effect on IgG production by B2 cells from young BWF1 mice (Figure 6C). Anti-ssDNA Ab activity was also detected in the culture supernatants when B1 cells were cultured with CD4 T cells from aged BWF1 mice (data not shown). Furthermore, IgG antibody production by B1 cells was enhanced when B1 cells were cultured with $CXCR5^+$, but not $CXCR5^-$ CD4 T cells (Figure 6D).

9. IMPAIRED MUCOSAL IMMUNITY IN THE GUT IN AGED BWF1 MICE

Kroese *et al.* (31, 32) reported that approximately half of IgA^+ cells in the intestinal lamina propria were derived from B1 cells. It is, therefore, expected that aberrant B1 cell trafficking during the development of lupus in BWF1 mice would result in decreased IgA secretion in the gut. In fact, the level of fecal IgA was dramatically decreased in aged BWF1 mice developing lupus nephritis (Figure 7A) (33). IgA levels in similarly aged NZB and NZW mice remained unchanged (33). All cellular components including CD4 and CD8 T cells, and B cells in Peyer's patches (PPs) were markedly decreased in aged BWF1 mice. Furthermore, aged BWF1 mice showed increased susceptibility to pathogenic bacterial infection (Figure 7B). It was further demonstrated that induction of oral tolerance was impaired and orally administered-antigen induced systemic allergic T cell sensitization in aged BWF1 mice (33).

10. DISCUSSION

Our findings in serial studies suggest that aberrant high expression of BLC/CXCL13 in aged BWF1 mice result in abnormal B1 cell trafficking, activation of self-reactive CD4 T cells and production of IgG autoantibody in the presence of $CXCR5^+CD4$ T cells during the development of murine lupus. Thus, cell trafficking and localization as well as function itself can be an important factor to understand the pathological significance of B1 cells in murine lupus.

Datta and his colleagues (34) previously reported the presence of nucleosome-specific T cells in lupus-prone mice including BWF1 mice, and their helper activity on anti-DNA and histone IgG antibody production. It has been recently reported that nucleosome specific T cells engineered by TCR gene transfer were activated by splenic DCs in BWF1 mice and that the antigen specific regulatory T cells engineered to express CTLA-4 delayed the onset of lupus nephritis (35). On the other hand, autoreactive B1 cells specific for the ribonuclear protein Sm are tolerant or ignorant in the peritoneal cavity (36, 37). Honjo and his colleagues (38) also demonstrated that anti-SRBC B1 cells escape from deletion in the peritoneal cavity in anti-SRBC Ig transgenic mice. These results suggest that the peritoneal cavity is a privileged site for autoreactive B cells to escape from immunological tolerance. Where else it could occur? Interestingly, the same group further demonstrated that anti-SRBC B1 cells did survive in the lamina propria in the gut of anti-SRBC Ig transgenic mice. It is therefore possible that autoreactive B1 cells migrated to the target organs expressing ectopic BLC/CXCL13 escape from tolerance and that they present autoantigens to self–reactive T cells and then are activated by those T cells to produce

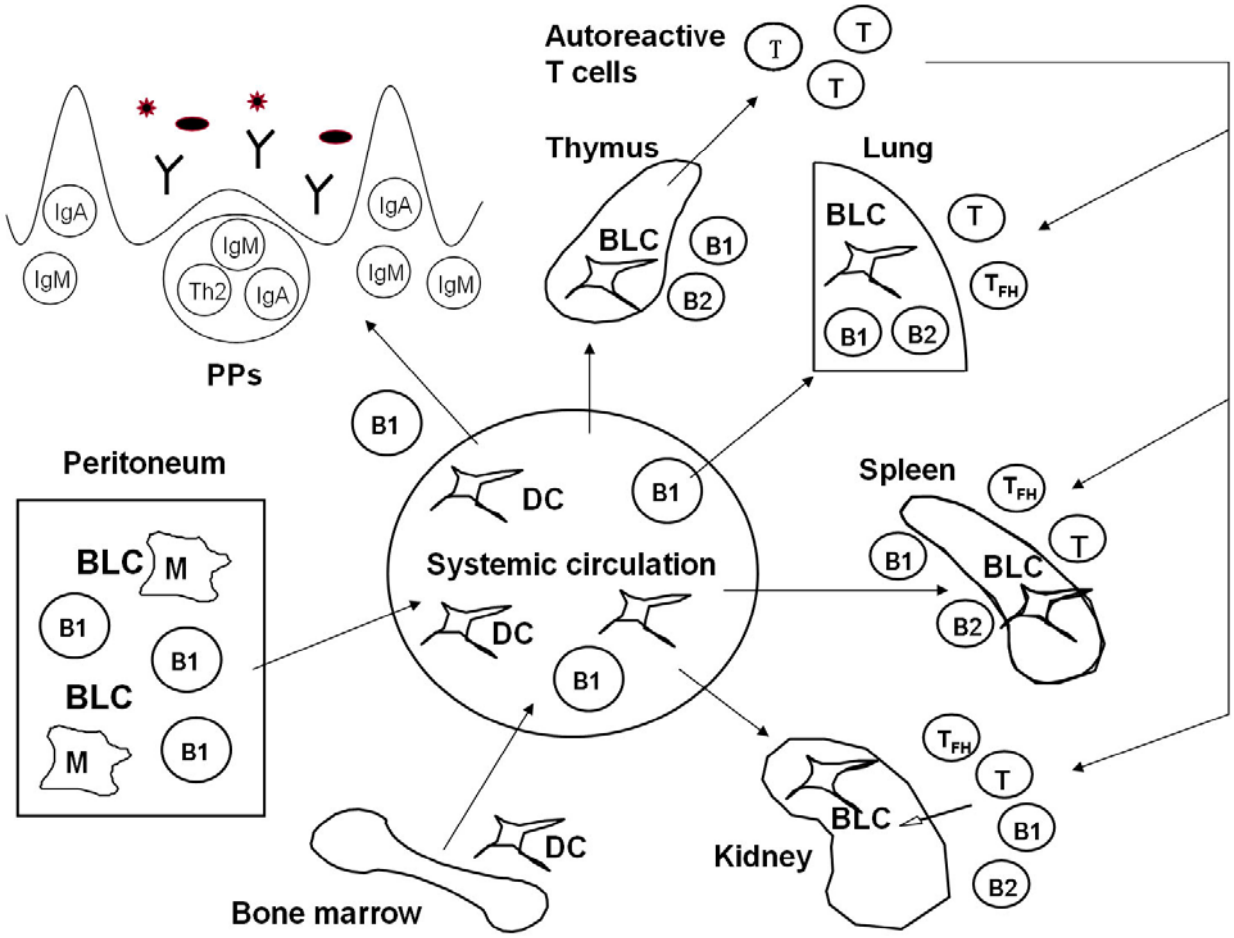

Figure 8. Hypothetical model for pathogenic roles of B1 cells in murine lupus. Aberrant B1 cell trafficking due to ectopic high expression of BLC in aged BWF1 mice results in activation of autoreactive CD4 T cells, IgG autoantibody production in the presence of follicular helper T cells (T_{FH}). DC: dendritic cells, PPs: Peyer's patches, M: macrophages.

autoantibodies. In this context, it is of great interest that B1 cells activate thymic CD4 T cells in the presence of IL-2 although antigenic peptides presented by B1 cells remain to be elucidated. We hypothesize that ectopic high expression of BLC by DCs chemo-attracts B1 and $CXCR5^+$ autoreactive CD4 T cells (T_{FH}-ike?) to form an immunological platform for IgG autoantibody production in the target organs (Figure 8).

Aberrant B1 cell trafficking may also be involved in impaired mucosal immunity in the gut of aged BWF1 mice because B1 cells are the major source of secreted IgA (31,32). IgA production by B1 cells is T cell-independent and requires the presence of commensal microflora (39). Furthermore, commensal bacteria bind mostly B1 cell-derived intestinal IgA, and less so B-2 cell-derived IgA (40). These results favor the idea that immunological stimulation of B1 cells by commensal bacteria at neonatal stage induce IgA production by B1 cells and at the same time develop the mucosal immune system in the gut. Therefore, decreased IgA secretion in aged BWF1 mice may result in penetration of commensal bacteia into systemic immune system. Penetration of commensal bacterial antigen would provoke a vigorous immune response by B1 cells which are specific for polysaccharides such as phosphorylcholine, lipids, and proteins of bacterial components. Unlike mammalian DNA, bacterial DNA has potent immunologic effects that lead to polyclonal B cell activation as well as the production of specific Abs in mice (41). It is also reported that bacterial DNA induces anti-dsDNA antibody cross-reactive to mammalian dsDNA in autoimmune prone mice such as BWF1 mice (42). It is, therefore, tempting to speculate that impaired IgA secretion in the gut lumen and low level of IgM natural antibodies in aged BWF1 mice due to aberrant B1 cell trafficking may result in penetration of commensal bacteria into systemic immune system and induction of vigorous anti-bacterial DNA antibody production which cross-reacts to mammalian DNA. On the other hand, breakdown of oral tolerance in aged BWF1 mice may be attributed to abnormal DC trafficking or antigen trafficking in the intestinal mucosa during the development of lupus.

It is also of interest whether or not stimulation of thymic CD4 T cells by B1 cells in the presence of IL-2 abrogate Treg activity of $CD25^+CD4$ T cells. It is well established that depletion of $CD25^+CD4$ T cells from the thymus or spleen results in autoimmunity in various organs

including the stomach and ovary (43). Although $CD25^+$ CD4 T cells were increased in aged BWF1 mice, their pathological significance in the development of murine lupus remains to be elucidated.

Recent studies have revealed that type-I IFN produced by plasmacytoid DCs (pDCs) play a pivotal role for the pathogenesis of SLE (44, 45). Consistent with findings in SLE patients, it has been also suggested that type-I IFN is involved in the pathogenesis of lupus in BWF1 mice (46, 47). However, the frequency of pDCs in the peripheral blood is very low in aged BWF1 mice (unpublished data), consistent with the study by Farcas *et al.* (48) who demonstrated that pDCs accumulated in the skin in SLE patients while the frequency of pDCs was low in the peripheral blood. It has been also described that pDCs induce plasma cell differentiation through IFN-α and IL-6 (49). A critical role of pDCs for CTL generation has been also documented (50-52). We recently demonstrated a pivotal role of CXCR3/CXCL9 interaction for pDC-transmigration into inflamed LNs through HEVs in bacteria-induced acute hepatitis model (53). Since CXCL9 and CXCL10 expression is also elevated in the target organs in aged BWEF1 mice (unpublished data), it is possible that pDCs migrate to the inflamed target organs and participate in the formation of immunological platform together with B1, T_{FH}, and mDCs.

It still remains unknown what kind of stimuli initiate autoimmune responses in BWF1 mice. Endogenous retroviral activation may be involved in the first step in the development of lupus in BWF1 mice. Lupus-prone mice including BWF1 mice expressed a novel 8.4 kb full-length retroviral transcript corresponding to an endogenous mink cell focus-forming (MCF) env-related provirus (54). Expression of thymic 8.4 kb MCF was detected soon after birth and was determined by bone marrow derived-cells rather than thymic epithelial cells (55,56). Since central tolerance or TCR repertoire formation is completed at the end of gestation, the expression of MCF env-related provirus by bone marrow-derived cells in the thymus just after birth may result in incomplete clonal elimination of MCF virus-specific T cells. It is, therefore, possible that activation of the virus-specific T cells would trigger systemic inflammatory responses including production of inflammatory cytokines/chemokines and generation of apoptotic cells. Plasmacytoid DCs would also produce a large amount of IFN-α at this stage. However, the particular retroviral expression is not enough to induce autoimmune disorders in BWF1 mice because the viral expression is also observed in NZB and NZW mice that do not develop lupus nephritis.

Recent genome-wide searches for susceptibility genes in murine and human SLE have revealed important similarities in the genetic mechanisms mediating SLE in BWF1 mice and humans (57-59). Mohan *et al.* (60) reported that Sle2 on mouse chromosome 4 led to expansion of B1 cells although Sle2 alone on normal B6 background was insufficient to generate anti-nuclear Ab and glomerulonephritis. In consistence with this study, a weak suggestive linkage with syntenic region on human chromosome 9 is reported (61).

Although CD5 molecule is commonly used for discrimination of B1 cells from B2 cells, CD5 may not be the best cell surface marker for B1 cells of human or other species. In human, CD5 is expressed on up to 30 % of B cells in PBL and lymph nodes while in mice, $CD5^+$ B cells are virtually absent from lymph nodes and PBL (18). Although it was demonstrated that human $CD5^+$ B cells selectively expressed a V_H4 subfamily of immunoglobulin genes (62), IgM antibodies bind autoantigens irrespective of CD5 expression (63). In rabbits, all B cells express CD5 molecules (64). These facts may indicate that CD5 expression on B cells in other species does not necessarily mean the same functional B1 subset described in mice. On the other hand, it is reported that unique $RP105^-$ B cells are increased in SLE patients and that the level of these B cells are closely correlated with the disease activity (65). $RP105^-$ B cells lack CD5 expression, but express CD86 and CD38 molecules on their cell surfaces and approximately 50 % of $RP105^-$ B cells express intracytoplasmic IgG although tissue localization, antigen presenting activity, BCR repertoire of $RP105^-$ B cells are not addressed yet. It is, therefore, possible that functional B1 cells with distinct phenotypic characteristics from those in mice are involved in the pathogenesis in human SLE.

11. SUMMARY AND PERSPECTIVE

Aberrant B1 cell trafficking and localization due to ectopic high expression of BLC/CXCL13 may play an important role in the pathogenesis of murine lupus. Pathological significance of $CXCR5^+$ T cells for IgG autoantibody production by B1 cells, of B1 cells for activation of autoreactive CD4 T cells, and of impaired mucosal immunity in the gut for systemic allergic responses in BWF1 mice will be clarified in more details in the near future. Our findings would provide a new insight for pathological significance of B1 cells in the development of SLE and a useful approach to regulate autoimmune responses by targeting B1 cell trafficking.

12. ACKNOWLEDGMENT

This work was supported by SORST (Solution Oriented Research for Science and Technology) by Japan Science and Technology Corporation and LRI (The Long-ranged Research Initiative) by Japan Chemical Industry Association.

13. REFERENCES

1. Hayakawa, K., R.R. Hardy, D.R. Parks & L.A. Herzenberg: The "Ly-1 B" cell subpopulations in normal, immunodefective, and autoimmune mice. *J Exp Med* 157, 202-218 (1983)
2. Hertzenberg, L.A. :The Ly-1 B cell lineage: *Immunol Rev* 93, 81-102. (1986)
3. Hayakawa, K. & R. R. Hardy: Development and function of B-1 cells. *Curr Opin Immunol* 12, 346-353 (2000)

4. Murakami, M., H. Yoshioka, T. Shirai, T. Tsubata, & T. Honjo: Prevention of autoimmune symptoms in autoimmune-prone mice by elimination of B-1 cells. *Int Immunol* 7, 877-882 (1995)
5. Ishida, H., R. Hastings, J. Kearney & M. C. Howard: Continuous anti-interleukin 10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. *J Exp Med* 175, 1213-1220 (1992)
6. Ishida, H., T. Muchamuel, S. Sakaguchi, S. Andrade, S. Menon, S & M. Howard: Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J Exp Med* 179, 305-310 (1994)
7. Hayakawa K, R. R. Hardy, M. Honda, L. A. Herzenberg & A. D. Steinberg: Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc Natl Acad Sci* USA 81, 2494-2498 (1984)
8. Mantovani L, R. L. Wilder & P. Casali: Human rheumatoid B-1a ($CD5^+$ B) cells make somatically hyper-mutated high affinity IgM rheumatoid factors. *J Immunol* 151, 473-489 (1993)
9. Arnold L. W, T. A. Gradina, A. C. Whitmore & G. Haughton: Ig isotype switching in B lymphocytes: isolation and characterization of clonal variants of the murine $Ly\text{-}1^+$ B cell lymphoma, CH12, expressing isotypes other than IgM. *J Immunol* 140, 4355-4363 (1988)
10. Mohan C, L. Morel, P. Yang, & E. K. Wakeland: Accumulation of splenic B1a cells with potent antigen-presenting capability in NZM2410 lupus-prone mice. *Arthritis Rheum* 41, 1652-1662 (1998)
11. Sato T, S. Ishikawa, K. Akadegawa, T. Ito, H. Yurino, M. Kitabatake, H. Yoneyama & K. Matsushima: Aberrant B1 cell migration into the thymus results in activation of CD4 T cells through its potent antigen presenting activity in the development of murine lupus. *Eur J Immunol* 34, 3346-3358 (2004)
12. Lanzavecchia, A.: Antigen-specific interaction between T and B cells. *Nature* 314, 537-539 (1985)
13. Dauphinee, M., Z. Tovar, & N. B. Talal: B cells expressing CD5 are increased in Sjogren's syndrome. *Arthritis Rheum* 31, 642-647 (1998)
14. Plater-Zyberk C., R. N. Maini, K. Lam, T. D. Kennedy, & G. A. Janossy: A rheumatoid arthritis B cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. *Arthritis Rheum.* 28, 971-976 (1985)
15. Smith H.R. & R. R. Olson: $CD5^+$ B lymphcytes in systemic lupus erythematosus and rheumatoid arthritis. *J Rheumatol* 17, 853-855 (1990)
16. Casali P., S. E. Burastero, J.E. Balow & A. L. Notkins: High affinity antibodies to ssDNA are produced by $CD5^-$ B cells in systemic lupus erythematosus patients. *J Immunol* 143, 3476-3483 (1989)
17. Becker H, C. Weber, S. Storch & K. Federlin: Relationship between $CD5^+$ B lymphocytes and the activity of systemic autoimmunity. *Clin Immunol Immunopathol* 56, 219-225 (1990)
18. Hardy R. R. & K. Hayakawa: Development and physiology of Ly-1 B and its human analog, Leu-1 B. *Immunol Rev* 93. 53-79 (1986)
19. Cong Y. Z., E. Rabin & H. H. Wortis: Treatment of murine $CD5^-$ B cells with anti-Ig, but not LPS, induces surface CD5: two B cell activation pathways. *Int Immunol* 3, 467 476 (1991)
20. Theofilopoulos, A. N.: Murine models of systemic lupus erythematosus. *Adv Immunol.* 37, 269-390 (1985)
21. Shirai T., S. Hirose, T. Okada & H. Nishimura: Immunology and immunopathology of the autoimmune disease of NZB and related mouse strains. In Immunological Disorders in Mice. E. B. Rihova and V. Vetvicka, editors. CRC Press Inc., Boca Raton, p95-136 (1991)
22. Ishikawa S, T. Sato, M. Abe, S. Nagai, N. Onai, H. Yoneyama, Y-Y. Zhang, T. Suzuki, S. Hahismoto, T. Shirai, M. Lipp & K. Matsushima: Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) in the development of murine lupus and preferential chemotaxis of B1 cells towards BLC. *J Exp Med* 193, 1393-1402 (2001)
23. Forster, R., A.E. Mattis, E. Kremmer, E. Wolf, G. Brem, & M. Lipp: A putative chemokine receptor, BLR1, directs B cell migration to defined lymohoid organs and specific anatomic compartment of the spleen. *Cell* 87, 1037-1047 (1996)
24. Gunn, M.D., V. N. Ngo, K. M. Ansel, E. H. Ekland, J. G. Cyster & L. T. Williams: A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 391, 799-803 (1998)
25. Ishikawa S, S, Nagai, T. Sato, K. Akadegawa, H. Yoneyama, Y-Y. Zhang, N. Onai & K. Matsushima: Increased circulating $CD11b^+CD11c^+$ dendritic cells in aged BWF1 mice which can be matured by TNF-□ into BLC/CXCL13 producing-dendritic cells. *Eur J Immunol* 32,1881-1887 (2002)
26. Brennan D. C, M. A. Yui, R. P. Wuthrich & V. E. Kelly: Tumor necrosis factor and IL-1 in New Zealand Black/White mice: Enhanced gene expression and acceleration of renal injury. *J Immunol* 143, 3470 3475 (1989)
27. Ansel KM, R. B. Harris & J. G. Cyster: CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity. *Immunity* 16, 67-76 (2002)
28. Ito T, S. Ishikawa, T. Sato, K. Akadegawa, H. Yurino, M. Kitabatake, S. Hontsu, T. Ezaki, H. Kimura & K. Matsushima: Defective B1 cell homing to the peritoneal cavity and preferential recruitment of B1 cells in the target organs in a murine model for SLE. *J Immunol* 172, 3628-3634 (2004)
29. Scharli P, K. Willimann, A. B. Lang, M. Lipp, P. Loetscher & B. Moser: CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J Exp Med* 192, 1553-1562 (2000)
30. Breitfeld D, L. Ohl, E. Kremmer, J. Ellwart, F. Sallusto, M. Lipp & R. Forster: Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J Exp Med* 192, 1545-1552 (2000)
31. Kroese F. G. M, W. A. Ammerlaan & A. B. Kantor. Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity. *Int Immunol* 1, 75-84 (1989)
32. Kroese F. G. M., W. A. Ammerlaan & A. B. Kantor: Evidence that intestinal IgA plasma cells in transgenic mice

are derived from B-1 (Ly-1 B) cells. *Int Immunol* 5, 1317-1327 (1993)
33. Akadegawa K, S, Ishikawa, T. Sato, J. Suzuki, H. Yurino, M. Kitabatake, T. Ito, T. Kuriyama & K. Matsushima. Breakdown of mucosal immunity in the gut and resultant systemic sensitization by oral antigens in a murine model for SLE. *J Immunol* 174, 5499-5506 (2005)
34. Mohan C., S. Adams, V. Stanik, & S. K. Datta. Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J Exp Med* 177, 1367-1381 (1999)
35. Fujio K., A. Okamoto, H. Tahara, M. Abe, Y. Jiang, T. Kitamura, S. Hirose, & K. Yamamoto. Nucleosome-specific regulatory T cells engineered by triple gene transfer suppress a systemic autoimmune disease. *J Immunol* 173, 2118-2125 (2004)
36. Qian Y., H. Wang, & S. H. Clarke. Impaired clearance of apoptotic cells induces the activation of autoreactive anti-Sm marginal zone and B-1 B cells. *J Immunol* 172, 625-635 (2004)
37. Qian Y., C. Santiago, M. Borrero, T. F. Tedder & S. H. Clarke. Lupus-specific anti-ribonucleoprotein B cell tolerance in non-autoimmune mice is maintained by differentiation to B-1 and governed by B cell receptor signaling thresholds. *J Immunol* 166, 2412-2419 (2001)
38. Murakami M., T. Tsubata, R. Shinkura, S. Nishitani, M. Okamoto, H. Yoshioka, T. Usui, S. Miyawaki, & T. Honjo. Oral administration of lipopolysaccharides activates B-1 cells in the peritoneal cavity and lamina propria of the gut and induces autoimmune symptoms in an autoantibody transgenic mouse. *J Exp Med* 180, 111-121 (1994)
39. Macpherson A. J., D. Gatto, E. Sainsbury, G. R. Harriman, H. Hengartner & R. M. Zinkernagel: A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 288, 2222-2226 (2000)
40. Bos N. A., J. C. Bun, S. H. Popma, E. R. Cebra, G. J. Deenen, M. J. van der Cammen, F. G. Kroese & J. J. Cebra: Monoclonal immunoglobulin A derived from peritoneal B cells is encoded by both germline and somatically mutated VH genes and is selective with commensal bacteria. *Infect Immun* 64: 616-623 (1996)
41. Gilkeson, G. S., J. Grudier & D. S. Pisetky: Specificity of anti-DNA antibodies induced in normal mice by immunization with bacterial DNA *Clin Immunol Immunopathol* 59, 288-300 (1991)
42. Gilkeson, G. S., M. M. Pippen, and D. S. Pisetky: Induction of cross-reactive anti-DNA antibodies in preautoimmune NZB/NZW mice by immunization with bacterial DNA. *J Clin Invest* 95, 1398-1402 (1995)
43. Sakaguchi S. Regulatory T cells: key controllers in immunologic self-tolerance. Cell 101, 455-458 (2000)
44. Blanco P. A. K. Palucka, M. Gill, V. Pascual, & J. Banchereau. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* 294, 1540-1543 (2001)
45. Pascual V., J. Banchereau, & A. K. Palucka. The central role of dendritic cells and interferon-a in SLE. *Curr Opin Immunol* 15, 548-556 (2003)
46. Santiago-Raber M.-L., R. Baccala, K. M. Haraldsson, D. Choubey, T. A. Stewart, D. H. Kono, & A. N. Theophilopoulos. Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. *J Exp Med* 197, 777-788 (2003)
47. Mathian A., A. Weinberg, M. Gallegos, J. Banchereau, & S. Koutouzov. IFN-a induces early lethal lupus in preautoimmune (New Zealand Black x New Zealand White) F1 but not in BALB/c mice. *J Immunol* 174, 2499-2506 (2005)
48. Farcas L., K. Beiske, F. Lund-Jahnsen, P. Brandtzaeg, & F. L. Jahnsen. Plasmacytoid dendritic cells (natural interferon-α/β-producing cells) accumulate in cutaneous lupus erythematosus lesions. *Am J Pathol* 159, 237-243 (2001)
49. Jego G., A. K. Palucka, J-P. Blanck, C. Chalouni, V. Pascual, & J. Banchereau. *Immunity* 19, 225-234 (2003)
50. Yoneyama H., K. Matsuno, E. Toda, T. Nishiwaki, N. Matsuo, A. Nakano, S. Narumi, B. Lu, C. Gerald, S. Ishikawa, K. Matsushima. Plasmacytoid dendritic cells help lymph node dendritic cells to induce anti-HSV CTLs. *J Exp Med* 202, 425-435 (2005)
51. Allan, R. S., C. M. Smith, G. T. Belz, A. L. von Lint, L. M. Walin, W. R. Heath, & F. R. Carbone. Epidermal viral immunity induced by CD8alpha+ dendritic cells but not Langerhans cells. *Science* 301, 1925-1928 (2003)
52. Krug A., O. Rosorius, F. Kratzer, G. Sterz, C. Cuhnt, G. Schulera, J. Hauber, & M. Cella. Interferon-producing cells fail to induce proliferation of naïve T cells but can promote expansion of antigen-experienced unpolarized T cells. *J Exp Med* 197, 899-906 (2003)
53. Yoneyama H., K. Matsuno, Y-Y. Zhang, T. Nichiwaki, M. Kitabatake, S. Ueha, S. Narumi, S. Morikawa, T. Ezaki, B. Lu, S. Ishikawa, & K. Matsushima. Evidence for recruitment of plasmacytoid dendritic cell precursors to inflamed lymph nodes through high endothelial venules. *Int Immunol.* 16, 915-928 (2004)
54. Krieg, A. M., A. D. Steinberg, A & A. S. Khan: Increased expression of novel full length endogenous mink cell-focus-forming-related transcripts in autoimmune prone mouse strains. *Virology* 162, 274-276 (1988)
55. Krieg A. M., W. C. Gause, M. F. Courley, & A. D. Steinberg: A role for endogenous retroviral sequences in the regulation of lymphocyte activation. *J Immunol* 143, 2448-2451 (1989)
56. Krieg A. M., M. F. Courley, & A. D. Steinberg. Association of murine lupus and thymic full length endogenous retroviral expression maps to a bone marrow stem cell. *J Immunol* 146, 3002-3005 (1991)
57. Morel L., U. H. Rudofsky, J. A. Longmate, K. Schiffenbauer & E. K. Wakeland: Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1, 219-229 (1994)
58. Kotzin, B. L. Systemic lupus erythematosus. *Cell* 85, 303-306 (1996)
59. Wakeland E. K., A. E. Wandstrat, K. Liu & L. Morel: Genetic dissection of systemic lupus erythematosus. *Curr Opin Immunol* 11, 701-707 (1999)
60. Mohan C, L. Morel, P. Yang & E. K. Wakeland: Genetic dissection of systemic lupus erythematosus pathogenesis; Sle2 on murine chromosome 4 leads to B cell heperactivity. *J Immunol* 159, 454-465 (1997)
61. Nath S. K., A. I. Quintero-Del-Rio, J. Kilpatrick, L. Feo, M. Ballesteros & J. B. Haley: Linkage at 12q24 with systemic lupus erythematosus (SLE) is established and

confirmed in Hispanic and European American families. *Am J Hum Genet* 74, 73-82 (2004)
62. Mageed R.A., L. MacKenzie, F. K. Stevenson, B. Yuksel, F. Shokri, B. R. Maziak, R. Jefferis & P. M. Lydyard: Selective expression of VHIV subfamily of immunoglobulin genes in human $CD5^+$ B lymphocytes from cord blood. *J Exp Med* 174, 109-113 (1991)
63. Deane M, L. MacKenzie, F. K. Stevenson, P.Youinou, P. M. Lydyard & R. A. Mageed: The genetic basis of human V_H4 gene family-associated cross-reactive idiotype expression in $CD5^+$ and $CD5^-$ cord blood B lymphocyte clones. *Scand J Immunnol* 38, 348-358 (1993)
64. Raman C & K.L. Knight: $CD5^+$ B cells predominate in peripheral tissues of rabbit. *J Immunol* 149, 3858-3864 (1992)
65. Koarada S, Y. Tada, O. Ushiyama, F. Morito, N. Suzuki, A. Ohta, K. Miyake, M. Kimoto & K. Nagasawa: B cells lacking RP105, a novel B cell antigen, in systemic lupus erythematosus. *Arthritis Rhemat* 42, 2593-2600 (1999)

Key Words: Systemic Lupus Erythematosus, SLE, chemokine, B cell, Immune System, Autoantibody, Review

Send correspondence to: Sho Ishikawa, PhD, MD, Associate Professor, Department of Molecular Preventive Medicine, School of Medicine, The University of Tokyo, Tokyo , 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, Tel: 81-3-5841-3677, Fax: 81-3-5841-3393, E-mail: yamasho@m.u-tokyo.ac.jp