CHARACTERIZATION OF THE B CELLS IN LUPUS-PRONE AND RESISTANT MOUSE MODELS

By

BIYAN DUAN

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

Copyright 2006

by

Biyan Duan

This document is dedicated to my parents and my sister.

ACKNOWLEDGMENTS

I would like thank my dissertation committee members, Dr. Michael J. Clare-Salzler, Dr. William L. Castleman, Byron P. Croker, Dr. Eric S. Sobel and my mentor, Dr. Laurence Morel, for their encouragement, support, and excellent suggestions. Their advice was crucial to my progress in this research.

TABLE OF CONTENTS

TABLE

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

CHARACTERIZATION OF THE B CELLS IN LUPUS-PRONE AND RESISTANT MOUSE MODELS

By

Biyan Duan

May 2006

Chair: Laurence Morel
Major Department: Pathology, Immunology and Laboratory Medicine

The general hypothesis is that in the models of lupus-prone and resistant mice, B cells function differently and contribute to the phenotypes and pathogenesis. Thus, the studies of B-lymphocytes in these models may help to understand the role of B-cells and to identify involved genes.Defect splenic marginal zone (MZ) has been found in the lupus-prone NZM2410 and B6.Sle1/2/3 (TC), but not in the lupus resistant NZM.TAN (TAN) mice. I hypothesize that this defect is due to the abnormal functions of marginal zone B cells and contributes to the pathogenesis. Immuno-fluorescence study revealed that lupus MZ B cells were trans-located inside the follicle. MZ macrophages (MZM) were also found defect only in the lupus mice. Reciprocal bone marrow transfer indicated that the lack of MZM was not the reason of MZB translocation, and MZB miss-location was determined by both bone marrow and stroma-derived factors. Functional studies revealed that MZ B cells in TC and TAN mice were abnormal.

The second goal of the research was to study the phenotypes and functions of the peritoneal cavity (PerC) B cells in these strains. Both lupus-prone and resistant mouse models have increased PerC B cells, especially B1 cells. Studies showed TAN PerC B1

cells had lower activation and higher resistance to activation induced cell death, while B1 cells from NZM2410 and TC had higher proliferation. Finally TAN B1 cells produce little IL-6 and IL-10, while TC B1 cells make more IL-6 and a normal levels of IL-10, suggesting a regulatory role of TC B1 cells in immune response.

The third goal was to study the properties of splenic B cells. Both strains have increased Transitional 1 and Transitional 2 populations and less mature follicular B cells. In vitro stimulations revealed the B cells from lupus mice had a lower activation threshold and higher proliferation/differentiation abilities, while TAN B cells showed the opposite directions. Lupus B cells also had abnormal BAFF-R/TACI expressions, indicating the mechanism behind the phenotypes. Overall these results suggest that B-cell populations in lupus-prone and resistant mice are quite different and may play an important role in the pathogenesis.

CHAPTER 1
INTRODUCTION

The general hypothesis of the study is that in the NZM models of lupus-prone and resistant mice, B cells function differently and contribute to the phenotypes and development of the disease. Thus, the studies of B-lymphocytes in these murine models may help to understand the role of B-cell in lupus development and to identify the genes responsible

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease that affects multiple organs and leads to end-organ damage. SLE is characterized by the production of large amounts of antibodies against a wide spectrum of auto-antigens, including ssDNA, dsDNA and RNPs. B cells have long been recognized as effectors to receive specific T-cell help and to secrete these pathogenic auto-antibodies which result in tissue damage. Recent studies suggest that B-cell may play a role other than the autoantibody producer. In the lupus-prone MRL^{lpr-} mice, modification of B-cell that blocking the antibody secretion does not prevent disease development(1). In the NZB/W derived lupus model NZM2410, genetic dissection shows that the expression of Sle susceptible locus on B cells is essential for the development of autoimmunity(2). T-cell hyper-activation has been found in lupus(3-6), while in MRL^{lpr-}, T-cell activation is greatly dependent on B cells (7), suggesting the role of B-cell as (auto)antigen presenting cells. Furthermore, B-cell depleting treatments lead to remission of SLE symptoms in patients(8-11). All of these findings demonstrate the pivotal role played by B cells in pathogenesis.

B cells are heterogeneous and include multiple subpopulations with distinct

functions and properties. However, the exact role of each subpopulation in the pathogenesis of lupus is still not clear. This study is focused on the characteristics and functions of different B-cell subsets in lupus prone and resistant mouse models.

Peripheral B-Cell Populations

Based on their location, surface marker expression, and functions, the peripheral mature B cells are now divided into three distinct populations: B1 cells, B2 (follicular B, FoB) cells and marginal zone B (MZB) cells(12).

B1 Cells

Based on the expression of the pan-T cell surface glycoprotein-CD5, B cells were originally divided into two populations: B1 (CD5+) and B2 (CD5-) cells(13). Further studies defined B-1 cells as a population possessing a distinct pattern of surface markers: $B220^{lo}$, IgM^{hi}, IgD^{lo}, $CD9^{+}$, $CD43^{+}$, $CD23^{lo}$, and conventional circulating B-2 cells as $B220^{hi}$, $IgM^{hi/lo}$, IgD^{+}, $CD9^{-}$, $CD43^{-}$, $CD23^{hi}$ (14;15). B1 B cells are also larger and exhibit more side scatter than do conventional B-2 cells by flow cytometry. Subsequently, B1 cells were divided into two populations: B1a (CD5+) and B1b(CD5-), i.e. they share all other surface markers and properties except CD5. It is still not clear whether these subsets are two distinct cell types or different development stages of one population. B1b cells contribute only to a small proportion of the B1 population. Recent studies show that they have distinct functions from B1a cells. B1b cells are responsible for long-term T-independent immunity specific for *B. hersmii* (16) and are critical in producing adaptive pneumococcal polysaccharide antibodies (17). For practical reasons, most studies performed on B1 cells are based on results of CD5+ B1a cells. Most B1 cells resides in body cavities such as peritoneal (PerC) and pleural cavities, where they comprise the majority of the local B-cell population. While in the spleen and lymph nodes, B1 cells is

the minority of the local B cells and most of B cells are CD5-. Finally, B1 cells are normally absent from peripheral blood(14).

Compared to B2 cells, B1 cells are long-lived, self-renewing and non-circulating B cells with limited B-cell receptor (BCR) diversity and affinity(18;19). Adoptive transfer suggested that B1 cells originated from fetal liver and thus belong to a developmental lineage different from B2 cells (14). When transferred into irradiated mice, fetal liver cells could reconstitute both the B1 and B2 compartments, while adult bone marrow only generated B-2 cells (20;21).

B1 cells are refractory to activation through B cell receptor (BCR) ligation, and do not undergo somatic hypermutation after stimulation (22;23). B1 cells mainly participate in T-cell independent responses. They respond to LPS much quicker than B2 cells (24) and uptake TI type II antigen dextran, but not ficoll (25). Recent studies also reveal that B1 cells from the spleen are phenotypically and functionally different than those obtained from peritoneal cavity (24;26): Unlike peritoneal B1 cells, splenic B1 cells do not express the myeloid marker CD11b (Mac 1) and have much lower surface IgM and CD80 levels. In addition, they make little natural IgM. Transcriptional factors Notch1 and Notch2 are expressed at high levels by splenic but not peritoneal B1 and B2 cells. Finally, peritoneal B1 cells respond to phorbol eater *in vitro*, while B2 and splenic B1 cells need the presence of the calcium ionophore ionomycin to proliferate. Due to the low concentration of splenic B1, most B1-cell studies are focused on peritoneal B1 cells.

CD5 has been recognized as a negative regulator of BCR signals in B1 cells (27;28). Its cytoplasmic tail contains a docking site for SH2-phosphatase SHP-1, which is critical for diminishing BCR signaling after antigen ligation(29). SHP-1 deficient

“motheaten” mice have lymphocyte over-activation and suffer from severe autoimmune symptoms(30;31). Studies on intracellular signaling pathways reveal that B1 cells have constitutive ERK and NF-AT activation, but lack of NFkapaB induction upon BCR cross-linking(32), which is similar to tolerant B cells(33;34). It has been suggested that CD5 expression by B1 cells occurs after auto-antigen exposure to down-regulate their activation status(35). Targeted deletion of CD5 leads to the activation of anergic B cells and results in the loss of tolerance (36;37), indicating the important role of CD5 expression on B1 cells.

B1 cells are the major producer of IgM natural antibodies, which are poly-reactive and weakly auto-reactive(38;39). Through specific up-regulation of the transcription factor Blimp-1, which was believed to be plasma cell-specific, B1 cells spontaneously secrete a large amount of natural IgM (40). Natural antibodies recognize a broad range of antigens from many bacterial/viral pathogens prior to the exposure. Thus, they are very important for the early response to bacterial and viral infections(41). Mice lacking natural antibodies suffer from higher susceptibility to influenza infections and have increased mortality(42). On the other hand, natural IgM can also bind to constituents of self, such as phosphorylcholine (PC) (16), phosphatidyl choline (PtC) (13;43) and oxidized low-density lipoprotein (LDL)(44). The production of auto-reactive natural antibodies has implicated B1 cells a potential contributors to the development of autoimmune diseases, such as lupus. In fact, elevated B1 cells have been observed in autoimmune patients with Sjogren’s syndrome, SLE and rheumatoid arthritis (RA) as well as in mouse models of lupus(14;45-47). The (NZB x NZW) F1 lupus mouse and its derivative NZM2410 have large numbers of B-1 cells which have accumulated in the peritoneal cavity and, to a

lesser extent, in the spleen(48). Elimination of B1 cells by hypotonic shock with repeated water injections on NZB and (NZB x NZW) F1 mice decreased autoantibody production and reduced kidney pathology(49), suggesting that B1 cells may play a role by producing pathological autoantibodies. While some other studies do not support this notion. For example, in the well-characterized FAS-deficient MRL^{lpr} mouse model, B2, but not B-1a cells are responsible for the autoantibody production (50). Furthermore, over-expression of IL-5 in the (NZB x NZW)F1 model greatly increased the number of B-1a cells, but significantly reduced anti-dsDNA antibody production and the incidence of nephritis (51). These results suggest that the role of B1 cells as autoantibody producer is context-dependent.

In addition to antibody production, B-1a cells express high levels of co-stimulatory molecules B7-1, B7-2 and display enhanced antigen presentation capabilities (48). In aged (NZB x NZW)F1 mice, target organs such as kidney and thymus are found to contain infiltrated myeloid cells with over-expressed CXCL13 (BLC) (52;53). B1a cells are attracted to the target organs by the high local BLC levels and defective in homing to peritoneal cavity. The accumulated B1a cells thus can activate autoreactive T cells through their potent antigen presentation capability and contribute to the damage of target organs (53;54).

B-1a cells are the main source of B cell-derived IL-6 and IL-10 (55), indicating their regulatory role in immune response. IL-6 promotes B-cell survival and strongly induces differentiation of B-cell to plasma cell (56). It also promotes T-cell growth through augmentation of IL-2 production and IL-2 receptor expression (57;58), and rescues T-cell from apoptosis (59;60). IL-6 has been linked to the development of

autoimmune diseases, including lupus and RA (61-64). In the lupus mouse models MRL^{lpr} and (NZB x NZW)F1, increased IL-6 levels has been found (62;65). Furthermore, administration of exogenous IL-6 to NZB/W mice increased anti-DNA autoantibody production and accelerated glomerulonephritis progression (66-68). Studies on human SLE patients have also found elevated IL-6 levels that correlate with disease activity (69;70). In the kidney, increased IL-6 can induces the proliferation of mesangial cells and is involved in the development of glomerulonephritis (71;72). IL-6 blockade treatment dampened the progression of SLE and decreased the severity (58;73). These data show a pivotal role for IL-6 in lupus pathogenesis and also suggest the role of IL-6 producing B1a cells in lupus.

IL-10 is an anti-immflamatory cytokine that strongly inhibits the activation of myeloid cells including monocytes, dendritic cells and macrophages(74-76). IL-10 can promote B cell differentiation, proliferation, and antibody production (77). IL-10 is also involved in regulatory T-cell differentiation and functions(78;79). The role of IL-10 in SLE is still controversial, as it has been shown to both inhibit (80) and exacerbate (81) disease in animal models as well as in patients (82;83). The effect of IL-10 maybe follow a time-dependent fashion, since early increased levels of IL-10 leads to decreased disease severity (Morel Lab, unpublished data).

Marginal Zone and Follicular B (B2) Cells

Conventional B2 cells are the major B-cell participants T-dependent immune responses. It has been shown that in the SLE, B2 cells are responsible for the bulk of high affinity autoantibodies(50;84). Apart from the production of autoantibodies, the exact role of B2 cells in lupus is still not clear.

The splenic CD5- B cells are composed of two populations, follicular B (FoB) and

marginal zone B (MZB) cells, based on their phenotypes, functions and anatomical locations. The FoB cells are IgM^{lo}, IgD^{hi}, $CD1d^{-}$, $CD21^{lo}$, $CD23^{hi}$, while MZB cells are IgM^{hi}, IgD^{lo}, $CD1d^{hi}$, $CD21^{hi}$, $CD23^{lo}$, and are CD9+ (as are B1 cells) (85;86). FoB cells make up the majority of the splenic B-cell compartment, and are short lived circulating cells with a highly diverse BCR repertoire. MZB cells are the major cell population in the marginal zone (MZ), an anatomically distinctive region surrounding the follicles in the spleen. In rodents, the marginal venous sinus additionally lies between the MZ and the follicle (Figure 1-1). Most of the spleen blood flow exits the circulation through the marginal sinus, and then the marginal zone. In addition to B cells, the MZ also contains specialized macrophages that express the scavenger receptor SIGN-R1, called marginal zone macrophages (MZM), stromal cells called reticular cells, and very few T cells(87-89). The location of MZB cells makes them the first-line to encounter blood-borne pathogens, and they have evolved properties and functions to fit this position(90;91). MZ B cells are long-lived and non-circulating cells, and respond to a wide spectrum of T-dependent and T-independent antigens (88;92). After encountering with the cognate TD antigens, MZ B cells migrate into the follicle toward the T-cell area, where they can activate naive T cells more efficiently than FoB cells and quickly differentiate to plasma cells(93;94). Furthermore, the MZB population has a biased BCR repertoire which contains a large amount of auto-reactive clones(95-97). These properties also suggest that they maybe contributors to autoimmunity. Expansion of the MZB population has been found in NZB/W and estrogen-induced lupus mice (98-100). On the other hand, sequestration of autoreactive B cells into the MZ area has also been proposed to be a mechanism to prevent autoimmunity (101;102), and this process is inefficient in

autoimmune MRL^{lpr} mice (103).

The location and migration of B cells are controlled by many factors including chemokines and integrins. As indicated by figure 1-1, re-circulating B cells express high levels CXCR5 and are directed to the follicle B-cell area, where CXCL13, the CXCR5 ligand, is highly expressed(104;105). T cells expressing CCR7 are attracted to the T-cell zone by CCL19/21(104). It has been shown that upon the stimulation of antigens, FoB cells up-regulate CCR7 and migrate to the T-B border of the follicle(106). The situation for MZ B cells is more complicated. MZB cells also express the B-cell zone chemokine receptor CXCR5, which by itself can direct MZB to enter into follicles. However, MZB cells are retained in the MZ by the integrin ligands ICAM-1 and VCAM-1 through their surface expression of LFA-1 and alpha-4/beta-1(107;108). The sphingosine 1-phosphate (S1P) receptors S1P1 and possible S1P3 also participate in the retention of MZB cells (109). S1P is produced by sphingosine kinase-mediated phosphorylation of sphingosine, and present abundantly in peripheral blood (110). MZB cells express much higher levels of S1P1 and S1P3 than FoB cells. Upon antigen encounter, MZ B cells quickly down-regulate the levels of S1P1 and S1P3, and tip the balance toward follicular migration(109). Marginal zone macrophages are also found to be involved in MZB cells retention through the contact of its scavenger receptor MARCO to MZB cells (111). Loss of MZM or blocking MARCO binding by antibodies allows the MZB cells to migrate to the follicle (111). There is also evidence to show that the normal development and maintenance of the marginal zone is dependent upon B-cell, especially MZB cells. Following total B cells depletion by Ig-alpha deletion or CD70 over-expression, both marginal zone macrophages and marginal metallophilic macrophages are lost and the MZ

area is missing (112).

Peripheral B-Cell Development

B cells that are newly emerged from bone marrow contain many self-reactive clones and must undergo peripheral negative selections before the maturation(113). Based on previous studies, B-cell peripheral maturation was divided into 2 or 3 transitional (T) stages according to classification by different surface markers(114;115). In the 2-stage transitional system, the T1 stage is IgM^{hi}, IgD-, $CD21^{lo}$, CD23- CD24+, and the T2 stage is IgM^{hi}, IgD+, $CD21^{hi}$, $CD23^{hi}$, CD24+. In contrast, the MZB cells are IgM^{hi}, IgD-, $CD21^{hi}$, CD23-, CD24-, and the FoB cells are IgM^{lo}, IgD+, $CD21^{med}$, $CD23^{med}$, CD24- (116). Furthermore, T2 cells are circulating and contain the precursors of both MZB and FoB cells(116) (Figure 1-2 A). In contrast, the 3-transitional stage scheme proposed by others was based on the immature B-cell marker AA4.1(117). The T1 cells are IgM^{hi}, $CD21^{-/lo}$, CD23-, AA4.1+. T2 cells are -IgM^{hi}, $CD21^{med}$, CD23+, AA4.1+, and T3 cells are IgM^{hi}, $CD21^{hi}$, CD23+, AA4.1+(117). In a subsequent refinement, the population which is IgM^{hi}, $CD21^{hi}$, CD23+, AA4.1+ was postulated to be the precursors of MZB cells (118) (Figure 1-2 B).

Regardless of the classification scheme utilized, it has been shown that the T1 stage contains large amounts of auto-reactive clones, most of which are removed during the transition from T1 to T2 by negative selection and receptor editing (119). Thus the accumulation of T1 cells, which could increase the workload of this check-point or a defect of this check-point per se could allow the maturation of auto-reactive clones. In addition to negative selection, other studies have indicated that specificity-based positive selection also occurs (120-123). All of these suggest that check points during the B-cell

peripheral development are important for the formation of normal mature B-cell repertoire.

BAFF and Receptors for BAFF

B-lymphocyte stimulator(BLys/BAFF/THANK/TNFSF13B) is a TNF superfamily member. TNF family members are type II transmembrane proteins that form homotrimers as membrane-bond or soluble ligands to their cognate receptors(124). BAFF has been identified as a trophic factor critical for B-cell development, growth and survival(125). The major producers of BAFF are the peripheral blood mononuclear cells (126;127). Macrophages, monocytes, dendritic cells(DC), follicular dendritic cells (FDC) and even B cells can also make BAFF in the context of antigen encounter and activation(127-129). In addition to promoting the peripheral B-cell maturation from the T1 to the T2 stage, BAFF also enhances the survival of immature T1, T2 cells as well as mature B cells(130-132). BAFF and its receptors have been implicated in autoimmunity. High levels of BAFF have been observed in patients with autoimmune diseases such as systemic lupus erythematosis (SLE) and rheumatoid arthritis (RA)(133;134). In animal models, BAFF deficient mice lack the mature B cells, while BAFF transgenic mice show elevated B-cell numbers, especially T2 and MZB cells, and develop autoimmune disorders (135). Furthermore, the lupus-prone models NZB/W F1 and MRL^{lpr} mice have high levels of BAFF in the periphery(136). Experiments on B-cell receptor transgenic mice showed that B cells compete with each other for the binding to BAFF, and that the self-reactive clones have reduced competitiveness, which results in increased susceptibility to deletion(137). In contrast, excess amounts of BAFF can rescue auto-reactive clones from this process and allows them to enter the follicle and marginal zone (138).

Three receptors have been identified for BAFF and its homologue APRIL(a

proliferation-inducing ligand): BAFF receptor (BAFF-R, BR3), B-cell maturation antigen (BCMA) and transmembrane activator and CAML interactor (TACI). The BAFF-R is the only receptor exclusively for BAFF(132;139), while both BAFF and APRIL can bind BCMA and TACI(136;140) (Figure 1-3). BAFF-R is predominantly expressed by all peripheral B cells, but is down-regulated when the cells are activated and become germinal center (GC) B cells(141;142). BCMA is expressed at high levels by plasma cells, plasmablasts and germinal center B cells(141;143). Research has shown that BCMA plays an important role in plasma cell survival rather than B-cell maturation(143). TACI is expressed predominately by marginal zone and activated B cells, but not GC B cells. Signals through TACI is essential for T-independent type II responses (144). However, lack of TACI also resulted into increased B-cell survival, activation, and the development of autoimmune disorders, suggesting that TACI has a role as a negative regulator of T-dependent responses (144-146).

The BAFF/APRIL ligands and their receptors compose a delicate system that regulates B-cell development, activation and homeostasis. Increased BAFF has been implicated in autoimmunity, while abnormal expressions of the receptors could also tip the balance of positive and negative signals and lead to the same outcome.

The NZB/W Derived Mouse Models for Lupus Prone and Resistance

The NZM2410 (NZM) is one of 27 inbred strains derived from an intercross between the NZW and NZB strains(147). NZM2410 mice develop lupus nephritis spontaneously, with 80% of the animals from both sexes affected by 6 months of age and have the histological features that closely resemble those of human patients (148). Analysis of backcrosses between NZM2410 and C57/BL6 (B6) identified 4 genomic intervals containing lupus susceptible loci Sle1~4 on NZM2410 chromosomes 1, 4, 7 and

17(149). Subsequently four congenic strains, B6.NZM*Sle1, -Sle2, -Sle3*, and -*Sle4* were produced by the backcross, each carrying one of the corresponding NZM2410-derived genomic intervals on the B6 genome (Figure 1-4) (149). Phenotypic analysis of these congenic strains revealed each of the loci contribute to the lupus phenotypes. *Sle1* leads to the break of tolerance to nuclear antigens, and results in abnormal phenotypes in both B and T cells(150-153). *Sle2* leads to B cell hyperactivity and increased B1 cells(154;155). *Sle3* leads to decreased activated induced cell death in CD4+ T cells and affects immunoglobulin heavy chain diversity (156-158). SLE pathogenesis can be fully reconstituted by recombining the *Sle1-3* loci on the B6 genomic background (i.e. B6.Sle1/2/3) (159). The B6.Sle1/2/3 (TC) mice display severe splenomegaly, full penetrance of SLE and lupus nephritis at an early age (159). Another strain derived from NZW and NZB, TAN, share the lupus susceptible loci *Sle1, Sle2,* and *Sle3* common to both the NZM2410 and TC strains. However, TAN mice display a dominant resistance to SLE (table 1-1). Although they develop the comparable levels of splenomegaly, TAN mice produce less anti-nuclear antibodies, and do not develop lupus nephritis when followed to 12 month of age. In contrast, aged TAN mice develop a high incidence of marginal zone lymphoma at old age (Morel et al. unpublished data). In this study, TAN mice were used as a lupus-resistant model as compared to NZM and TC mice.

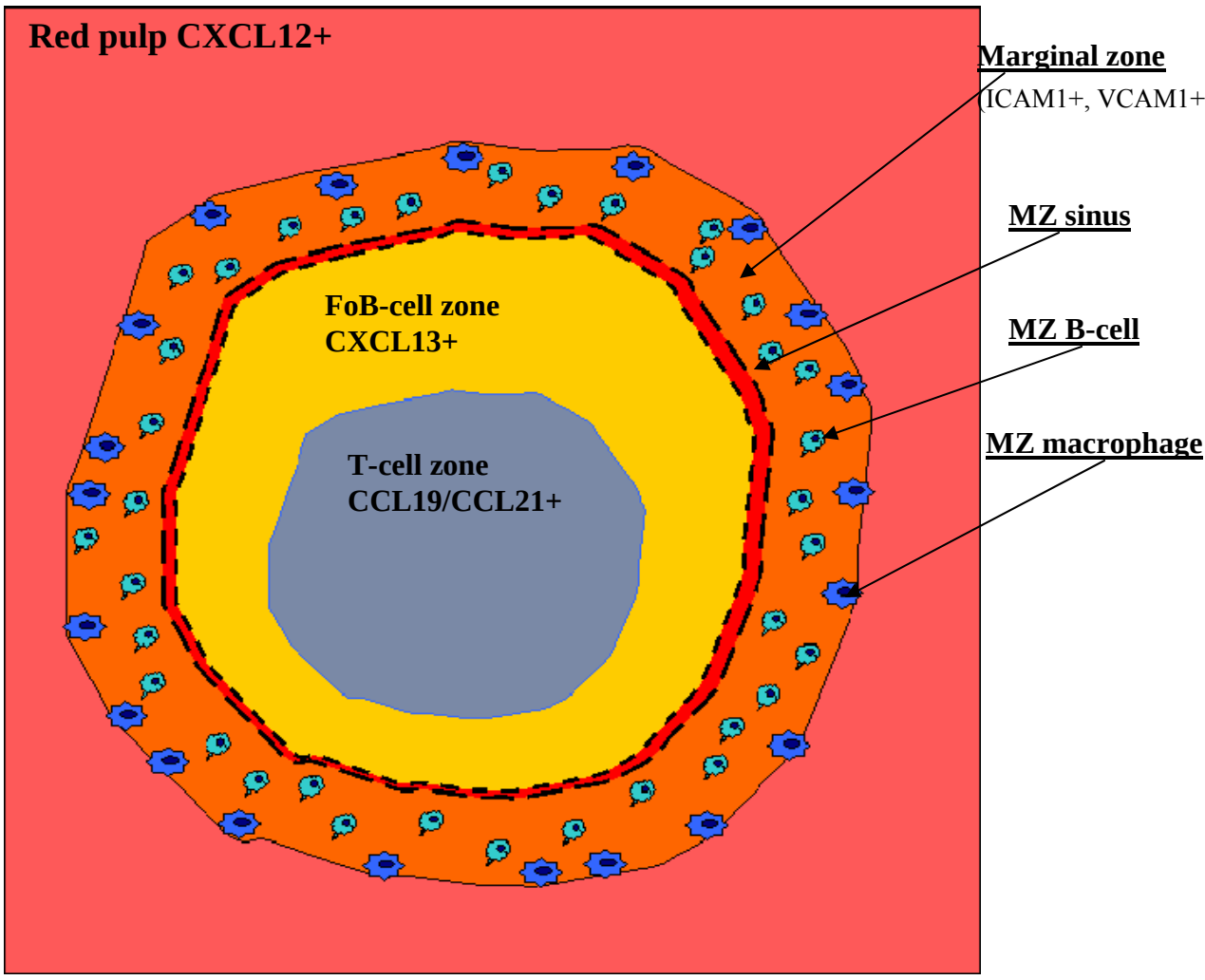

Figure 1-1 Diagram of white pulp and red pulp.

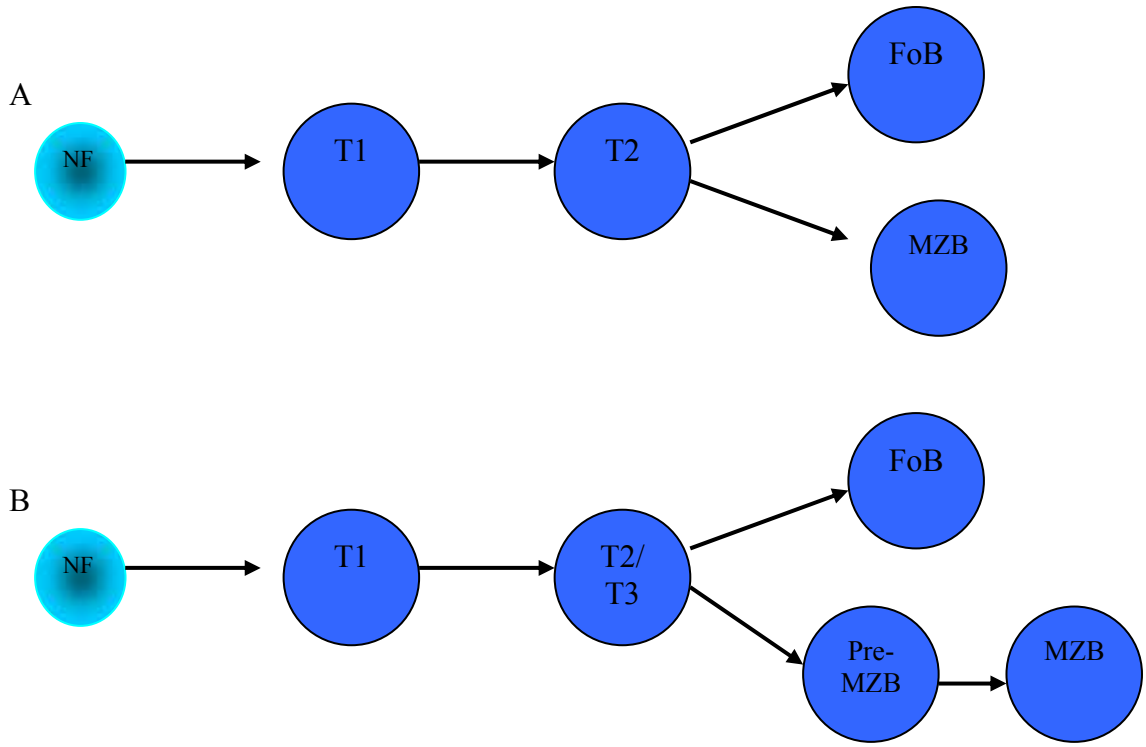

Figure 1-2 Peripheral B-cell development stages. (A) Two-transitional stage scheme. (B) Three-transitional stage scheme. NF, newly formed immature B-cell from bone marrow. T1/T2/T3, transitional 1/2/3 B cells. Pre-MZB, MZB precursors.

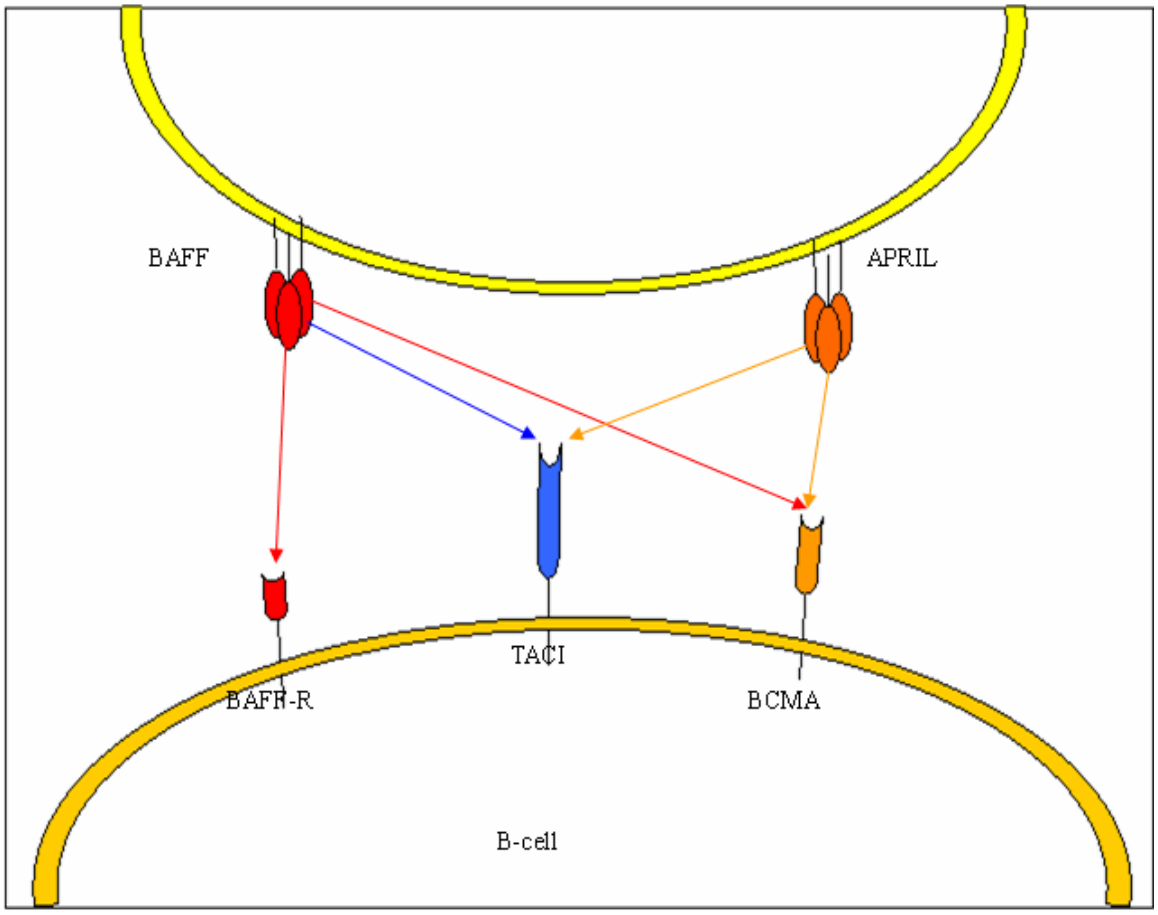

Figure 1-3. BAFF/APRIL and their receptors. Soluble or membrane bonded BAFF and APRIL are trimers. BAFF can bind all three receptors, BAFF-R, TACI and BCMA, while APRIL does not bind to BAFF-R. After ligation, the receptor trimerizes and transduces signals into the cell.

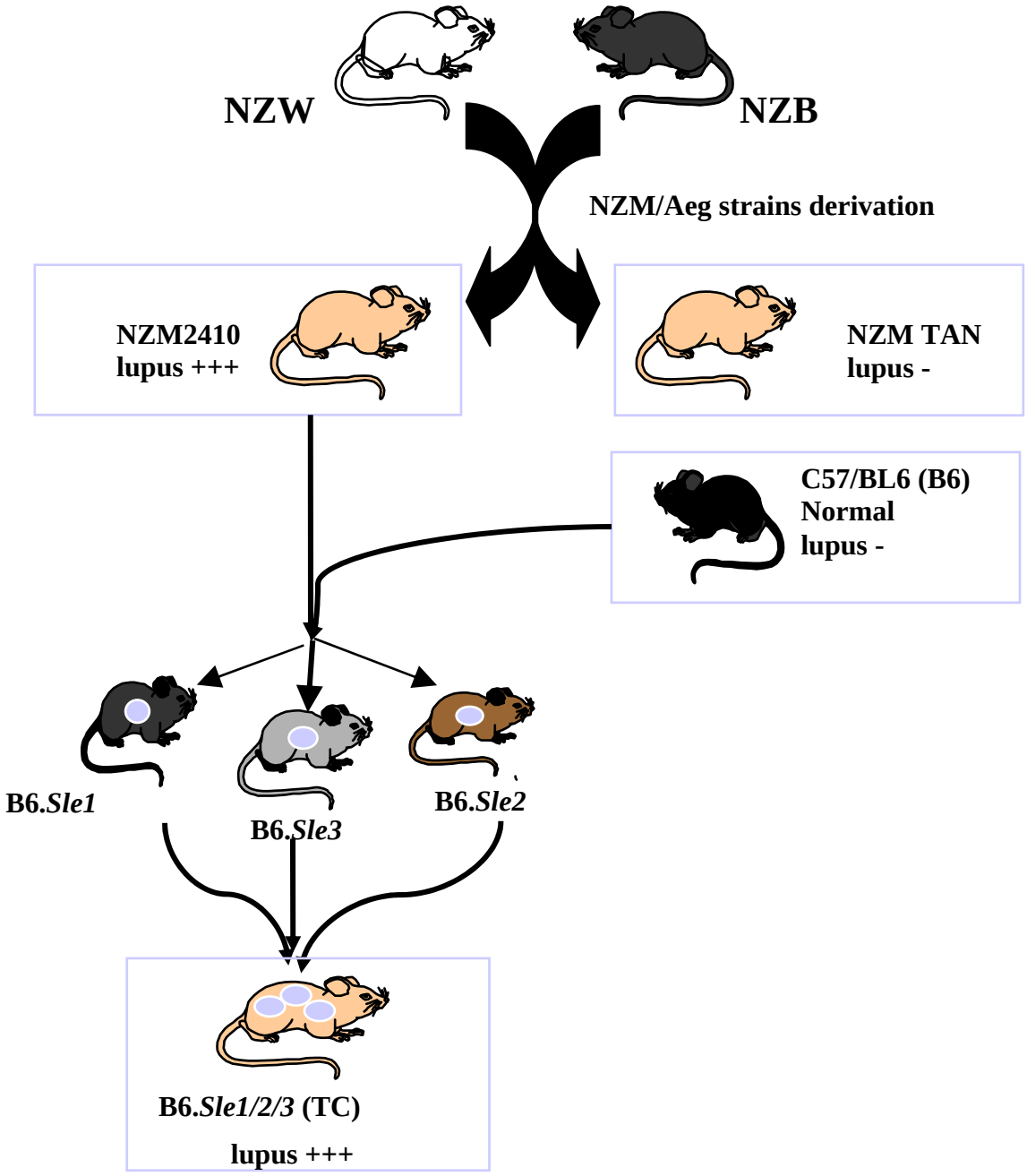

Figure 1-4. NZM models of lupus prone and resistance mice

Table 1-1. Pathological phenotypes of mouse models

	TAN	NZM	TC
Mortality 12mo	10%	85%	100%
Proliferative GN	0%	55%	72%
Anti-chromatin	39%	86%	88%
Anti-dsDNA	35%	85%	78%

CHAPTER 2
MATERIALS AND METHODS

Experimental Animals

TAN mice were maintained in a conventional colony (after several failed attempts to re-derive this strain as specific pathogen free (SPF)). All other strains, NZM2410, TC, B6 and B6.Ly5a (purchased from Jackson Lab, Bar Harbor, ME) were kept in SPF housing as specified by the University of Florida Animal Care Services. Our studies have shown that the housing conditions (conventional versus SPF) did not switch the lupus susceptibility (data not shown). B6.Ly5a mice express a different allotype of CD45 on their leukocytes than that of B6 mice (CD45.1 on B6.Ly5a versus CD45.2 on B6), which can be distinguished by monoclonal antibodies. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida.

Immunofluorescence Staining

Fresh tissues were embedded in OCT, then snap-frozen and stored at –80^{o}C. All samples were cut at –20^{o}C with a cryostat into sections of 6-7um in thickness. Sections were fixed in cold acetone for 10 minutes, briefly air-dried and kept in –80^{o}C until staining. For staining, sections were first blocked with blocking buffer containing 10% rat serum in PBS for 20 min, then stained with fluorochrome-conjugated monoclonal antibodies for 30 min. Anti-mouse MOMA1-FITC was purchased from Serotec (UK), SIGN-R1 (ER-TR9)-biotin was from BMA (Switzerland), while B220 (RA3-6B2)-APC, IgM (160)-APC, CD1d-biotin, and anti-TNP-Biotin were purchased from BD Pharmingen (San Diego, CA). Biotinylated antibodies were further detected with

streptavidin-Alexia 568 from Molecular Probes (Carlsbad, CA). Sections were finally washed, mounted with Prolong Gold media from Molecular Probes and analyzed with a Zeiss Axiom fluorescent microscope.

B-Cell Isolation and Cell Culture

Splenic CD43- naive B cells were purified with the B-cell isolation kit (Miltenyi Biotech, Auburn, CA) according to the manufacture's instructions. Purified B cells were counted with cell counter and adjusted to a concentration of 10^6/ml in complete RPMI1640 containing 10% FCS, then cultured at 37^{o}C, 5% CO_2 with different stimulation conditions. Peritoneal cells were pre-incubated for 3hr in the same conditions to remove adherent macrophages. All cell culture were conducted using 6-, 12- or 24-well tissue culture plate (Corning Life Sciences, Acton, MA). For B-cell stimulations, cells were treated with goat anti mouse IgM $F(ab)_2$ (Jackson Lab, Bar Harbor, ME) at 0, 1 and 10μg/ml or LPS (Sigma, St. Louis, MO) at 1μg/ml.

Flow Cytometry

Single cell suspensions from spleen or peritoneal lavage were treated with FcRgamma Blocker (anti-CD16/32, clone 2.4G2) in flow cytometry buffer (5% FCS in PBS) for 20 min on ice. For the in vitro TNP-Ficoll binding assay, splenocytes suspensions were incubate with TNP-ficoll (Biosearch, Novato CA) at different concentrations for 30 minutes at 37^{o}C. Samples were then stained for 20 min with fluorochrome- or biotin- conjugated monoclonal antibodies against mouse CD1d, CD5 (53-7.3), CD11a (M17/4), CD19 (1D3), CD23 (B3B4), CD45.1 (A20), CD45.2 (104), CD80 (16-10A), CD86 (GL1), IgM (II/41) anti-TNP (G235-2356) (all from BD Pharmingen, San Diego, CA), and CD21 (7E9) (Purified from a hybridoma clone provided by Dr. Boackle, University of Colorado, Denver, CO.). Since all of the strains

that carry Sle1 allele has mutation on extracellular domain of CD21, the commercially available anti-CD21 clone 7G6 binds poorly to the mutated CD21, while clone 7E9 binds well. Biotinylated antibodies were further detected with Strepavidin PerCP-Cy5.5. For intracellular cytokine stains, cells were further fixed and permeabilized with Cytofix/Cytoperm solution for 30 min on ice,. then stained with anti-IL-6, or -IL-10 antibodies according to manufacturer's instructions. Samples were finally analyzed with BD FACS Calibur machine, and at least 60,000 cells were counted.

Bone Marrow Transfers

Three days prior to transfer, recipient mice were started on drinking water containing 40mg/L of the antibiotic Septra (Sulfamethoxazole and Trimethoprim, Hi-Tech Pharmacal CO, Amityville, NY). One day before the experiment, recipient mice were irradiated twice at 525 Rads, each dose separated by 4 hours. On the day of transfer, donor mice were euthanized and bone marrow was flushed with Hank's Solution. Single cell suspension was prepared and washed with cytotoxicity media (0.3% BSA, 0.025M HEPES, 1x Pen/Strep in RPMI1640), and adjust to a concentration of $5x10^7$ cells/ml. To remove T cells, the bone marrow cell suspension was incubated with 1:100 anti-Thy1, anti-CD4 10μg/ml, anti-CD8 10μg/ml (Accurate Chemical, Westbury, NY) for 1 hour at 4^oC. Cells were washed and incubated twice with 1:10 Guinea Pig complement (Accurate Chemical, Westbury, NY) twice for 60 and 30 minutes respectively. Finally, the cells were washed and adjusted to a concentration of $2x10^7$ cells/ml, and a volume of 0.5ml was injected into a tail vein intravenously.

After bone marrow transfer, recipient mice were continued on antibiotic drinking water for three days, and maintained under SPF conditions.

Treatment of Mice with TI Antigens

Mice of 7~9 month old from different strains were injected i.p. with T-independent antigen LPS or Ficoll. For LPS treatment, mice were injected intra-peritoneal with 100ug LPS (Sigma, St. Louis, MO) for 3 hours. For TI-2 antigen ficoll treatment, mice were injected intra-peritoneal with 30ug TNP-Ficoll (Biosearch, Novato CA) for 30 minutes. Mice were sacrificed after the treatment and spleen samples were snap-frozen and stored at -80^{o}C for future Immunofluorescent assay.

Western Blotting Assay

Cultured cells were collected and lysed in RIPA buffer containing protease and phosphatase inhibitor (Santa Cruz Biotechnology, Santa Cruz, CA) for about 15 minutes on ice. Protein concentration was determined by Bio-Rad Protein Assay (Bio-Rad, Hercules, CA). Equal amount of protein (10ug) was resolved on Bio-Rad 12.5% SDS-PAGE gels by electrophoresis, and then transferred to PVDF membrane (Amersham, Piscataway, NJ) by electro-blotting. Membranes were blocked overnight at 4^{o}C with blocking buffer (0.2% Tween 20, 5% non-fat dry milk in PBS) and then probed with primary antibody for 1 hour at room temperature. Rabbit anti-mouse ERK1/2, phosphorylated ERK1/2 (Cell Signaling, Danvers, MA) was used as primary antibody at 1:1000 dilution in blocking buffer. The goat anti-rabbit IgG HRP (Cell Signaling, Danvers, MA) was used as second antibody at 1:2000. Finally, detection was performed with the ECL plus system (Amersham, Piscataway, NJ) and exposed to X-ray film.

Statistics

All data were analyzed by ANOVA. Statistical significance was obtained when $P \leq 0.05$.

CHAPTER 3
RESULTS AND DISCUSSION: ABNORMAL MARGINAL ZONE AND MARGINAL ZONE B CELLS ARE FOUND IN BOTH LUPUS-PRONE AND LUPUS RESISTANT MICE

Diminished splenic marginal zones (MZ) have been found in the lupus-prone NZM2410 and B6.Sle1/2/3 (TC), while lupus resistant NZM.TAN (TAN) mice have enlarged MZ (B.P. Croker, unpublished observations). I hypothesized that these phenotypes are due to the abnormal functions of marginal zone B cells in lupus-prone and lupus-resistant mice. I also hypothesize that the different MZ phenotypes of these strains contributes to their lupus susceptibilities.

Different Marginal Zone Phenotypes Between Lupus Lupus-Prone and Resistant Resistant-Mice

Histological examination of the spleen revealed the age- dependent changes of the marginal zone among the NZM, TC and TAN mice. From 5 or 6 months of age, significantly reduced or absent splenic marginal zone areas and IgM^{hi} MZB cells were observed in both NZM and TC mice. In contrast, the lupus- resistant strain TAN showed a markedly enlarged marginal zones with accumulation of IgM^{hi}, CD5+ cells (Figure 3-1). Flow cytometry analysis did not show a corresponding reduction or enlargement of MZB population in the spleen of lupus-prone or resistant mice (Figure 3-2). This inconsistence indicated the possibility of an altered location instead of the loss of MZB cells in the lupus-prone mice. It also suggested that the B cells present in the MZ of TAN mice could have different characteristics from normal MZB cells.

Further immunofluorescent studies using another MZB cell marker, CD1d,

revealed that the MZB cells in the lupus TC and NZM mice were in fact translocated inside the follicles (Figure 3-3). Chemokine receptor CXCR5 is expressed on mature B cells and is essential for directing B cells into the follicular B-cell area (52;161). Flow cytometry studies revealed that lupus mice MZB cells expressed a higher CXCR5 levels than that of B6, indicating thesuggesting a reason forof their translocation. The TAN MZB cells, on the other hand, did not showed increased CXCR5 levels, but had a significantly high percentage of CD5 expression (Figure 3-4). This is consistent with previous observation by histology showing that TAN MZ area was occupied by CD5+ cells. Thus the B1- type MZB cells were unique for TAN mice. Since CD5 is a negative regulator of B-cell receptor signaling, the CD5+ MZB cells in TAN mice may have different phenotypes from that of other strains.

Subsequently, the number of ER-TR9+ MZ macrophages (MZM) were was also found to be reduced in the lupus mice but was normal in the TAN mice, while the Moma-1 expressing metalophillic macrophages were intact in all strains (Figure3-5). MZMs has have been suggested to interact with MZB cells directly and is are important for the retention of MZB cells (111). These results indicate suggested that the loss of MZM may at least partially responsible for the diminished marginal zone in lupus mice.

Interactions between integrin ligands VCAM-1, ICAM-1 and receptors LFA-1, αalpha-4/βbeta-1 integrin on the cell surface are also critical for the retention of MZB cells (107;108). So we studied the expression levelss of these molecules on the MZB cells and on the stroma cells in of the follicles. Flow cytometry analysis for the levels of LFA-1 on TC MZB cells did not find significant changes (data not shown). Since it is hard to isolate stromal cells, we used an iImmunofluorescencet approach. Studies on the

frozen sections revealed a slightly lower VCAM-1 levels on the MZ area of TC spleen, but not on that of TAN and B6 spleen (figure Figure 3-6). Since lowerLower VCAM-1 levels indicates a lowerless binding for the integrin and a weaker retention factor, which may tip the balance toward follicular migration. This suggests that the stroma cells in TC MZ area may also contribute to the phenotype of MZB cell trans-location.

Reciprocal Bone Marrow Transfers Indicates MZB Translocation is Determined by Multiple Factors

The defective MZ phenotype of lupus mice can be determinominated by either stroma-derived factors, i.e. stromal cell produced cytokines and, integrin ligands, or by bone marrow derived factors, i.e. MZB cell itself, or myeloid cells like MZMs. Since stromal cells are radiation-resistant, and all bone marrow- derived cells are sensitive to irradiation, we conduct reciprocal bone marrow transfers on irradiated B6 and TC mice to study evaluate these possibilities. The B6 and TC recipient mice were lethally irradiated and transferred with bone marrow from untreated TC or B6 mice. The rationale is if the TC MZB cell phenotype was dominant by bone-marrow derived factors, the expected results would be a normal MZ in TC mice that received B6 bone marrow (B6 → TC), and an intra-follicular MZ in B6 mice transferred with TC bone marrow (TC → B6). The reverse results would be expected if stromal cells are solely responsible for the phenotype.

Three months after transfer, mice were sacrificed and spleen sections were examined as mentioned before. Unexpectedly, both B6 recipients that received TC bone marrow and TC recipients transferred with B6 bone marrow showed normal MZ (Figure 3-7).

We also look at the presence of ER-TR9+ marginal zone macrophages on bone marrow transferred mice. None-the-less, the TC- derived bone marrow could not give rise

to a normal amount of marginal zone macrophages, while in the TC recipients transferred with B6 bone marrow, the MZMs rim was present normally (Figure3-8). To verify the origin of the MZB and MZM and rule out the possibility of recipient bone marrow cell contamination, We we also did performed reciprocal bone marrow transfers between B6.Ly5a and TC (allotype Ly5b) mice and determine the cell origin by antibodies that discriminate the allotypic marker Ly5a and Ly5b. Results showed that the MZMs and MZB cells observed are both donor bone marrow derived. And recipient mice have the same manifestation on MZB cells and MZMs (data not shown). Furthermore, flow cytometry analysis on 3 months after reciprocal bone marrow transfer revealed the reconstitution of B-cell phenotypes and functions according to the derived by donor bone marrow origin, i.e. the TC → B6 mice have abnormal B-cell subsets and B6 → TC mice showed similar phenotype as unmanipulated B6 (data showed and discussed ion Chapter 5). This suggested first firsthat the deficiency fect of in MZM number was intrinsic tofor the TC bone marrow, although its pathological significance is still not clear. Second, , these results indicate that the lack of MZMs was not the determining factor of MZB cells' translocation into the follicles in TC mice, and vice versa, thus both the MZB -cells per se and the stromal cells in the marginal zone are most likely to contributed to this phenotype.

Functional Studies of MZB Cells

MZ B cells are the major responders to T-independent antigens (12). To study their functions in the lupus-prone and resistant strains, mice were challenged with the TI antigens TNP-ficoll and LPS. The responses of MZ B cells were assayed by immunofluorescence and flow cytometry.

MZB Respond to TI-2 Antigen Ficoll.

Mice were injected i.p. with 30 μg TNP-Ficoll each, then sacrificed after 30

minutes. Frozen spleen sections were produced, and the uptake of ficoll by MZB cells was revealed by using anti-TNP and anti-IgM antibodies. MZB cells that bind TNP-Ficoll are double positive. Results showed less ficoll binding by TC MZB cells compared to B6 and TAN MZB cells. And In addition, a small amount of ficoll-binding cells present in the follicle of TC mice (Figure 3-9).

To rule out the possibility that the low binding of TC MZB cells to ficoll was due to the limited access resulting from their translocation into follicles, in vitro studies were conducted. Fresh splenic single cell suspensions were taken from mice without any treatment, then incubated with various amounts of TNP-ficoll for 30 minutes. The binding of ficoll was determined by flow cytometry with anti-TNP antibody and gated on MZB cells. We confirmed that non-MZB splenic cells were negative for TNP-binding (data not shown). The results showed that both TAN and TC mice MZ B cells had different degree of impaired binding capability to the TI-2 antigen-Ficoll. The TAN MZB cells had similar binding ability as B6 at low Ficoll concentration ($\leq$ 5 μug/ml). At higher concentrations, they seemed to be saturated and can not bind any more of ficoll as of B6 MZB. In contrast, TC MZB cells have severe defect of ficoll uptake even at lowest concentration tested (Figure 3-10).

MZB Migration in Response to TI-1 Antigen LPS.

It has been shown that the MZB cells migrate into the follicles when they encounter the TI-1 type antigen LPS (162). To determine MZB cell function in response to TI-1 antigen, mice at 7~9 month old were injected i.p. with 100ug LPS. After 3 hrs, the spleens were sectioned and stained with MZB cell markers to reveal their location. The results show that after 3 hrs of LPS treatment, the TAN MZB cells were still stayed located in the MZ area, while MZB cells in TC and normal B6 mice were localized inside

the follicles (Figure 3-11). This suggests that the response to TI-1 type antigen for TAN MZB cells is impaired in terms of migration in response to LPS. It should be noted that most TC MZB cells were already inside the follicule before LPS exposure, and therefore it cannot be determined whether or not they responded to LPS.

Discussion

Both the lupus-prone NZM, TC and resistant TAN mice have abnormal lymphoid micro-structures. This is at least partially due to the fact that they all carry the lupus susceptible locus *Sle3* (156). We have also shown that lupus-prone mice accumulate plasmablasts and plasma cells in the spleen at the expense of their normal migration to the bone marrow (163). The studies here show that the phenotype of the MZ cells correlate to the lupus susceptibility of the mouse strains. The lupus-prone strains MZN and TC have missingmiss the marginal zone area, with MZB cells trans-localized inside the follicles and lack of marginal zone macrophages. The lupus-resistant TAN mice, on the other hand, have an enlarged marginal zone, with a MZ arrestednon-migrating MZB cells having a large proportion of CD5 expression and normal marginal zone macrophages.

The localization and migration of MZB cells is controlled by multiple factors and is a balanced outcome (164). In this study, we conducted reciprocal bone marrow transfers to dissect the reasons for the MZB cell miss-aberrant location in lupus prone mice. The results showing normal of MZ recovery in both directions of the transfers could be explained as the process was not long enough for the manifestation of MZ defect on the experiment animals. Although it can not be totally excluded at this point, This this is, although can not be totally excluded, not likely the case, since flow-cytometric analysis of bone marrow transferred mice have foundshowed the reconstitution of donor

phenotypes, i.e. abnormal B-cell subsets and functions in TC→ B6 mice, which is were identical toreminiscent to unmanipulated adult TC mice (see Chapter 4), indicating the development of lupus phenotype. Still further Additional transfers with longer procedure time before sacrifice are to be conducted to verify this notion. The alternative explanation is that both stroma cells and lymphocyte/myeloid cells are necessary but not sufficient to cause the TC MZB-cell phenotype. Early studies have shown the interactions between integrin receptors and ligands are important for the organization of follicular structures (164;165). Specifically for the B cells, the integrins LFA-1 (CD11a) and α4β1alpha-4beta-1 expressed by B cells interact with their respective ligands ICAM-1 and VCAM-1 expressed by the stromal cells to direct B cells' entry to the spleen white pulp (165). Marginal zone B cells express higher levelss of LFA-1 and α4β1alpha-4beta-1 than follicular B cells to facilitate their entering to the MZ, and this interaction is also critical for their retention in this area (107). Ablation of integrin binding by neutralizing antibodies caused the releasing of marginal zoneMZ B cells from marginal zone (107). We speculated the changes of integrin levelss on either MZB or stromal cells may be involved in the MZB- cell translocation in lupus mice. Till now noNo difference on LFA-1 levels was found between MZB cells from TC and B6, while immunofluorescent study revealed that in TC mice spleen, the MZ has a slightly may have lowerless VCAM-1 levels than the surrounding area, indicating a reduced VCAM-1 expression by their MZ stromal cells. In contrast, TAN and B6 MZ showed a the similarly higher VCAM-1 levels. In addition, alterations of other integrin molecules e.g. α4β1 alpha-4beta-1 and ICAM-1 are not excluded and will be assayed in the future. In short, this suggests that the less

diminished interactions between MZB cells and stromal cells may participate in the MZ phenotype in lupus mice.

Flow cytometry studies have found increased levels of the chemokine receptor CXCR5 on TC and NZM MZB cells, which may also contribute to this phenotype. CXCR5 is the receptor for chemokine CXCL13 (BLC), and it is well known that FoB cells that express CXCR5 enter follicles following the gradient of CXCL13 established by the follicular stroma cells (166). MZB cells also express CXCR5, but other factors that counter the CXCL13 chemoattractant skew the balance to their retention in the marginal zone. One of these factors is are the integrins and their ligands just mentioned above. The receptors for the lysophospholipid sphingosine-1 phosphate (S1P) are also shown important in this process (109). S1P is found in abundant concentrations in the peripheral blood (167). MZ B cells express higher levelss of S1P1 and S1P3 than the follicular B cells, which prevent them from entering the follicles (109). Antigen exposure results to the down regulation of S1P receptors, and treatment with FTY720, an inhibitor for S1P receptor functions, can both abolish the S1P retention and allow the marginal zone B cells to migrate following the CXCL13 gradient (109;168). In the mice lacking of both CXCL13 and S1P1, the marginal zoneMZ B cells stayed in the marginal zone in spite of antigen stimulation (109), suggesting the importance of balance between S1P and CXCL13 chemoattractant. Thus in the case of MZB cells in lupus prone NZM and TC mice, higher CXCR5 levels is likely to tip the balance toward the follicular migration. However, in vitro chemotaxis assay did not show increased migration in response to CXCL13 by MZB cell from lupus mice (data not shown). It is possible that the slightly increased CXCR5 levels itself is not significant enough to cause the increased migration

of MZB cells, and other factors discussed here may also contribute to the MZ phenotype. This notion is consistent with the reciprocal bone marrow transfer results mentioned above. In particular, the ligand CXCL13 may also play a role in this process, as higher CXCL13 expression has been found in peripheral organs of lupus-prone NZB/W F1 mice, which leads to the accumulation of attracted B1 cells in targeted organs (52;169;170). So in the following follow-up studies, the follicular CXCL13 levels in the lupus prone and resistance mice will be assayed. Furthermore, the involvement of S1P-mediated MZB cell retention can not be excluded. It is not known that if the expression of S1P receptors and/or the down-stream signaling are compromised in the lupus mice MZB cells. Due to the lack of commercially available antibodies for S1P receptors, we still can not assay their expression levelss in vivo. The A chemotaxis assay on S1P will be conducted in future studies. Furthermore, we still cannot exclude the role of activation in the function and location of MZB cells, since TC mice with CD86 knocked out showed less disease with little or no MZB translocations (Morel Lab, unpublished data).

MZMs is constitute another major cell population in the marginal zone (111). MZMs express type A scavenger receptor MARCO and C-type lectin SIGN-R1 and are important for capture and clearance of blood-borne pathogens (171;172). Early studies have suggested that MZMs were involved in the retention MZB cells through the contact of the surface receptor MARCO to MZB cells, and MARCO- blocking antibody treatment leads to MZB MZB-cell migration (111). Our studies on unmanipulated and reciprocal bone marrow transferred mice suggested a correlation between MZM defect deficiency and lupus susceptibility. However, the bone marrow transfer studies also show ed that the MZM defect is not the cause of MZB cell translocation in lupus mice. It is

possible that unlike the normal MZB cells in B6, lupus mice MZB cells (gradually) lose their dependence on the contact with MARCO for their retention. Consistent with this notion, the MARCO knockout on B6 mice resulted a diminished of MZB cell population and MZ area (173), while in our lupus-prone NZM and TC mice, the MZB population is not significantly changed in spite of lacking MZM (Figure 3-2). Reciprocal MZB cell transfer between TC and B6 mice with MARCO blocking treatment may help to verify this notion. Besides, the significance of MZM defect in lupus pathogenesis is still not clear.

Recent studies using conditional knockout models have shown that B cells, especially the MZB cells itself themself may also play a pivotal role in the development and maintenance of normal marginal zone (112). Lymphotoxin expressed by B cells have been found to be necessary for the differentiation and maturation of stromal cells, and further inducing their production of chemokine in the white pulp (174-178). Depletion of B cells results to thein defects of the marginal zone and the loss of marginal zone macrophages (112). Thus in the case of NZM and TC mice, the missing MZ and ER-TR9+ MZMs could be the consequence, rather than the cause of MZB MZB-cell translocation.

MZB cells have been implicated in lupus autoimmunity (101;179). Specially, increased number of MZB cells has been found in NZB/W F1 mice (98). Compared to FoB cells, MZB cells respond to TD antigens more rapidly and are more potent T-cell activators (94). Besides, early studies have shown that autoreactive B cells could be positively selected into MZ, which is a postulated mechanism to prevent autoimmunity (100;101;180). In consistent consistence with this notion, the theory of follicular

exclusion also states that auto-reactive B cells are excluded from follicles to prevent their activation, proliferation and differentiation, and finally the excluded B cells would undergo anergy or apoptosis (181-184). In the case of lupus prone NZM and TC mice, although we did not observed higher number of MZB cells and higher MZB MZB-cell activation indicated by CD86 levels (data not shown), their intra-follicular localization may already give them a privilege to participate in follicular responses, which does not occur for MZB cells than the counterpart in TAN and B6 mice, unless they are specifically activated by their antigen or by LPS. In addition, the lupus prone NZM and TC mice have activated auto-reactive T-cell clones and less regulatory T cells ((185) and unpublished data), which may further facilitate the trans-located MZB cells to initiate autoimmune response in the follicles.

On the other hand, the CD5+ MZB cells in TAN mice may also contribute to their resistant phenotype. CD5 is a negative regulator for BCR signaling (29;36;186). The expression of CD5 thus can increase the activation threshold of TAN MZB cells, leading to their poor responsiveness to TI antigens and the impaired ability to participate TD responses. The reason for the CD5 expression on TAN MZB cells is still not clear. Besides, only TAN mice develop high instance of marginal zone lymphoma at 12 month old (Morel et al, unpublished), and the tumor cells are all CD5+ , indicating the MZB cells in TAN have distinct properties. To directly test the role of MZB cells in the lupus susceptibility of these strains, we planed to conduct reciprocal MZB cell transfer and in vitro characterization on purified MZB cells. However, our attempt to isolate MZB population was unsuccessful due to the lack of high-speed cell sorting facility.

The functions of marginal zone B cells in lupus mice were was also defective in terms of TI-2 antigen up-take. Together with the lack of MZMs, this indicates that the marginal zone of lupus mice is incompetent to react against blood bourn pathogens. It is possible that the ineffective clearance of blood–borne pathogens increase the chance of inflammatory damage of peripheral organs and the release of auto-antigens. The components of pathogen organelles may also activate multiple B-cell clones and elicit persistent adaptive responses, increasing the risk of auto-reactive cross-reaction and bystander activation. Further investigations are needed to verify this hypothesis.

In short, this study shows that the marginal zone and MZM phenotypes correlate to the lupus susceptibility but are separate from each other. And the defect on in lupus prone mice is due to the translocation of MZB cells into the follicle, results resulting from defective of itselfMZB cells and the stroma cells.

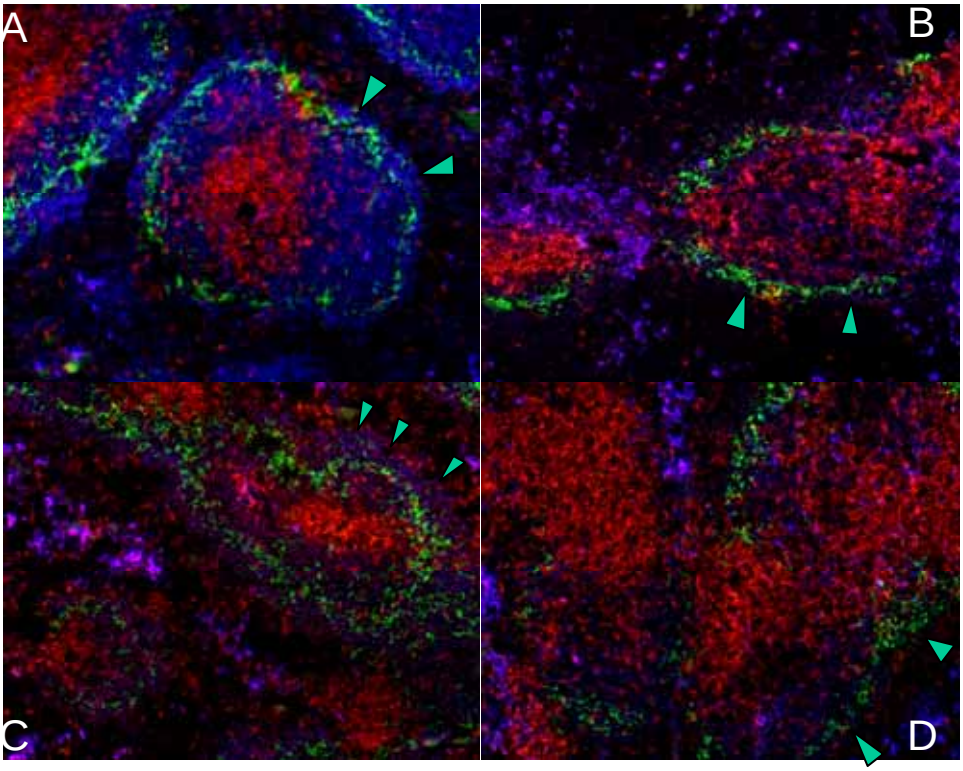

Figure 3-1. Reduced MZ in TC and NZM versus enlarged MZ in TAN mice. Representative slides from 3 mice of each strain at 7~9 month old. Spleen sections were stained with FITC conjugated Moma1 (green), CD5 PE (red) and APC-IgM (blue). MOMAMoma-1+ metallophillic macrophages mark the boundary of the follicle (A) B6. (B) TC. (C) TAN. (D) NZM. B6 follicles have a normal marginal zone. MZB cells are IgM ^{high}, CD5- and are located outside the green ring of Moma-1 stain. The CD5 ^{high} T-cell zone and IgM+ B-cell zone are well defined by the stain. (B) TC and (D) NZM mice showed reduced to absent marginal zone, and T-cell zone and B-cell zone are mixed together and poorly defined. (C) TAN mice have enlarged MZ area, and the cells in MZ express both IgM and CD5 (purple). Arrows indicate MZ area. (100x)

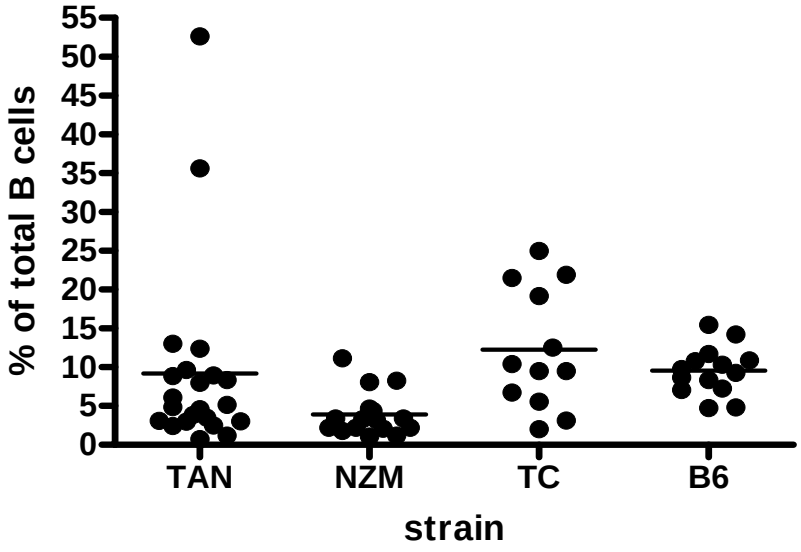

Figure3-2 Splenic MZB populations determined by flow cytometry. MZB cells were gated as B220+, CD21^{hi}, CD23$^{lo/-}$. No significant reduction (P>0.05) of MZB was found between, TAN, TC and NZM compared to normal B6 mice.

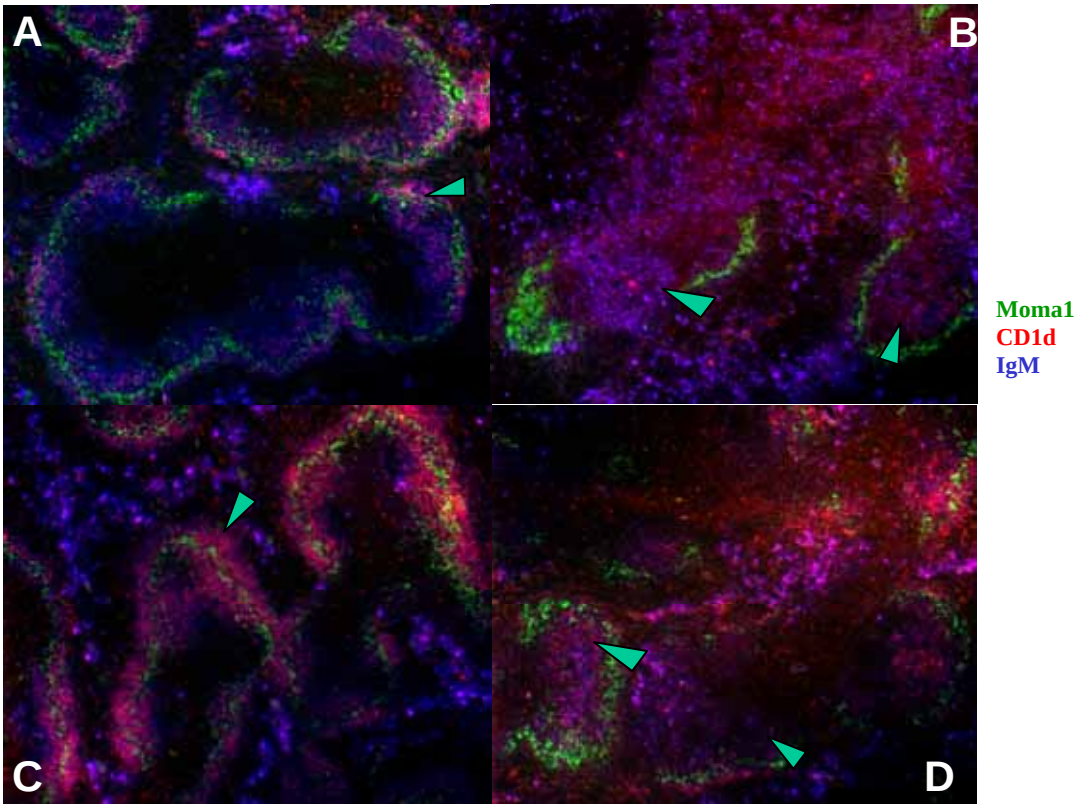

Figure 3-3. Intrafollicular location of MZB cells (IgM+, CD1d+) in the TC and NZM. Representative slides from 3 mice of each strain at 7~9 month old. (A) B6 spleen follicles with normal marginal zone. IgM+, CD1D+ MZB cells (purple color) localized outside the Moma-1 (green) positive rim. (C) TAN MZB cells localized on the both sides of Moma1 rim with increased cell layers. (100x)

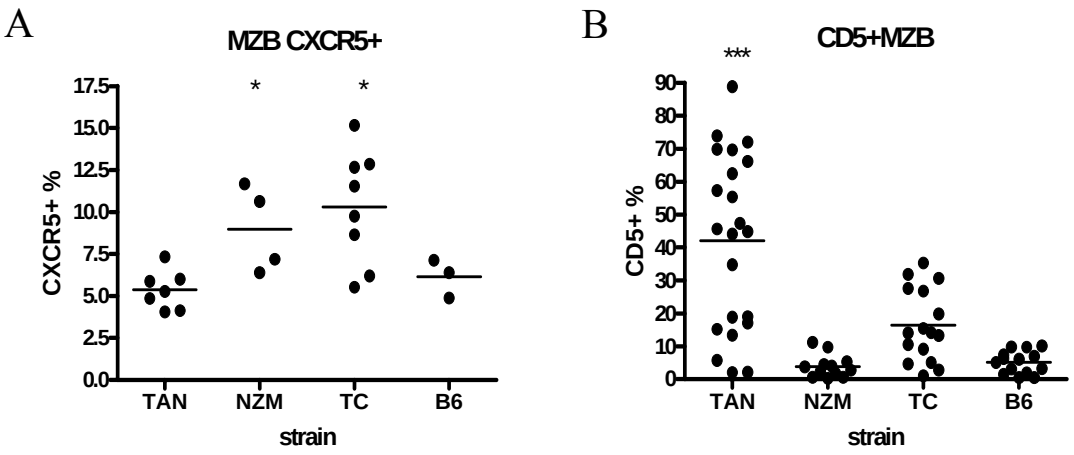

Figure 3-4. MZB cell phenotypes. Fresh splenic cells analyzed with by flow cytometryer. MZB cells are defined as IgM+, CD1d ^{high}. (A) MZB cells from both NZM and TC have express significantly higher levels of chemokine receptor CXCR5. (B) Significantly high proportion of MZB cells in TAN express B1 B-cell marker CD5. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

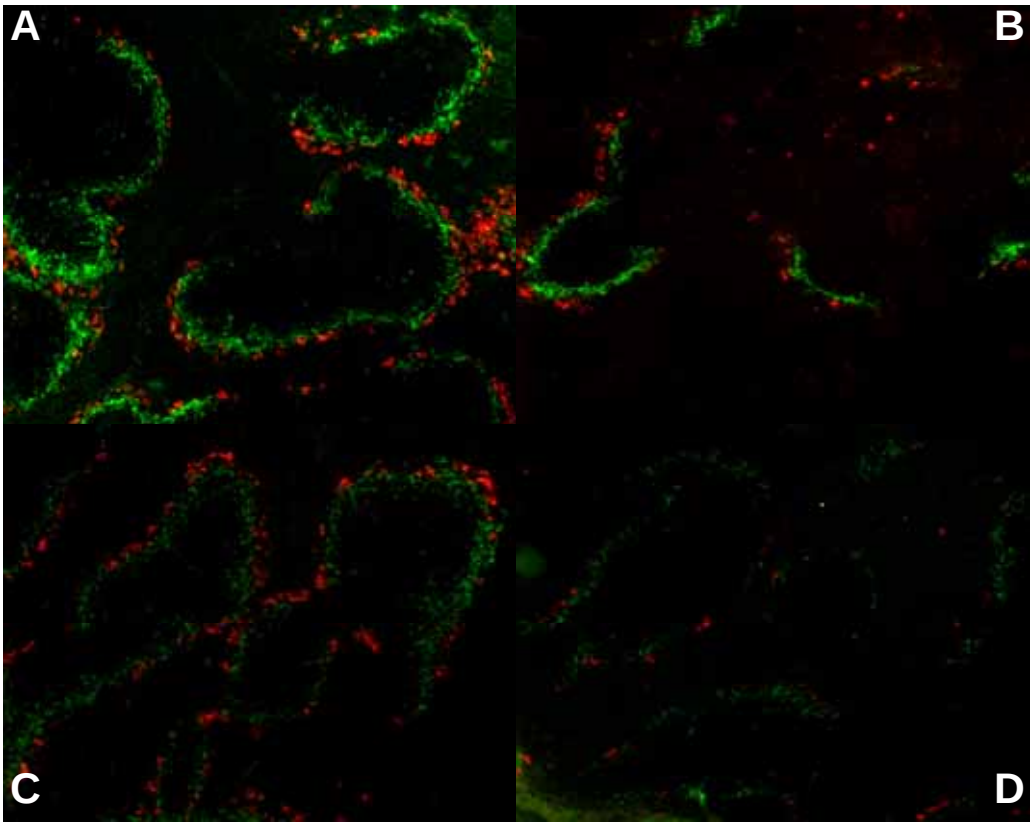

Figure 3-5. Greatly reduced MZMs in the TC and NZM mice. Representative spleen sections are from 3 mice of each strain. Mice were 7~9 month old. (A) B6 spleen follicles have a continuous layer of MZMs (ER-TR9+, red) distributed outside the Moma-1 rim (green). (B) TC had much less MZMs. (C) TAN follicles with a continuous MZM layer similar to B6. (D) NZM mice like TC, has have a defective MZM layer. MZM, marginal zone macrophage. (100x)

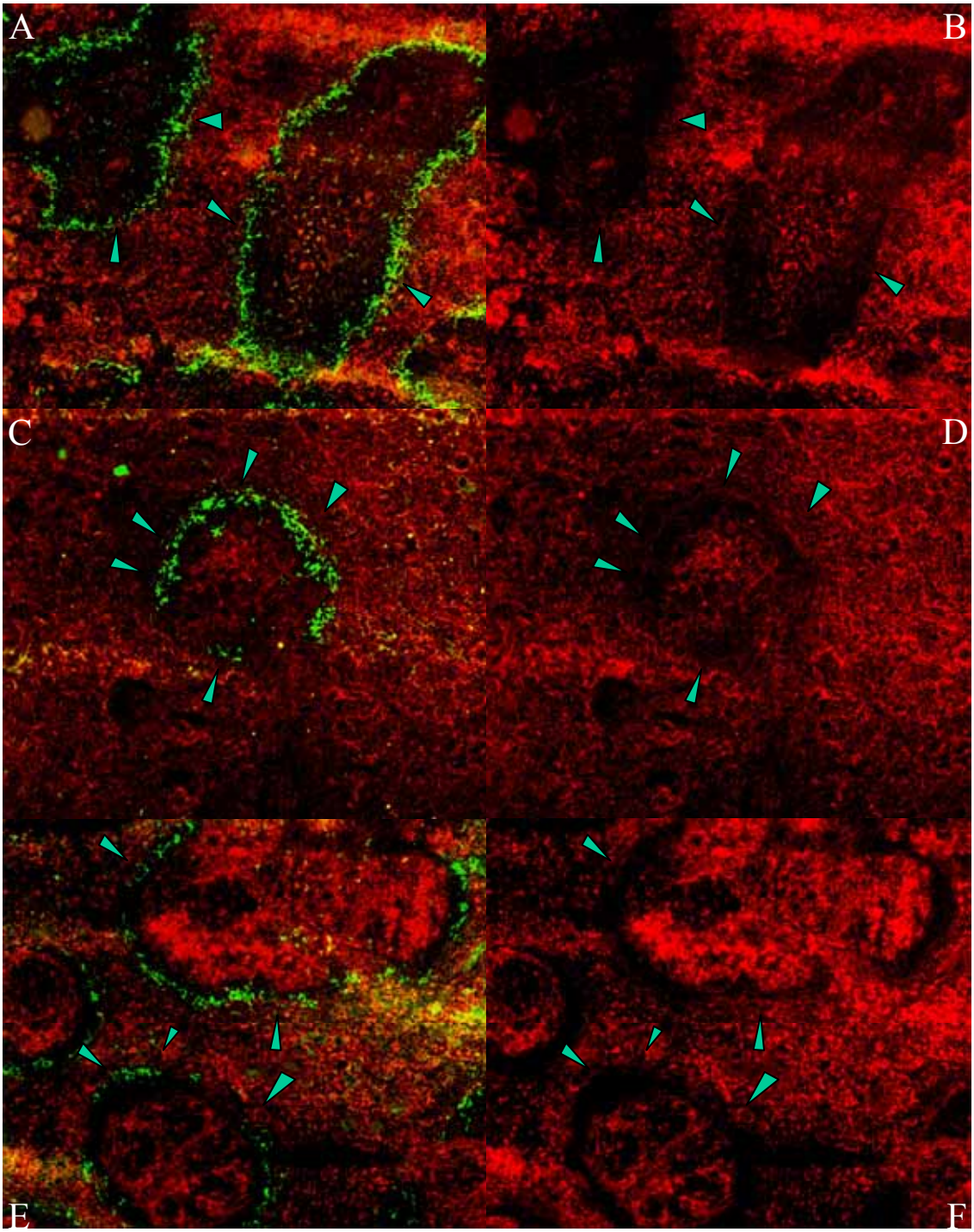

Figure 3-6. Reduced VCAM-1 levels on TC marginal zone. Representative spleen sections are from 3 mice of each strain. Mice were 7~10 month old. Representative spleen sections were stained with anti-mouse Moma-1 (green) and VCAM-1 (red). Arrows indicate the marginal zone area. (A) (B), B6. (C) (D), TC. (E)(F) TAN. TC spleen section show reduced VCAM-1 around marginal zone area. (100x)

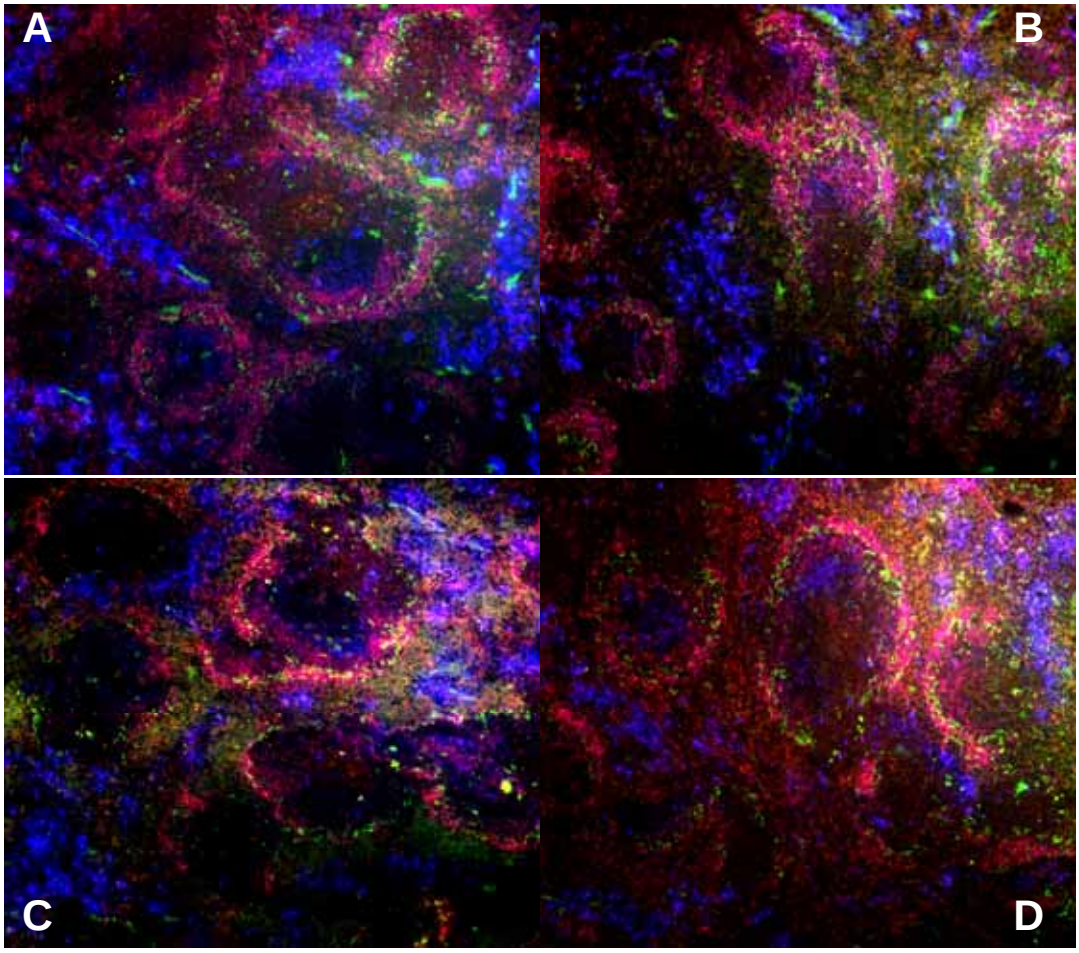

Figure 3-7. Marginal zone of TC and B6 reciprocal bone marrow transferred mice. Representative spleen sections are from 4 mice of each group. Mice about 3 month s old were lethally irradiated and conducted transferred with bone marrow transferfrom the opposite strain. Spleen sections were taken 3 months after transfer. Both B6 → TC and TC → B6 bone marrow chimeras show normal marginal zone. (A) (B) TC recipients received B6 bone marrow. (C) (D) B6 recipients received TC bone marrow. B6 recipients received TC bone marrow. All representative slides show MZ comparable to that of unmanipulated B6 spleen. (100x)

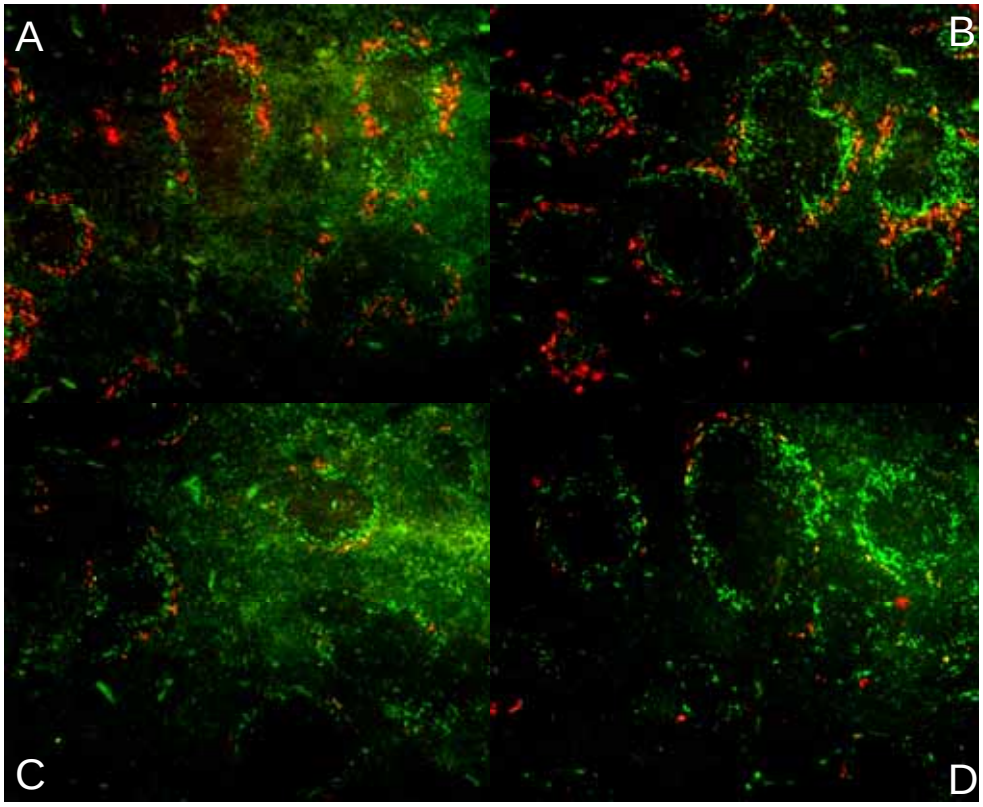

Figure 3-8. Defect of marginal zone macrophages in recipients receiving TC bone marrow. Representative spleen sections are from 4 mice of each group. (A) (B) TC recipients transferred with B6 bone marrow. The ER-TR9+ MZM layer was normal as in B6 mice. (C) (D) B6 recipients transferred with TC bone marrow. The MZM layer was defective as in unmanipulated TC mice. (100x)

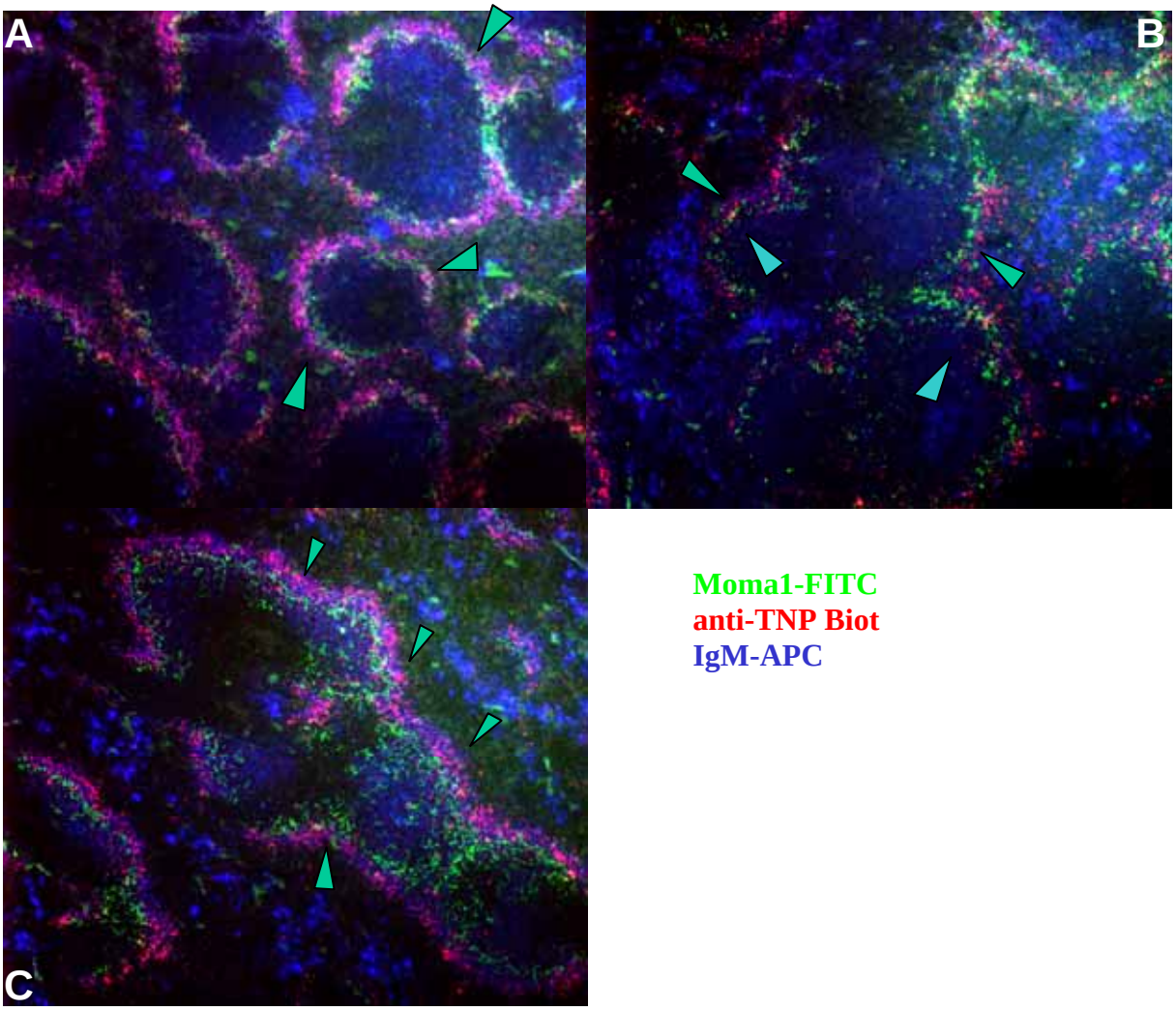

Figure 3-9. TNP-Ficoll up-take by splenic marginal zoneMZ B cells. Spleen sections were take produced 30minutes after TNP-Ficoll i.p. injection. TNP-Ficoll was exposed by anti-TNP (red). Representative spleen sections are from 3 mice of each strain. (A) B6 had an intense TNP+, IgM+ (purple) MZB cell layer surrounding Moma1+ circle (B) TC MZ shows much less Ficoll binding MZB cells in MZ. Also a few TNP+, IgM+ cells localized inside the follicles (red arrows). (C) TAN MZ also showed good TNP-ficoll binding in the MZ, but the presence of TNP negative MZB cells indicates less bind than in B6. (100x).

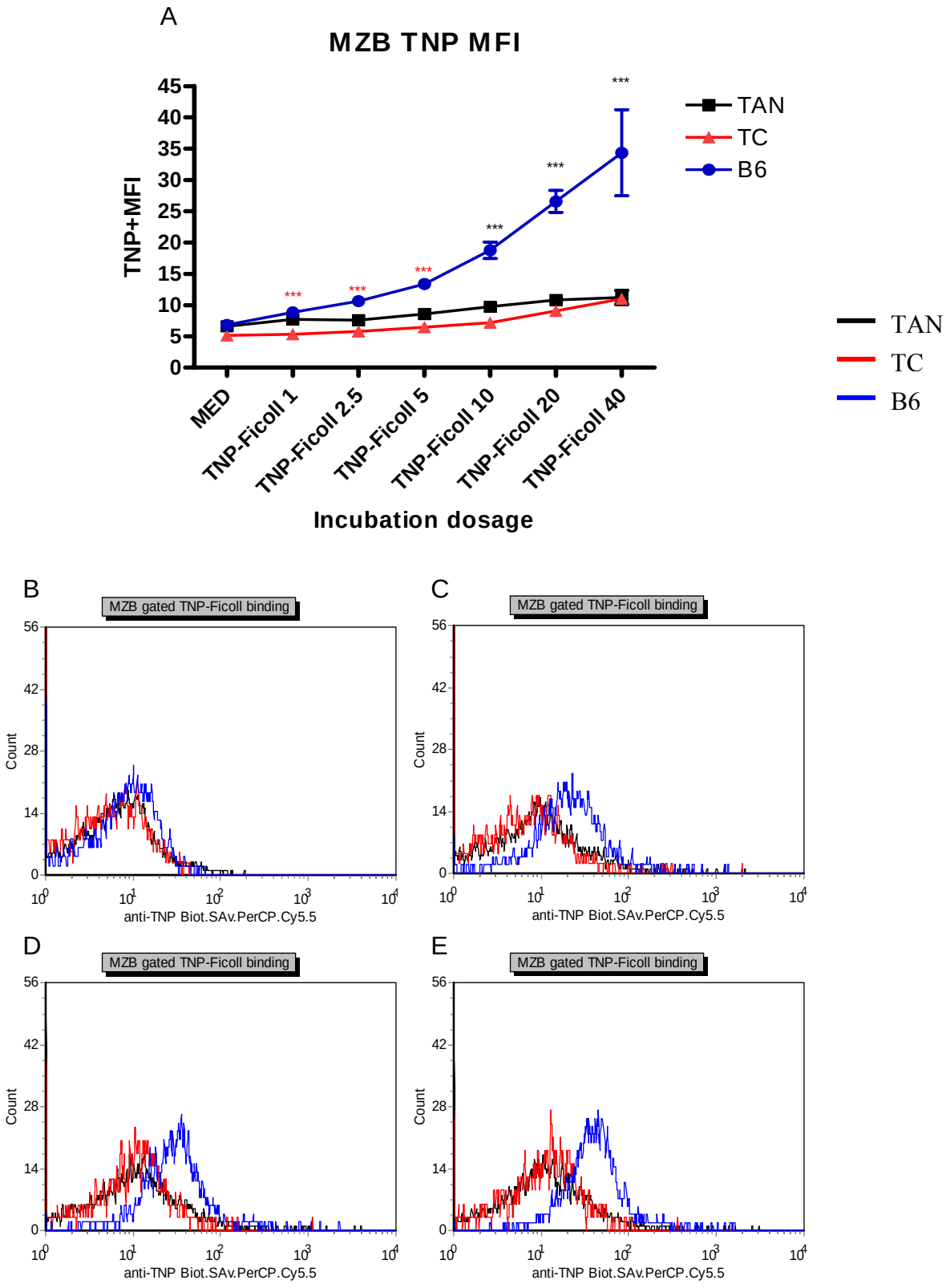
A
MZB TNP MFI
TNP+MFI
45
40
35
30
25
20
15
10
5
0
***
MED
TNP-Ficoll 1
TNP-Ficoll 2.5
TNP-Ficoll 5
TNP-Ficoll 10
TNP-Ficoll 20
TNP-Ficoll 40
Incubation dosage
TAN
TC
B6
B
C
D
E
MZB gated TNP-Ficoll binding
Count
anti-TNP Biot.SAv.PerCP.Cy5.5

Figure 3-10 In vitro TNP-Ficoll binding capability of MZ B cells. Fresh isolated splenocytes were incubated with TNP-Ficoll at different concentration for 30 minutes. The binding of TNP-Ficoll was assayed by flow cytometry with anti-TNP monoclonal antibody. Cells were gated on MZB markers (IgM+, CD1d high). (A) Impaired TNP-ficoll up-take by TC and TAN MZB cells as compared to B6. Representative histograms of TNP-ficoll binding by MZB cells from each strain shown on (B) Medium only. (C) TNP-Ficoll 10µg/ml. (D) TNP-Ficoll 20µg/ml. (E) TNP-Ficoll 40 µg/ml. * P<0.05, ** P<0.01, *** P<0.001.

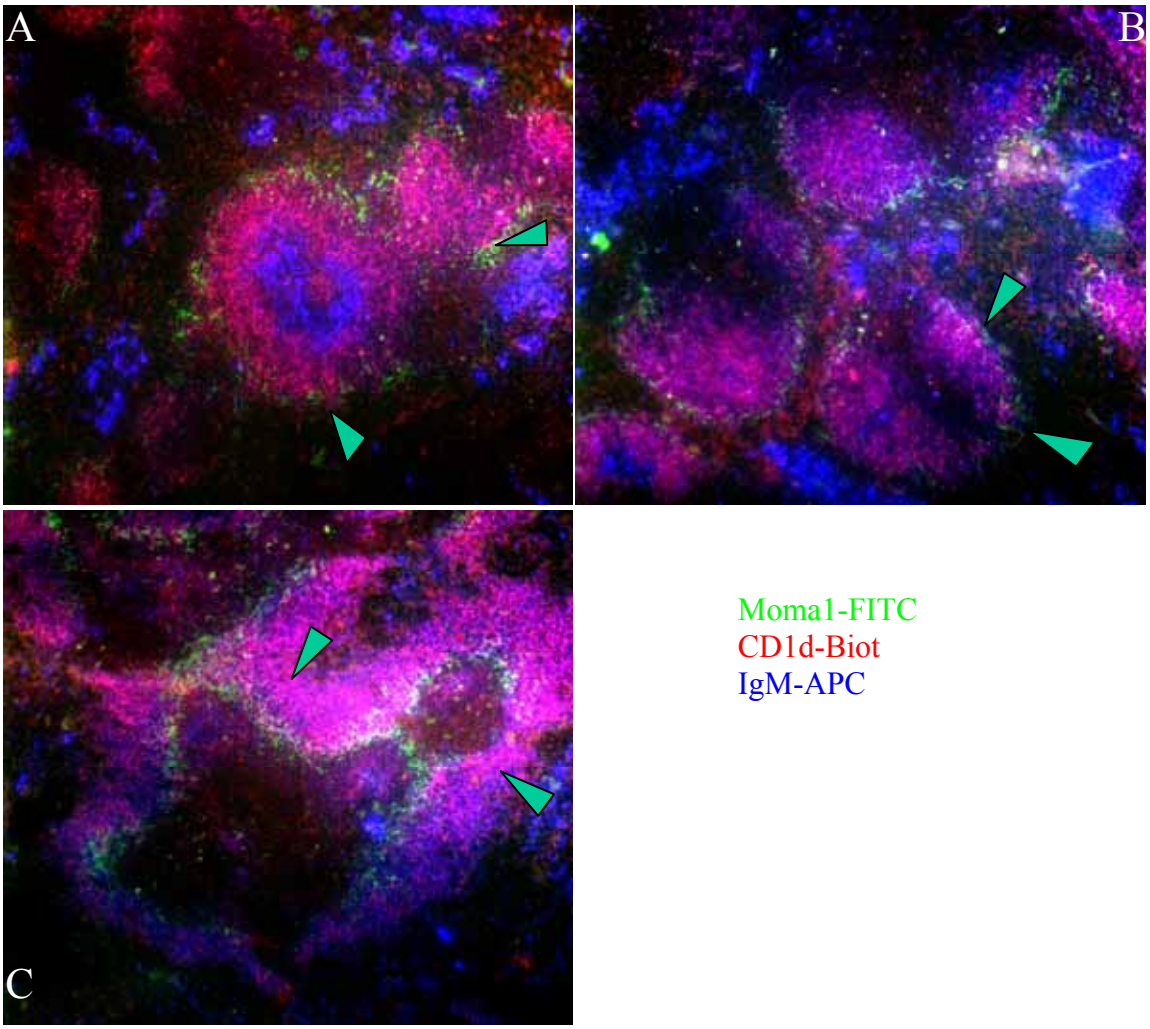

Figure 3-11 TAN MZB cells do not migrate after LPS treatment. Representative spleen sections are from 3 mice of each strain. Mice were ~7 month old. Mice were injected with 100 µug LPS i.p. for 3 hours. Spleen sections were taken and location of MZB cells (IgM+, CD1d ^{high}, purple) were assayed. (A) B6 and (B) TC. MZB cells are trans-located into follicle demarcated by green Moma-1 circle. (C) TAN. MZB cells do not migrate. (100x).

CHAPTER 4
RESULTS AND DISCUSSION: ROLE OF PERITONEAL CAVITY B CELLS IN LUPUS SUSCEPTIBILITY

Both lupus-prone TC, NZM and resistant TAN mice have increased total peritoneal cavity (PerC) cells as well as B1a B-cell population. Decreased numbers of B2 cells are also found in NZM and TAN, but not TC mice (Figure 4-1). This increase in PerC B1a cell corresponds to the expression of Sle2, which is present in TAN, NZM, and TC (155;187). No difference was found in expression levels of activation markers, e.g. B7, CD69, CD40 on PerC B1 and B2 cells between these strains.

Activation, Proliferation and Apoptosis

Both the lupus-prone and resistant strains have increased B1 cells. I speculate the B1 cells in lupus prone mice are different from their counterpart in lupus resistant mice, and they both contribute to the strain phenotypes. It is not clear if the B1 cells in lupus mice are more prone to activate and undergo differentiation than that of TAN and B6 mice upon stimulation. If this is true, it means the B1 cells may directly participate in the pathogenesis as effectors. Furthermore, the increased cell number in both strains can result from the enhanced proliferation and/or decreased apoptosis. To test these possibilities, experiments to assay the B1 cell activation, proliferation and apoptosis properties were conducted on these strains.

In Vivo Spontaneous Proliferation:

Adult mice from each strain were injected with 1mg BrdU i.p. After 4 days, the spontaneous proliferation of B cells indicated by BrdU incorporation was assayed by

flow cytometry. Results showed B1 cells from lupus-prone NZM and TC mice had more proliferation, while only B2 cells from TC mice had slightly higher proliferation (Figure 4-2).

In Vitro B-cell Apoptosis

Whole peritoneal cavity lavage cells from TAN, TC and B6 mice were incubated for 2 hrs at 37^0C to remove macrophages, then cultured with medium only, anti-IgM (10 μg/ml) or LPS (1 μg/ml) for 16 hrs. Early apoptosis was measured by flow cytometry to detect cleaved caspase-3. Results showed that the TAN mice B1 cells had significantly lower apoptosis rate, while no difference was found on TC B1 cells (Figure 4-3). On the PerC B2 cells, only TAN mice showed lower apoptosis when treated with LPS, and the TC did not show a difference with B6 mice (Figure 4-4)

Peritoneal Cavity B-Cell Activation

PerC cells were cultured as mentioned above for 48 hrs. B-cell activation was evaluated by the expression of CD80 and CD86 with flow cytometry. Results showed that TAN B1 cells showed significantly less activation capability in all three conditions. The same levels of activation was observed between TC and B6 B1a cells, except with LPS stimulation, which resulted in a significantly lower activation in TC B1a cells (Figures 4-5 and 4-6).

Cytokine Production

Peritoneal B1 cells are the major source of B-cell derived IL-10 (55), and also produce IL-6 (188). Both cytokines are important in regulating immune responses (189). In this study, we tested the production of IL-10 and IL-6 by PerC B cells in vitro. PerC cell suspensions were cultured for 72 hrs, and the intra-cellular cytokine levels were assayed by flow cytometry. Results showed that TAN B1 cells produce significantly less

IL-10 and IL-6 than either B6 and TC mice, while TC B1 cells make significantly more IL-6 when stimulated with anti-IgM (Figures 4-7, 4-8). Interestingly, B1a cells from the lupus prone TC mice do not produce more IL-10 on a per cell basis, but because TC mice have large amounts of B1 cells accumulated in the peritoneal cavity, the overall effect is an elevated IL-10 levels. This is consistent with the fact that lupus is associated with higher levels of circulating IL-10 (190).

Discussion

Increased levels of B1 cells has been observed in human autoimmune patient as well as some lupus mouse models(14;45-47). The role of B1 cells in the lupus pathogenesis has not been fully understood. In the NZM derived models, the lupus-prone NZM, TC and resistant TAN mice have increased peritoneal B1 cells. This is at least partially because they all carry the entire lupus susceptible locus *Sle2*, which leads to an age-dependent enlargement of the B1 compartment (155). Our studies here show that the peritoneal B1 cells from lupus prone and resistant mice have different functions and properties.

B1 cells from lupus prone NZM and TC mice show significantly higher rate of spontaneous proliferation than that of TAN and B6 mice, indicating an active cell cycle progression. Normal B1 cells do not actively cycle in vivo (191). The increased B1 cell proliferation in NZM and TC mice reflect the dominant functions of lupus susceptible locus *Sle2* carried in these strains, which have been found to cause elevated B1 cell proliferation (155). Interestingly, although TAN mice also carry *Sle2*, their B1 cells do not show the same high proliferation as that of lupus mice, suggesting other genes in TAN genome may suppress this function of *Sle2* locus, and this correlates with the lupus susceptibility. The exact reasons for high B1 cell proliferation caused by Sle2 is still

unknown (155). As B6 mice carrying Sle2 have heightened B cell responsiveness to in vitro stimuli and to in vivo antigenic challenge (154), the B1 cells from NZM and TC mice may have lower threshold in response to antigen exposure in the peritoneum, or on the other hand, they may up-regulate cell-cycle progression genes without stimulation. This hypothesis is to be tested with purified B1 cells in future studies.

Both B1 and B2 cells from TAN mice peritoneum showed higher resistance to activation induced cell death when stimulated in vitro. While the lupus-prone TC mice B1 and B2 cells showed similar apoptosis rate as that of normal B6. The Sle2 locus also promote the B1 cells survival when carried by B6 genome (155) This result suggests that on the Peritoneal B1 cells of TC mice, the proliferation-promoting function of Sle2 locus is dominant while its anti-apoptotic effect is not significant. The reason for this inconsistency is not clear, probably due to the interaction with other lupus loci. On the other hand, these data also indicated that the mechanism of B1 cells accumulation in the peritoneal cavity is different among these strains: with less apoptosis more survival in TAN and high proliferation rate in the lupus mice.

The responsiveness of TC peritoneal B cells to the stimulations could be the indication of their roles and functions in lupus pathogenesis. If they actively participate in the autoimmunity, the heightened activation response to stimulations is expected, and vice versa. The in vitro stimulation shows compared to that of B6, both B1 and B2 cells in the TC peritoneal cavity do not have significantly different activation in response to BCR cross-linking and to LPS. On the other hand, peritoneal B cells from TAN mice express much lower co-stimulatory molecules regardless of these stimulations. These results indicate peritoneal B1 cells in lupus TC mice may not contribute to the lupus

pathogenesis through more heightened activation to antigen. And the low responsiveness and low co-stimulatory molecule expression by TAN B1 cells may contribute to the lupus resistant phenotypes. The approach to purify B1 cell is under investigation and peritoneal B1 cell transfer will be conducted to verify this notion.

Peritoneal B1 cells is the major source of B-cell derived IL-10 (55), and also produce a lot of IL-6 (188), indicating their regulatory role in immune response. In vitro stimulation showed that TAN B1 cells produce much lower amount of both IL-10 and IL-6 regardless of stimulations. TC B1 cells produce similar amount of IL-10 as that of B6 B1 cell at a per cell level, and they produce more IL-6 after BCR cross-linking. Since TC mice have much more B1 cells than B6 mice, the overall effect will be a lot more total IL-10 and IL-6 production by PerC B1 cells. IL-10 is a Th2 cytokine that can promote B cell differentiation, proliferation, and antibody production (77). It has been shown to be involved in lupus pathogenesis (77). Higher levels of circulating IL-10 correlate with lupus development (190). Thus, this result suggests that B1 cells participate in lupus pathogenesis by producing IL-10.

Furthermore, IL-6 is also an important cytokine that regulate immune response. IL-6 promotes B-cell survival and strongly induce differentiation of B-cell to plasma cell (56). In human SLE patients, elevated IL-6 levels correlate with lupus activity and kidney pathology (69-72). Besides, IL-6 also blocks the suppression effect of regulatory T cells (192;193), which will exacerbate status of already low Treg in lupus.

Interestingly, the BCR crosslinking suggesting that upon antigen encounter, TC self-reactive B1 cells will make much more IL-6. In general, the elevated B1 cells in TC will results in more IL-6 entering the circulation and promote the disease development.

Overall, these studies suggest that the peritoneal B1 cells in lupus prone NZM and TC mice are different from that of lupus resistant TAN and B6 mice. The enhanced proliferation results in the accumulation of B1 cells in lupus mice. And their B1 cells contribute to lupus pathogenesis by the mass production of IL-6 and IL-10. B1 cells in TAN mice are more resistant to apoptosis and respond poorly to stimulations in terms of activation and cytokine production.

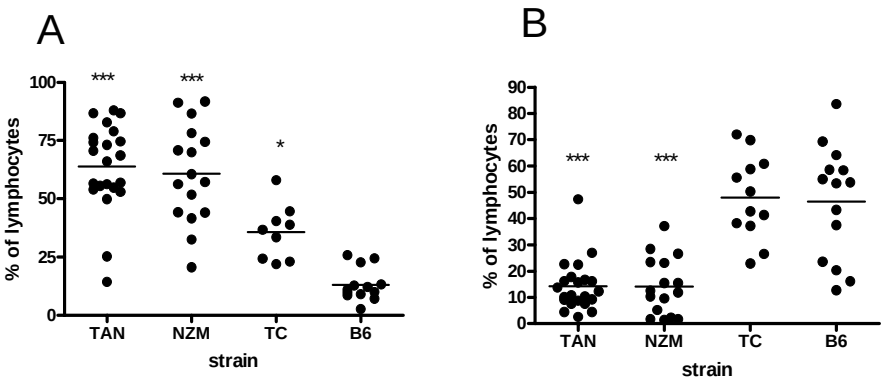

Figure 4-1 Peritoneal B1 and B2 populations by flow cytometry. Mice are 5~9 month of old. (A) Peritoneal cavity B1 cells. Significantly increased B1 B cells in TC, NZM and TAN mice. (B) Peritoneal B2 cells. Both TAN and NZM mice show lower percentage of B2 cells.

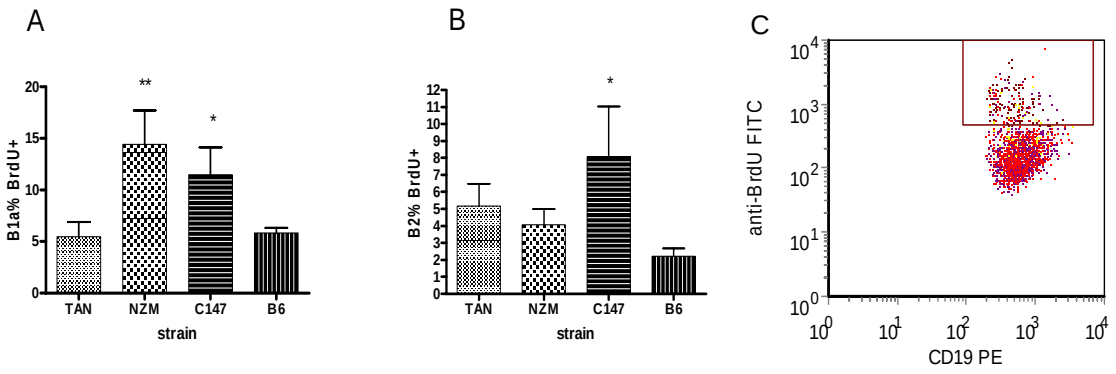

Figure 4-2 In vivo proliferation of peritoneal cavity B cells. Mice were injected with 1mg BrdU i.p. and proliferation indicated by BrdU incorporation was assayed 4 days later by flow cytometry with anti-BrdU antibody. (A) B1 cells in the lupus TC and NZM PerC showed higher spontaneous proliferation than TAN and B6 mice. (B) B2 cells in TC PerC showed higher proliferation rate. (C) Representative flow cytometry plot of BrdU incorporation. Bar graph shows mean ± SD. * P<0.05, ** P<0.01.

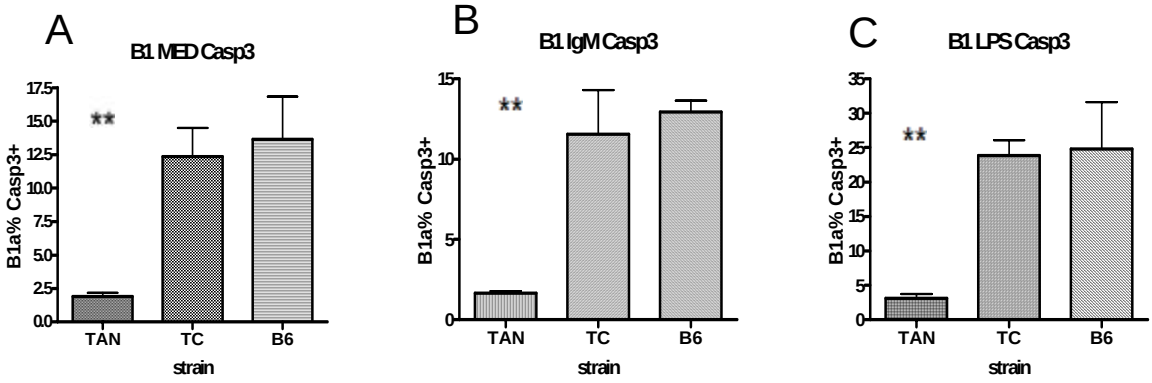

Figure 4-3 PerC B1 cells apoptosis. Cells from peritoneal lavages were collected and cultured with (A) Medium only, (B) 10 μg/ml anti-IgM, (C) 1 μg/ml LPS. Intracellular activated Caspase-3 was assayed by flow cytometry. TAN B1 cells showed lower apoptosis than TC and B6 in all 3 conditions. Bar graph shows mean ± SD. ** P<0.01.

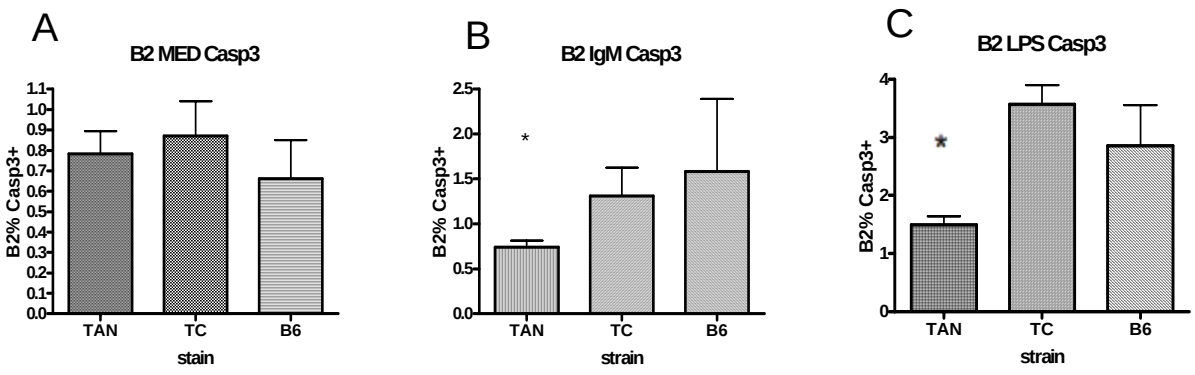

Figure 4-4 PerC B2 cells apoptosis. (A) Medium only. (B) Stimulated with anti-IgM 10 μg/ml (C) Treated with LPS at 1 μg/ml. Only TAN B2 cells showed lower apoptosis in response to stimulation. TC B2 cells did not show a significant difference from B6 in the culture. Bar graph shows mean ± SD. * P<0.05.

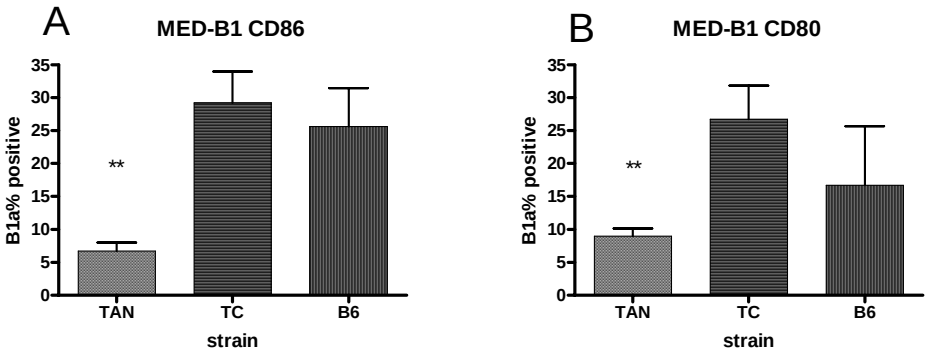

Figure 4-5 Cells from peritoneal lavage were collected and cultured for 48 hours in medium only. Expression levels of (A) CD86 and (B) CD80 were assayed by flow cytometry. Bar graph shows mean ± SD. * P<0.05.

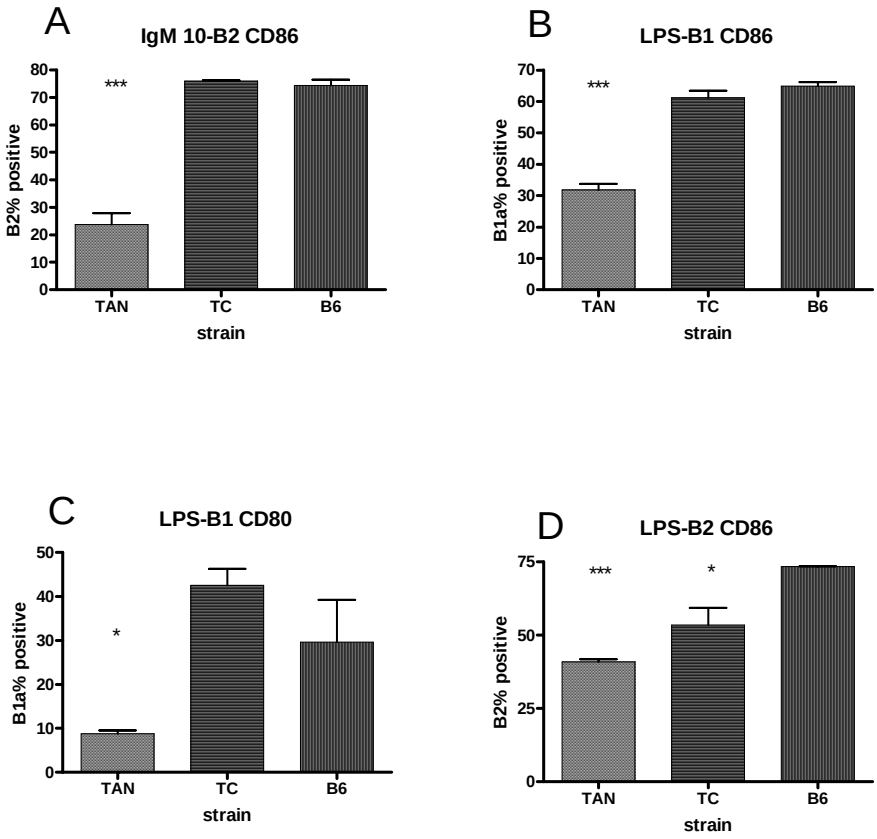

Figure 4-6 Peritoneal B-cell activation after 48 hrs of stimulation. (A) CD86 levels of PerC B2 cells stimulated with anti-IgM at 10 μg/ml. (B) (C) PerC B1 cells stimulated with LPS at 1 μg/ml. (D) B2 cells stimulated with LPS 1 μg/ml. Only stimulation conditions showing significant difference are shown. Both B1 and B2 cells in the TAN PerC had lower activation. Bar graph shows mean± SD. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

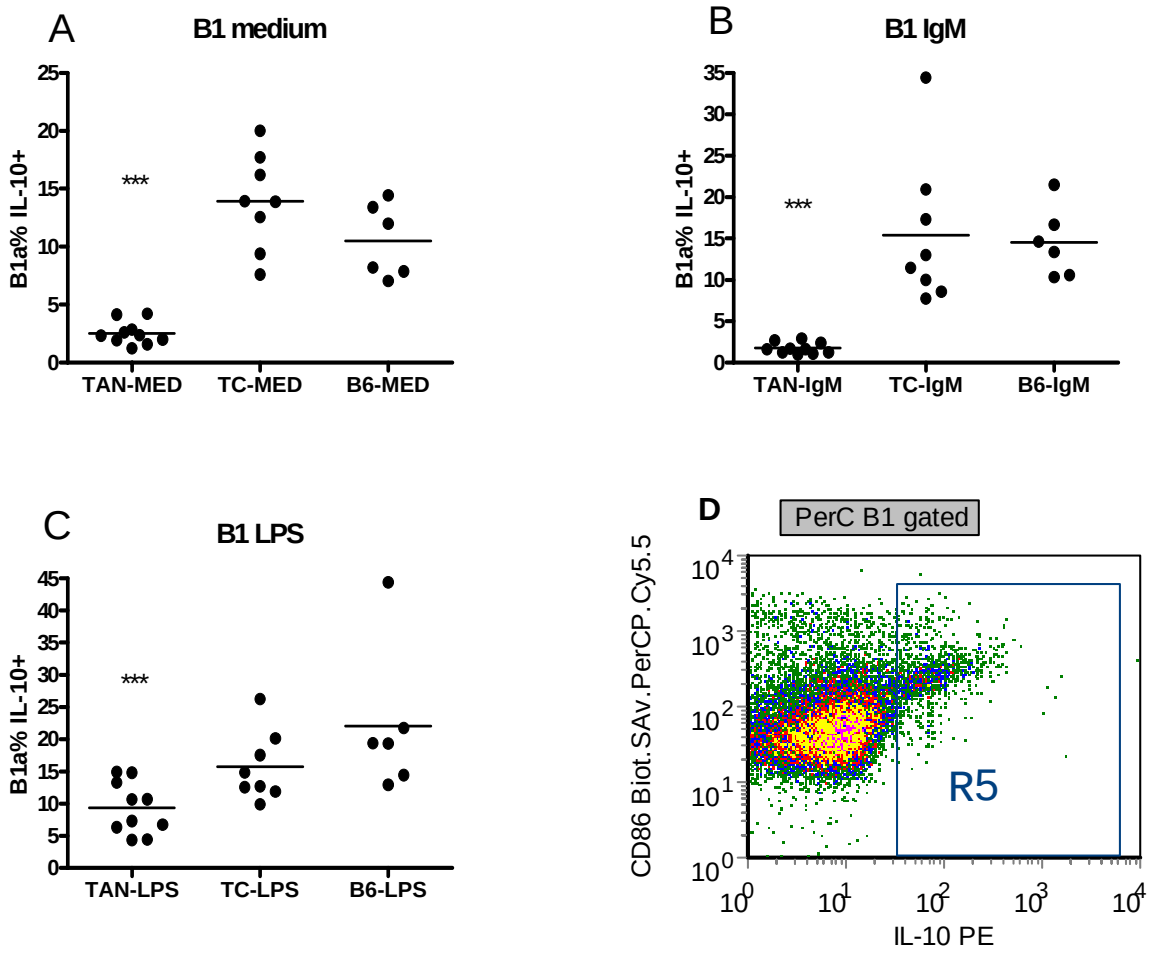

Figure 4-7. Intracellular IL-10 after 48 hrs of culture. Cells from peritoneal lavages were cultured for 48 hrs with (A) medium only. (B) anti-IgM 10 µg/ml. (C). LPS 1 µg/ml. (D) Representative plot of flow cytometry analysis for intracellular IL-10 levels. Cells were gated on B1a B-cell population. TAN PerC B1a cells produce significantly less IL-10 than TC and B6. * P<0.05, ** P<0.01, *** P<0.001.

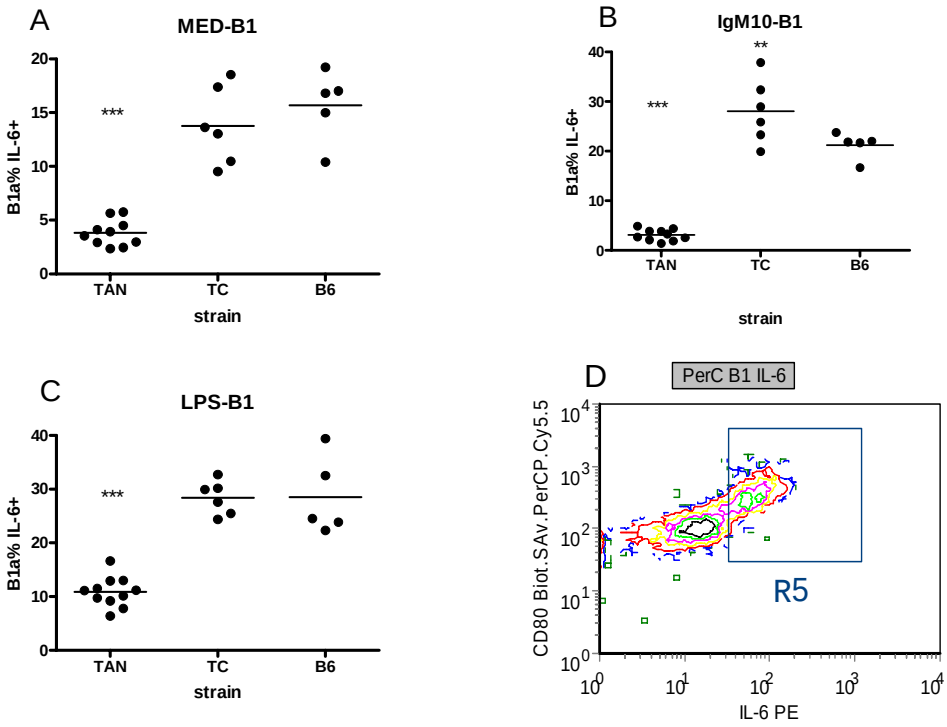

Figure 4-8. Intracellular IL-6 after 48 hrs of culture. Cells from peritoneal lavage were cultured for 48 hrs with (A) medium only, (B) 10 µg/ml anti-IgM. (C) 1 µg/ml LPS. (D) Representative plot of flow cytometry analysis for intracellular IL-6 levels. Cells were gated on B1a B-cell population. TAN PerC B1 cells make significantly less IL-6 in all 3 conditions, while TC B1 cells produce more IL-6 when treated with anti-IgM. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

CHAPTER 5
RESULTS AND DISCUSSION: SPLEEN B CELLS IN THE LUPUS –PRONE AND RESISTANT MICE

The hypothesis is that lupus prone mice have different splenic B cell functions and/or development stages and these differences reflect their roles in the pathogenesis.

Splenic B1 and B2 Cells

Flow cytometry studies on adult spleen showed increased B1 cells in both TAN and NZM, while proportionally less B2 cells were found in NZM, TC and TAN mice (Figure 5-1). Activation marker levels measurement found no significant differences of B7-1 and B7-2 expression on either TAN or lupus mice B-cell (data not shown). TC B2 cells showed however an increased of early activation marker CD69 and NZM B2 cells showed higher CD40 expression. In addition B-cells from the 3 strains express reduced levels of IgM on average as compared to B6, indicating that that these B cells have encountered antigens, presumably self-antigen (Figure 5-2).

Newly formed B cells that are just released from bone marrow undergo a series of development stages in the periphery (113). Based on CD21 and CD23 expression, the B-cell population in the spleen has been divided into 4 sub-groups: T1 (Transitional 1), T2 (Transitional 2), follicular (FoB) and marginal zone B (MZB) cells(116) (Figure 5-3).

Flow cytometry revealed that NZM, TC and TAN mice have accumulated T1 and less T2 and FoB cells than B6, suggesting a developmental arrest on T1 to T2 stage (Figure 5-4). No difference was observed for marginal zone B cells, as mentioned earlier.

B-cell development can be determined by bone marrow derived cells, including the

B cells themselves, or by non-bone marrow derived stromal cells. To identify whether abnormal B cell development in TC mice was determined by bone marrow derived cells, we performed reciprocal bone-marrow transfers between B6 and TC mice. The results of this experiment showed that the abnormal B cell subset distribution can be completely accounted for by TC bone marrow (Figure5-5).

Splenic Naive B-Cell Functional Properties

To study B-cell functional properties without the confounding factor of differential sub-population distribution, we analyzed the response of naïve CD43- B cells to stimulation. Splenic naive CD43- B cells were isolated with a B-cell isolation kit from Miltenyi Biotech. The output was >96% pure. Then purified B-cells were cultured with medium only, anti-IgM (low dose: 1 μg/ml or high dose: 10 μg/ml), or LPS (1 μg/ml).

B-Cell Proliferation after Stimulation

During the 72 hrs of culture in medium only, B cells from TAN and NZM, TC mice showed a higher spontaneous proliferation as compared to B6 (Figure 5-6). When stimulated with low dose of anti-IgM, B cells in lupus-prone mice showed an increased proliferation index but TAN had a decreased proliferation index. In the situation of high dose of anti-IgM stimulation, TAN mice B cells had a similar proliferation index as B6, while TC showed an even higher proliferation. Interestingly, no difference was observed with LPS treated B cells from either strain.

Activation-Induced Cell Death

To assess activation-induced cell death in B cells, CD43- B cells were treated as mentioned before for 16 hrs, and intracellular cleaved caspase-3 levels was assayed by flow cytometry. The results showed significantly less apoptosis in lupus-prone NZM and TC B-cells when treated with 10μg/ml anti-IgM. No differences were observed in B-cell

treated with medium only, low dose of anti-IgM and LPS (Figure 5-7).

B-Cell Activation and Differentiation After In Vitro Stimulation.

To test the activation and differentiation of cultured B cells, cells were treated as mentioned before for 48 hrs, and the expression of surface activation/differentiation markers was assayed by flow cytometry as the read-out. The results showed that when left in the medium only, all TAN, NZM and TC mice showed significant higher differentiation into germinal center (GC) B cells as shown by the high levels of GL-7 expression. Besides, a higher percentage of $CD19^{hi}$ population also present in the TAN and TC B cells (Figure 5-8). Since GL-7 is a germinal center marker, and activated B cells up-regulate CD19(194-196), a BCR co-receptor, these results suggested a higher spontaneous B-cell activation and differentiation among these strains. No difference was observed for the expression of early activation marker CD69 and co-stimulatory molecule B7-2 (CD86).

When treated with low dose of anti-IgM at 1μg/ml, the TAN B cells showed decreased CD86 and GL-7 levels. Expression of CD80 and CD19 were not significantly different (Figure-5-9).

In the situation of high dose of anti-IgM treatment (10μg/ml), the TAN B cells still showed significantly lower levels of CD86, although their GL-7 levels was now similar to B6, and their CD19hi B-cells were significantly less than B6. On the other hand, the B cells from lupus prone mice stimulated with high doses of IgM showed high levels of GL-7 and CD19 up-regulation (Figure 5-10). In contrast to these differences, Western blot assay shows that both TAN and TC B cells have similar elevated ERK phosphorylation compared to B6 B cells (Figure 5-11).

For the B cells stimulated with LPS, no significant difference of

activation/differentiation were found between the strains (data not shown), indicating that the differences in activation and differentiation reported above were specific for BCR stimulation.

Discussion

Although both TAN, NZM and TC carry the three lupus susceptible loci, the increased splenic B1 cells was not observed in TC mice, indicating the gene(s) responsible for high SP B1 cells map outside the three Sle loci. In addition, the levels of activation markers on splenic B1 cells from TAN and NZM were not different, suggesting that splenic B1 cell is not necessary for the development of lupus, either by their number of activation status.

Both the lupus-prone and resistant mice have abnormal splenic B cell development, displayed by the accumulated T1 cells as well as decreased T2 and follicular B cells, suggesting a development arrest between the T1 and T2 stages. Reciprocal bone marrow transfer suggests that this defect can be completely accounted for by the bone marrow i.e. either B cell itself and/or interaction between B and T/myeloid cells. The immature B cells that just emerged from bone marrow contain autoreactive clones and must undergo maturation in the periphery, during which these clones are removed, and checkpoints of negative selection during T1 and T2 stages has been suggested (113;119). Thus the accumulation of T1 cells may overload this checkpoint and increase the possibility of autoreactive clones maturation in periphery. Besides, recent studies have shown that transitional B cells can present antigens to CD4+ T cells and activate T cells if exogenous costimulation is provided (197). On the other hand, interactions with activated T cells can also protect immature B cells from negative selection (197). Since the lupus prone NZM and TC, but not the resistant TAN mice, have hyper-activated T cells (unpublished), the

accumulated immature B cells in these strains thus may have different outcome, and play an important role in disease development.

An age-dependent splenomegaly appears in TAN, NZM and TC mice. Thus although they have a lower B2 proportion of splenic lymphocytes, the actual B2 cell number is still higher. Consistent with this, naive B cells from all the strains show a significantly higher spontaneous proliferation in vitro. Besides, both strains also have higher proportion of B-cells expressing GL7 and up-regulating CD19 without stimulation. GL7 is an activation marker that is expressed only on germinal center B cells in the periphery (198). CD19 forms complex with CD21 and CD81 and is an essential co-receptor for BCR that functions to enhance BCR signaling (199). The cytoplasmic domain of CD19 recruits vav, PI-3 kinase and Src-family kinase Lyn after BCR ligation and activates down-stream signaling (195;200). Over-expression of CD19 leads to B cell hyper-activation and development of autoimmunity (201). These results indicate a higher spontaneous B-cell activation and differentiation in these strains.

Further studies were conducted using different dosage of anti-IgM $F(ab)_2$ to mimic different strengths of BCR ligation in vivo. Results showed that B cells from lupus-prone and resistant mice have different activation threshold. The TAN B cells responded poorly to low dose anti-IgM stimulation, even less than the medium only condition, while at this low levels, the lupus TC and NZM B cells showed significant activation and differentiation. At the high dose of anti-IgM stimulation, still, the lupus mice B cells exhibited higher proliferation and activation potential, while TAN B cells were similar as the B6 B cells. Especially TAN B cells did not show a significant up-regulation of CD19 upon stimulation. Furthermore, the lupus B cells have less activation induced cell death.

One interesting observation is that both TAN and TC B cells have a similarly elevated ERK phosphorylation upon low and high dose anti-IgM, suggesting the effects of the Sle loci they share. In fact, hyper-activation of ras-ERK has been mapped to Sle1ab sub-locus recently (202). This also indicate that signaling differences between TAN and TC B cell may exist downstream of ERK. Finally, flow cytometry study showed that both TAN and lupus mice B cells have similar levels of surface IgM, which is significantly lower than that of B6, thus the amount of surface IgM did not account for the different signaling phenomenon. In short, these results indicate that compared to normal B cells, lupus mice B-cells have lower threshold on BCR signaling pathway, while TAN B cell has elevated threshold, and their TLR4 signaling pathway is not changed.

It has been show that B cells are indispensable to prime T cells and to initiate lupus pathogenesis (1;7). The B-cell phenotypes and BCR signaling properties thus may contribute to the phenotypes of the strains. Both TAN and lupus mice B cells have high spontaneous activation, proliferation and activation in vitro. Accordingly, all these mice develop an age-dependent anti-chromatin and anti-dsDNA IgM autoantibody production. This can be due to the effects of 3 *Sle* loci they share (154;203-205). Breaking of the tolerance is not sufficient, however, for the fully development of autoimmune disease (152). The disease phenotypes of the strains correlate to and affected by their B-cell properties. As B cells from lupus prone mice have more potency to activation, proliferation and differentiation as well as resistance to apoptosis, the lupus mice generate large amount of germinal centers, long-lived plasma cells (unpublished data), class-switched high affinity IgG autoantibodies (206) and finally full development of the disease. TAN mice B cell respond poorly to low dose BCR cross-linking, and they do not

up-regulate CD19 expression even with strong BCR stimulation. Accordingly, their autoantibody production is significantly lower, and most of the isotype is IgM (unpublished data). Most importantly, TAN mice do not develop lupus nephritis. These facts also indicate the impacts of lupus suppressing genes carried by the TAN genome.

In summary, this study shows that in lupus prone mice, splenic B cells actively participate in the pathogenesis by the enhanced activation and differentiation to plasma cells in response to antigen stimulation. In lupus resistant TAN mice, the limited B cell activation likely contributes to the protection the host from lupus.

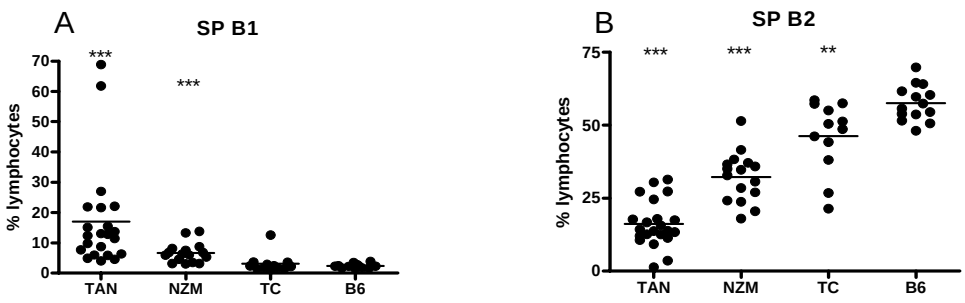

Figure 5-1. Splenic B cell populations. Freshly isolated splenocytes were assayed by flow cytometry for (A) B1 cells and (B) B2 cells. Both TAN and NZM have increased percentages of spleen B1 cells and decreased B2 cells, while TC has only a decreased percentage of B2 cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

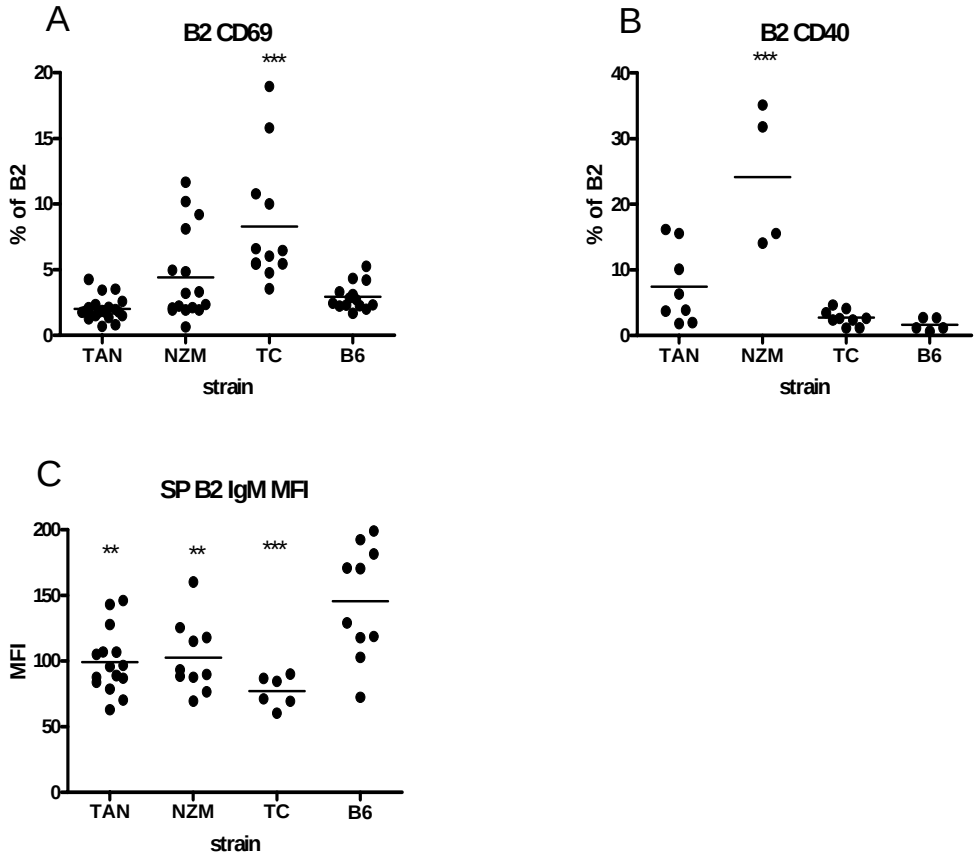

Figure 5-2. Activation marker expression on splenic B2 cells assayed by flow cytometry. (A) Significantly higher percentages of TC B2 cells express CD69. (B). Significantly higher percentages of NZM B2 cells express CD40. (C) Lower IgM mean fluorescence intensity on TAN, TC and NZM B2 cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

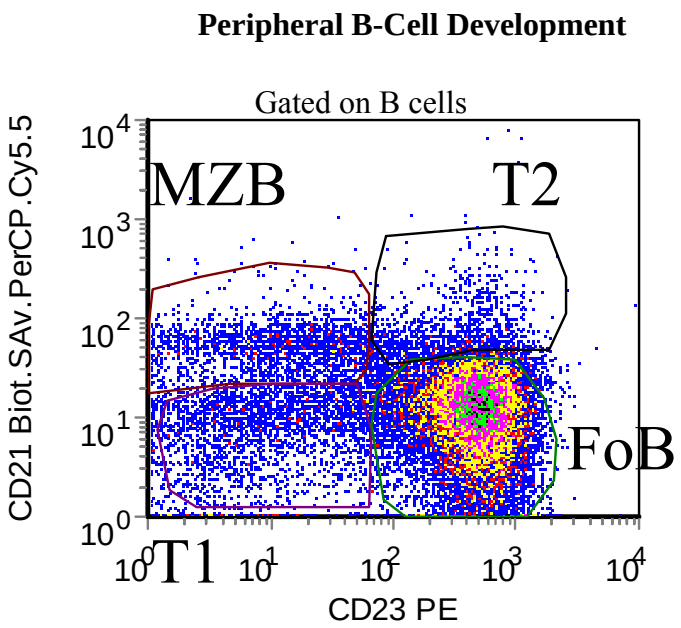

Figure 5-3. Definition of splenic B-cell developmental subpopulations with flow cytometry. Figure shows typical B-cell subpopulations from normal B6 mice spleen. Cells are gated on B220+. T1, transitional 1; T2, transitional 2; FoB, follicular B cells; MZB, marginal zone B cells.

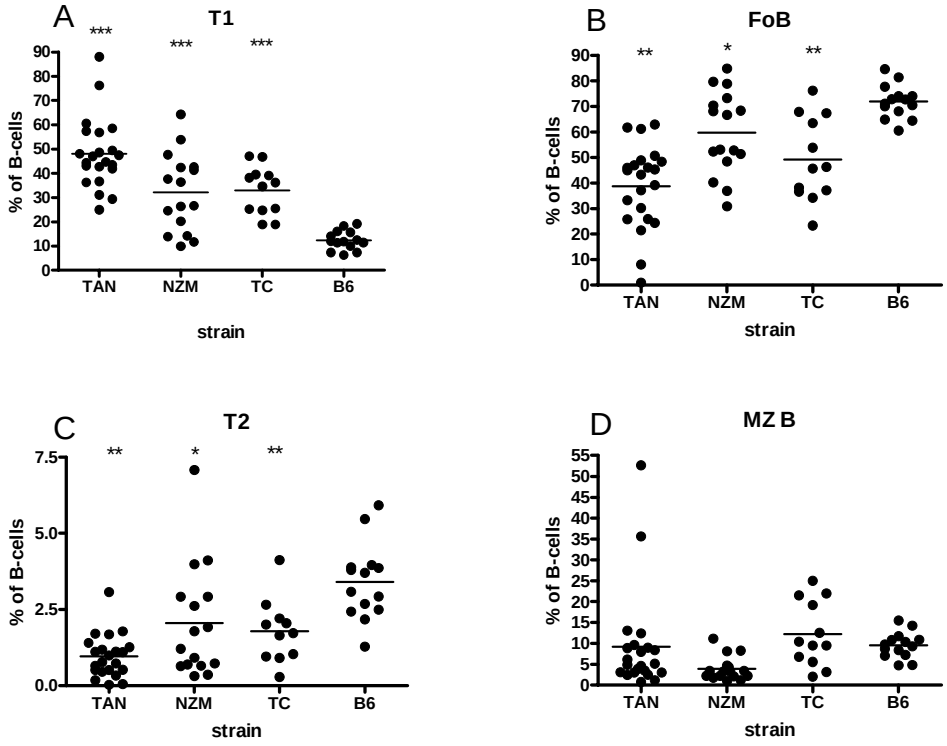

Figure 5-4. Peripheral B-cell development. Freshly isolated splenocytes were assayed by flow cytometry for (A) T1. (B) FoB. (C) T2 and (D) MZB cells. Both TAN and lupus-prone NZM and TC have accumulated T1, less T2 and follicular B cells. Populations were gated on B220+ B cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

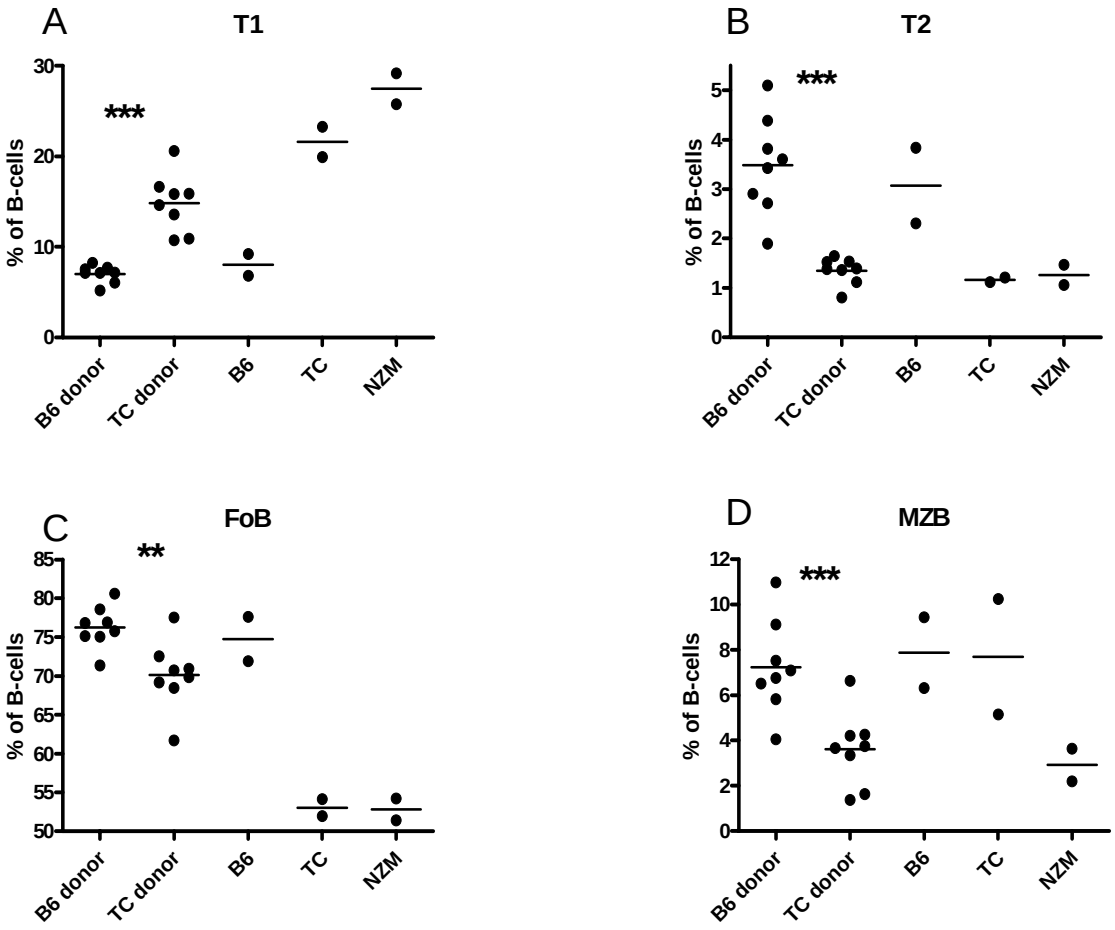

Figure 5-5 Splenic B cell populations in mice after reciprocal bone marrow transfer. Three month old B6 and TC mice were lethally irradiated and TC or B6 bone marrow was transferred. All mice were sacrificed after 3 months and splenic B cells were assayed for (A) T1, (B) T2, (C) FoB, and (D) MZB populations. Unmanipulated mice were used as controls. Splenic B-cell populations from bone marrow transferred mice were similar as the non-operated donor strains. Statistical comparisons were conducted between B6 donor and TC donor groups. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

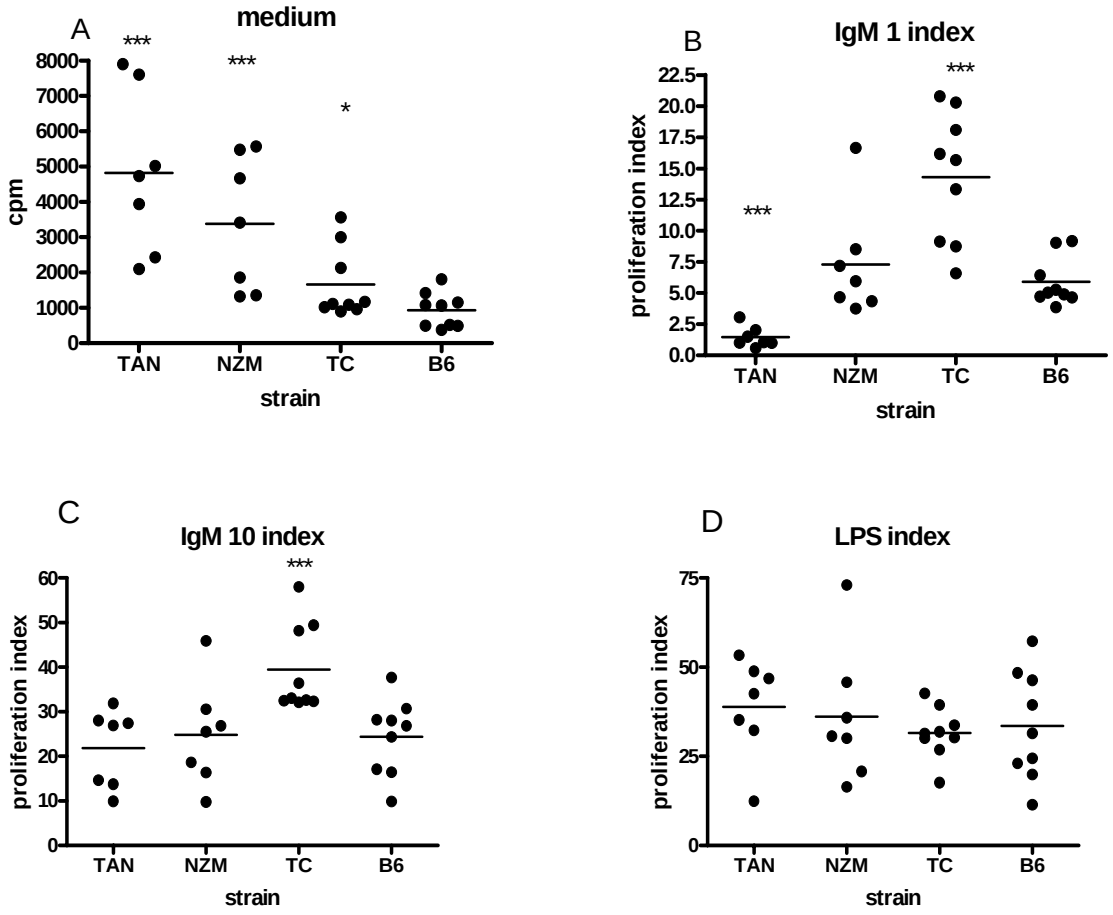

Figure 5-6. B-cell proliferation indicated by 3H-Thymidine incorporation. Splenic naive B cells were isolated and cultured for 72 hrs with (A) medium only, (B) anti-IgM at 1 μg/ml; (C) anti-IgM at 10 μg/ml (D) LPS at 1 μg/ml. Proliferation index was calculated by the relative ratio of stimulation cpm to the corresponding medium only cpm value. Without stimulation, both TAN and NZM, TC mice showed increased spontaneous proliferation. When stimulated with lose doses of anti-IgM, lupus-prone mice had significantly higher proliferation, while TAN mice show decreased proliferation as compared to B6. At higher doses of anti-IgM, TAN and NZM showed similar proliferation as B6, while TC had an even higher proliferation. Finally, no significant difference was observed in LPS-treated B cells. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

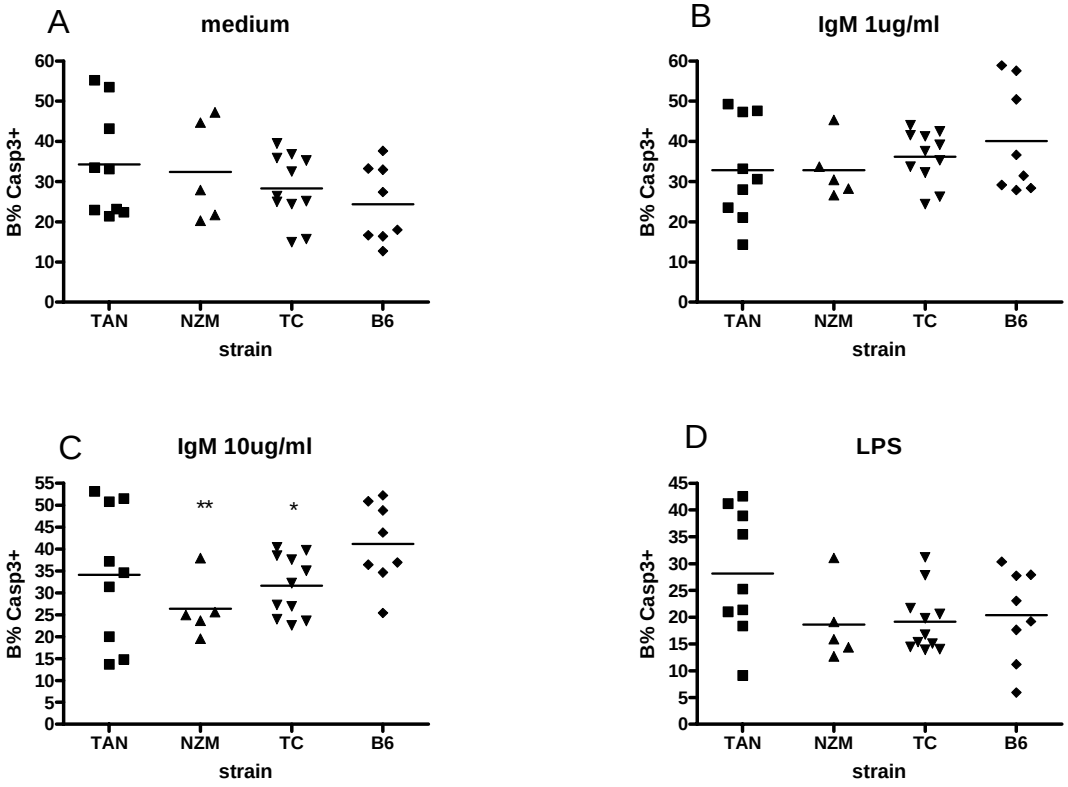

Figure 5-7. Apoptosis rate during of in vitro stimulation. Splenic naive B cells were isolated and cultured for 16 hrs with (A) medium only, (B) anti-IgM at 1 μg/ml, (C) anti-IgM at 10 μg/ml, (D) LPS at 1 μg/ml. Only when stimulated with high doses of anti-IgM, lupus prone TC and NZM B cells showed less cell death than TAN and B6. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

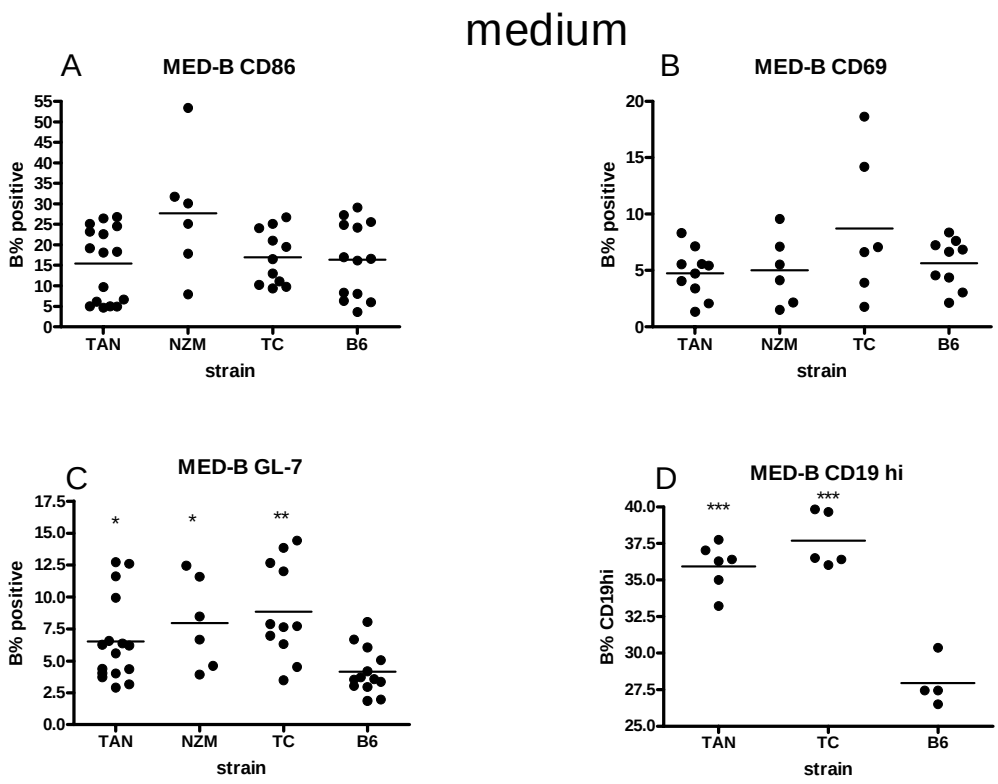

Figure 5-8 B-cell activation during in vitro culture. Splenic naive B cells were isolated and cultured for 48 hrs with medium only. Activation markers were assayed by flow cytometry for (A) CD86, (B) CD69, (C) GL-7 and (D) CD19. No significant difference was observed for CD86 and CD69. (C) TAN, NZM and TC showed higher GL-7 levels. (D) Both TC and TAN had much more B cells with up-regulated CD19. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

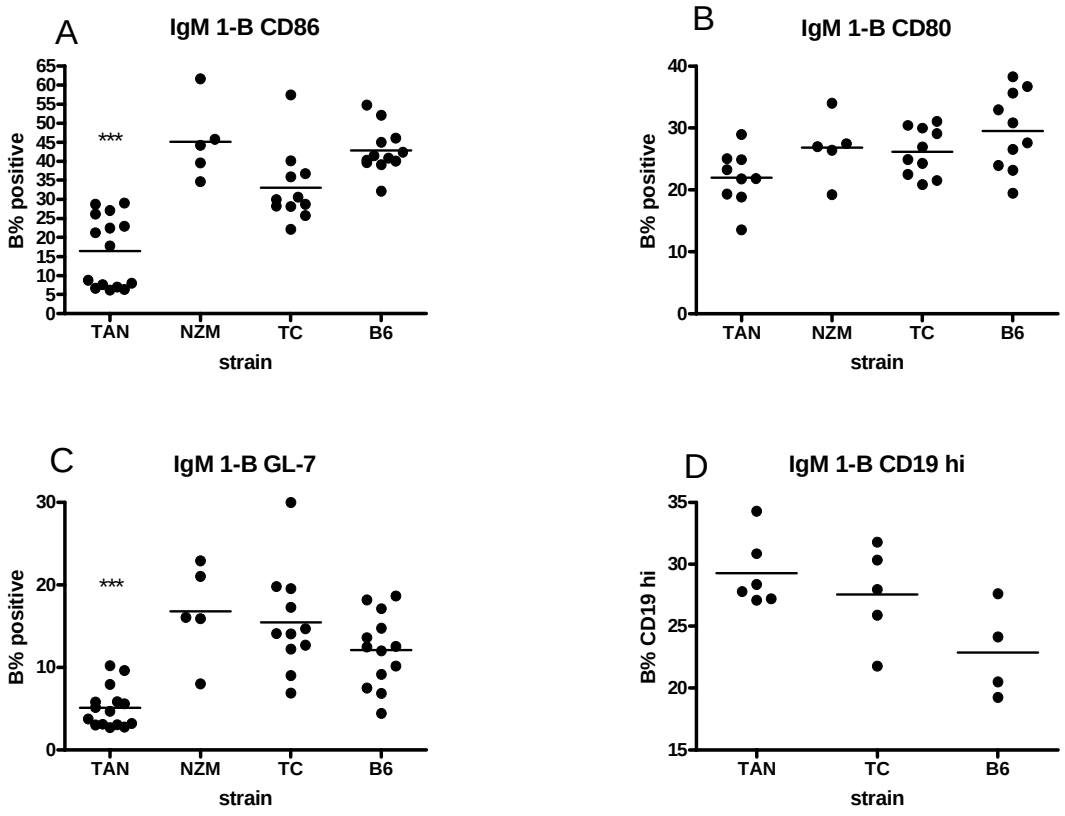

Figure 5-9. B-cell activation and differentiation with a low dose of anti-IgM stimulation. Splenic naive B cells were isolated and cultured for 48hrs with anti-IgM at 1μg/ml. Activation markers were assayed by flow cytometry for (A) CD86, (B) CD69, (C) GL-7 (D) CD19. TAN B-cell showed lower CD86 and GL-7 levels. No significant differences were observed among TC, NZM and B6 mice for all of these markers.* $P<0.05$, ** $P<0.01$, *** $P<0.001$.

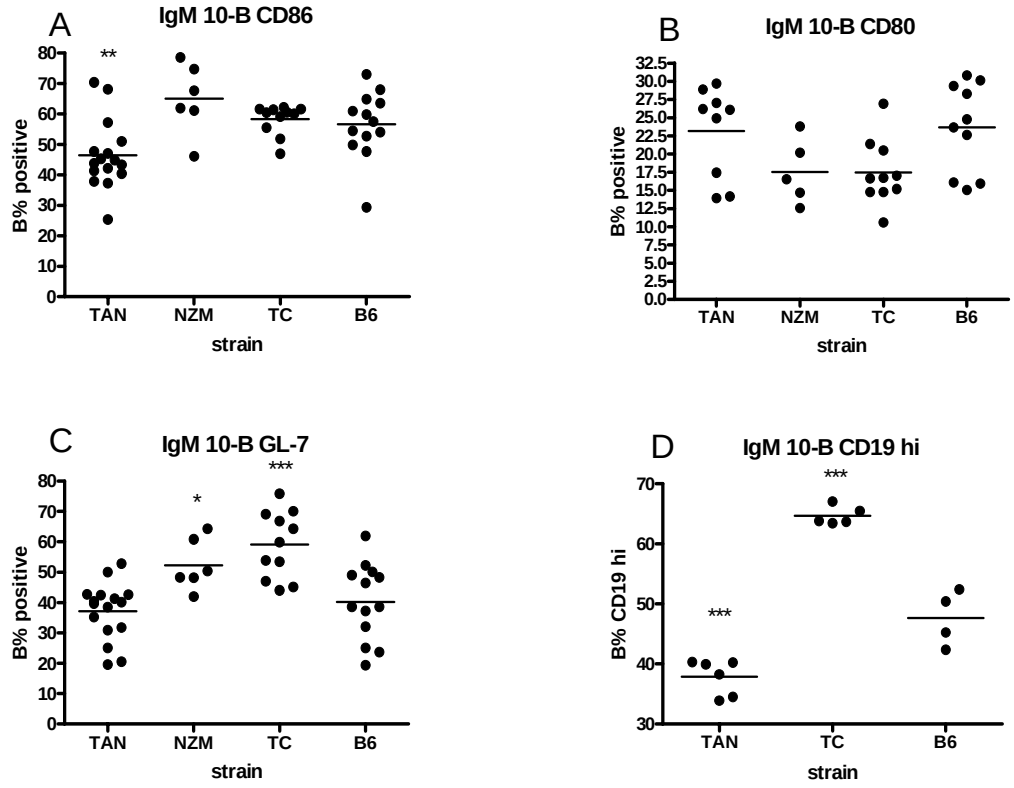

Figure 5-10. B-cell activation under treatment with high dose of BCR stimulation. Splenic naive B cells were isolated and cultured for 48 hrs with anti-IgM at 10μg/ml. Activation markers were assayed by flow cytometry for (A) CD86, (B) CD69, (C) GL-7 (D) CD19. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

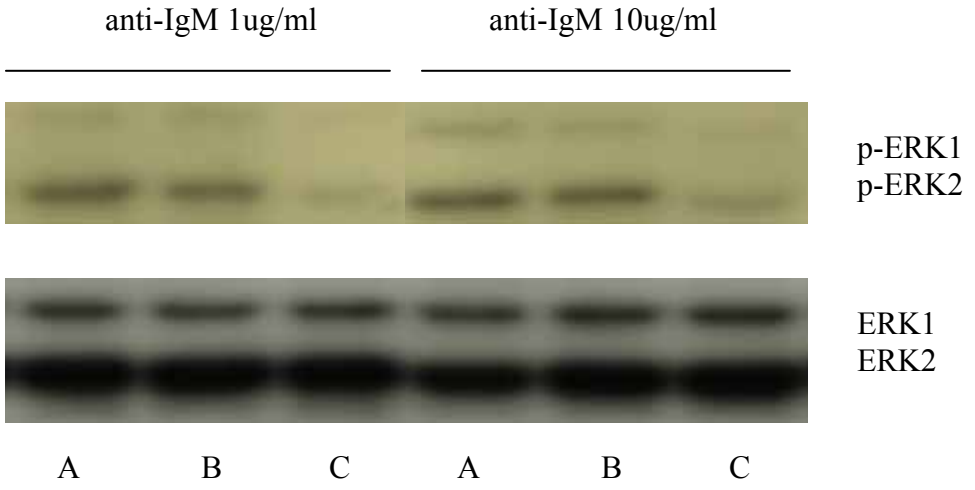

Figure 5-11 ERK phosphorylations in isolated naive B cell after 5 minutes of stimulation. (A) TAN (B) TC (C) B6. Both TAN and TC B cell have elevated ERK phosphorylation

.

CHAPTER 6
SUMMARY AND CONCLUSIONS

In this study, we have characterized the properties of B-cell populations in lupus prone and resistant mice. We found that the splenic MZB cell phenotype correlated with the lupus susceptibility. In the lupus-prone TC and NZM mice, we found a loss of marginal zones, missing marginal zone macrophages, and marginal zone B cells trans-located inside the follicles. In contrast, lupus-resistant TAN mice had enlarged MZ with CD5+ marginal zone B cells retained despite of stimulation. Besides, MZB cells of both strains have defective TI-2 antigen up-take. As the MZB cells have potent antigen presenting and rapid differentiation capabilities, and also contain auto-reactive clones, the trans-localized MZB cells acquire the privilege to present antigen to and to activate CD4 T cells and ultimately differentiate into (auto)antibody-producing plasma cells. In contrast, the expression of CD5 and MZ arrest of MZB cells in TAN mice may raise the activation threshold and prevent them from developing overt autoimmunity. Furthermore, the lack of marginal zone macrophages and defects in TI-2 antigen uptake by MZB cells suggests that lupus mice are ineffective in the clearance of blood-borne pathogens.

Secondly, B-cell responsiveness to BCR ligation was also correlated to the disease phenotype of these strains. The naive splenic B cells in lupus mice generally have lower activation thresholds, and higher rates of differentiation, while TAN B cells showed the opposite. All of our data strongly suggest that B cells in lupus mice actively participate in lupus pathogenesis by over-activation to antigen stimulation and differentiation to plasma cells.

In the peritoneal cavity, TC B1 cells produced overall more IL-6 and IL-10 upon BCR cross-linking. In contrast, peritoneal B cells from TAN mice still show the decreased responsiveness, and little IL-6 and IL-10 production with stimulations. Both IL-6 and IL-10 can promote B-cell proliferation, differentiation and antibody production, and they also play a positive role in lupus progression. These results suggest that peritoneal B1-cells in lupus mice participate in the pathogenesis of lupus by producing disease –promoting cytokine IL-6 and IL-10.

Taken together, these studies suggest that B-cell populations contribute to the development of lupus both through effector and regulatory mechanisms.

LIST OF REFERENCES

1. Chan OTM, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. J Exp Med 1999; 189(10):1639-1647.

2. Sobel ES, Mohan C, Morel L, Schiffenbauer J, Wakeland EK. Genetic dissection of SLE pathogenesis: Adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. J Immunology 1999; 162(4):2415-2421.

3. Niculescu F, Nguyen P, Niculescu T, Rus H, Rus V, Via CS. Pathogenic T cells in murine lupus exhibit spontaneous signaling activity through phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways. Arthritis and Rheumatism 2003; 48(4):1071-1079.

4. Singh RR, Hahn BH, Tsao BP, Ebling FM. Evidence for Multiple Mechanisms of Polyclonal T Cell Activation in Murine Lupus. J Clin Invest 1998; 102(10):1841-1849.

5. Rozzo SJ, Drake CG, Chiang BL, Gershwin ME, Kotzin BL. Evidence for Polyclonal T-Cell Activation in Murine Models of Systemic Lupus-Erythematosus. J Immunology 1994; 153(3):1340-1351.

6. Craft J, Peng S, Fujii T, Okada M, Fatenejad S. Autoreactive T cells in murine lupus - Origins and roles in autoantibody production. Immunologic Research 1999; 19(2-3):245-257.

7. Chan O, Shlomchik MJ. New role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-Ipr/Ipr mice. J Immunology 1998; 160(1):51-59.

8. Thatayatikom A, White AJ. Rituximab: A promising therapy in systemic lupus erythematosus. Autoimmunity Reviews 2006; 5(1):18-24.

9. Leandro MJ, Cambridge G, Edwards JC, Ehrenstein MR, Isenberg DA. B-cell depletion in the treatment of patients with systemic lupus erythematosus: a longitudinal analysis of 24 patients. Rheumatol 2005; 44(12):1542-1545.

10. Anolik JH, Barnard J, Cappione A, Pugh-Bernard AE, Felgar RE, Looney RJ et al. Rituximab improves peripheral B cell abnormalities in human systemic lupus erythematosus. Arthritis and Rheumatism 2004; 50(11):3580-3590.

11. Looney RJ, Anolik J, Sanz I. B lymphocytes in systemic lupus erythematosus: lessons from therapy targeting B cells. Lupus 2004; 13(5):381-390.

12. Martin F, Kearney JF. B-cell subsets and the mature preimmune repertoire. Marginal zone and B1B cells as part of a natural immune memory. Immunological Reviews 2000; 175:70-79.

13. Hayakawa K, Hardy RR, Honda M, Herzenberg LA, Steinberg AD, Herzenberg LA. Ly-1 B-Cells - Functionally Distinct Lymphocytes That Secrete Igm Autoantibodies. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences 1984; 81(8):2494-2498.

14. Berland R, Wortis HH. Origins and functions of B-1 cells with notes on the role of CD5. Ann Rev Immunol 2002; 20:253-300.

15. Won WJ, Kearney JF. CD9 is a unique marker for marginal zone B cells, B1 cells, and plasma cells in mice. J Immunology 2002; 168(11):5605-5611.

16. Alugupalli KR, Leong JM, Woodland RT, Muramatsu M, Honjo T, Gerstein RM. B1b Lymphocytes Confer T Cell-Independent Long-Lasting Immunity. Immunity 2004; 21(3):379-390.

17. 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(1):7-18.

18. Kantor AB, Merrill CE, Herzenberg LA, Hillson JL. An unbiased analysis of V-H-D-J(H) sequences from B-1a, B-1b, and conventional B cells. J Immunology 1997; 158(3):1175-1186.

19. Pennell CA, Mercolino TJ, Grdina TA, Arnold LW, Haughton G, Clarke SH. Biased Immunoglobulin Variable Region Gene-Expression by Ly-1 B-Cells Due to Clonal Selection. European Journal of Immunology 1989; 19(7):1289-1295.

20. Kantor AB, Herzenberg LA. Origin of murine B cell lineages. Annu Rev Immunol 1993; 11:501-538.

21. Hardy RR, Hayakawa K. Cd5 B-Cells, A Fetal B-Cell Lineage. Advances in Immunology, Vol 55 1994; 55:297-339.

22. Rothstein TL, Kolber DL. Anti-Ig Antibody Inhibits the Phorbol Ester-Induced Stimulation of Peritoneal B-Cells. J Immunology 1988; 141(12):4089-4093.

23. Morris DL, Rothstein TL. Abnormal Transcription Factor Induction Through the Surface Immunoglobulin-M Receptor of Lymphocytes-B-1. J Exp Med 1993; 177(3):857-861.

24. Tumang JR, Hastings WD, Bai C, Rothstein TL. Peritoneal and splenic B-1 cells are separable by phenotypic, functional, and transcriptomic characteristics. Eur J Immunol 2004; 34:2158-2167.

25. Mond JJ, Lees A, Snapper CM. T Cell-Independent Antigens Type 2. Ann Rev Immunol 1995; 13(1):655-692.

26. Fischer GM, Solt LA, Hastings WD, Yang KJ, Gerstein RM, Nikolajczyk BS et al. Splenic and peritoneal B-1 cells differ in terms of transcriptional and proliferative features that separate peritoneal B-1 from splenic B-2 cells. Cellular Immunology 2001; 213(1):62-71.

27. Bikah G, Carey J, Ciallella JR, Tarakhovsky A, Bondada S. CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. Sci 1996; 274(5294):1906-1909.

28. Bondada S, Bikah G, Robertson DA, Sen G. Role of CD5 in growth regulation of B-1 cells. Curr Top Microbiol Immunol 2000; 252:141-149.

29. Sen G, Bikah G, Venkataraman C, Bondada S. Negative regulation of antigen receptor-mediated signaling by constitutive association of CD5 with the SHP-1 protein tyrosine phosphatase in B-1B cells. European Journal of Immunology 1999; 29(10):3319-3328.

30. Khaled AR, Butfiloski EJ, Sobel ES, Schiffenbauer J. Functional consequences of the SHP-1 defect in motheaten viable mice: Role of NF-kappa B. Cellular Immunology 1998; 185(1):49-58.

31. Lyons BL, Lynes MA, Burzenski L, Joliat MJ, Hadjout N, Shultz LD. Mechanisms of anemia in SHP-1 protein tyrosine phosphatase-deficient "viable motheaten" mice. Experimental Hematology 2003; 31(3):234-243.

32. Wong SC, Chew WK, Tan JEL, Melendez AJ, Francis F, Lam KP. Peritoneal CD5(+) B-1 cells have signaling properties similar to tolerant B cells. J Biol Chem 2002; 277(34):30707-30715.

33. Healy JI, Dolmetsch RE, Timmerman LA, Cyster JG, Thomas ML, Crabtree GR et al. Different Nuclear Signals Are Activated by the B Cell Receptor during Positive Versus Negative Signaling. Immunity 1997; 6(4):419-428.

34. Healy JI, Dolmetsch RE, Lewis RS, Goodnow CC. Quantitative and qualitative control of antigen receptor signalling in tolerant B lymphocytes. Immunological Tolerance 1998; 215:137-144.

35. Fagarasan S, Watanabe N, Honjo T. Generation, expansion, migration and activation of mouse B1 cells. Immunological Reviews 2000; 176:205-215.

36. Hippen KL, Tze LE, Behrens TW. CD5 maintains tolerance in anergic B cells. J Exp Med 2000; 191(5):883-889.

37. Behrens T, Hippen K, Baness E, Tze L, Wortis H. Spontaneous loss of immunologic tolerance in anergic B cells lacking CD5. Arthritis and Rheumatism 1999; 42(9):S384.

38. Murakami M, Honjo T. B-1 cells and autoimmunity. Immunoglobulin Gene Expression in Development and Disease 1995; 764:402-409.

39. Berland R, Wortis HH. A model for autoantigen induction of natural antibody producing B-1a cells. B 1 Lymphocytes in B Cell Neoplasia 2000; 252:49-55.

40. Tumang JR, Frances R, Yeo SG, Rothstein TL. Cutting Edge: Spontaneously Ig-secreting B-1 cells violate the accepted paradigm for expression of differentiation-associated transcription factors. J Immunol 2005; 174(6):3173-3177.

41. Casali P, Schettino EW. Structure and function of natural antibodies. Curr Top Microbiol Immunol 1996; 210:167-179.

42. Baumgarth N, Herman OC, Jager GC, Brown LE, Herzenberg LA, Chen JZ. B-1 and B-2 cell-derived immunoglobulin M antibodies are nonredundant components of the protective response to influenza virus infection. J Exp Med 2000; 192(2):271-280.

43. Hardy RR, Carmack CE, Shinton SA, Riblet RJ, Hayakawa K. A Single Vh Gene Is Utilized Predominantly in Anti-Brmrbc Hybridomas Derived from Purified Ly-1 B-Cells - Definition of the Vh11 Family. J Immunology 1989; 142(10):3643-3651.

44. Shaw PX, Horkko S, Chang MK, Curtiss LK, Palinski W, Silverman GJ et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest 2000; 105(12):1731-1740.

45. Atencio S, Kotzin BL. The role of marginal zone and B1a B cell Populations in NZB-related lupus. Faseb Journal 2003; 17(7):C183.

46. Dauphinee M, Tovar Z, Talal N. B cells expressing CD5 are increased in Sjogren's syndrome. Arthritis Rheum 1988; 31(5):642-647.

47. Ebo D, DeClerck LS, Bridts CH, Stevens WJ. Expression of CD5 and CD23 on B cells of patients with rheumatoid arthritis, systemic lupus erythematosus and Sjogren's syndrome. Relationship with disease activity and treatment. In Vivo 1994; 8(4):577-580.

48. Mohan C, Morel L, Yang P, Wakeland EK. Accumulation of splenic B1a cells with potent antigen-presenting capability in NZM2410 lupus-prone mice. Arthritis and Rheumatism 1998; 41(9):1652-1662.

49. Murakami M, Yoshioka H, Shirai T, Tsubata T, Honjo T. Prevention of Autoimmune Symptoms in Autoimmune-Prone Mice by Elimination of B-1 Cells. Int Immunol 1995; 7(5):877-882.

50. Reap EA, Sobel ES, Cohen PL, Eisenberg RA. Conventional B-Cells, Not B-1-Cells, Are Responsible for Producing Autoantibodies in Lpr-Mice. J Exp Med 1993; 177(1):69-78.

51. Wen XS, Zhang DQ, Kikuchi Y, Yi J, Nakamura K, Yan X et al. Transgene-mediated hyper-expression of IL-5 inhibits autoimmune disease but increases the risk of B cell chronic lymphocytic leukemia in a model of murine lupus. European Journal of Immunology 2004; 34(10):2740-2749.

52. Ishikawa S, Sato T, Abe M, Nagai S, Onai N, Yoneyama H et al. Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b(+)CD11c(+) dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. J Exp Med 2001; 193(12):1393-1402.

53. Ito T, Ishikawa S, Sato T, Akadegawa K, Yurino H, Kitabatake M et al. Defective B1 cell homing to the peritoneal cavity and preferential recruitment of B1 cells in the target organs in a murine model for systemic lupus erythematosus. J Immunology 2004; 172(6):3628-3634.

54. Sato T, Ishikawa S, Akadegawa K, Ito T, Yurino H, Kitabatake M et al. 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. European Journal of Immunology 2004; 34(12):3346-3358.

55. O'Garra A, Chang R, Go N, Hastings R, Haughton G, Howard M et al. Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. Eur J Immunol 1992; 22:711-717.

56. Hirano T, Suematsu S, Matsusaka T, Matsuda T, Kishimoto T. The Role of Interleukin-6 in Plasmacytomagenesis. Ciba Foundation Symposia 1992; 167:188-200.

57. La Flamme AC, Pearce EJ. The Absence of IL-6 Does Not Affect Th2 Cell Development In Vivo, But Does Lead to Impaired Proliferation, IL-2 Receptor Expression, and B Cell Responses. J Immunol 1999; 162(10):5829-5837.

58. Tackey E, Lipsky PE, Illei GG. Rationale for interleukin-6 blockade in systemic lupus erythematosus. Lupus 2005; 13:339-343.

59. Teague TK, Marrack P, Kappler JW, Vella AT. IL-6 rescues resting mouse T cells from apoptosis. J Immunology 1997; 158(12):5791-5796.

60. Kovalovich K, Li W, DeAngelis R, Greenbaum LE, Ciliberto G, Taub R. Interleukin-6 protects against Fas-mediated death by establishing a critical level of anti-apoptotic hepatic proteins FLIP, Bcl-2, and Bcl-xL. J Biol Chem 2001; 276(28):26605-26613.

61. Kishimoto T. INTERLEUKIN-6: From Basic Science to Medicine40 Years in Immunology. Ann Rev Immunol 2005; 23(1):1-21.

62. McMurray RW, Hoffman RW, Nelson W, Walker SE. Cytokine mRNA Expression in the B/W Mouse Model of Systemic Lupus Erythematosus--Analyses of Strain, Gender, and Age Effects. Clin Imm and Immunopath 1997; 84(3):260-268.

63. Finck BK, Chan B, Wofsy D. Interleukin-6 Promotes Murine Lupus in Nzb/Nzw F1-Mice. J Clin Invest 1994; 94(2):585-591.

64. Koller MD. Targeted therapy in rheumatoid arthritis. Wien Med Wochenschr 2006; 156(1-2):53-60.

65. Tang B, Matsuda T, Akira S, Nagata N, Ikehara S, Hirano T et al. Age-Associated Increase in Interleukin-6 in Mrl Lpr Mice. Int Immunol 1991; 3(3):273-278.

66. Mihara M, Fukui H, Koishihara Y, Saito M, Ohsugi Y. Immunological Abnormality in Nzb/W F1-Mice - Thymus-Independent Expansion of B-Cells Responding to Interleukin-6. Clin Exp Immunol 1990; 82(3):533-537.

67. Mihara M, Ohsugi Y. Possible Role of Il-6 in Pathogenesis of Immune Complex-Mediated Glomerulonephritis in Nzb/W F1-Mice - Induction of Igg Class Anti-Dna Autoantibody Production. Int Arch Allergy Appl Immunol 1990; 93(1):89-92.

68. Ryffel B, Car BD, Gunn H, Roman D, Hiestand P, Mihatsch MJ. Interleukin-6 Exacerbates Glomerulonephritis in (Nzbxnzw)F-1 Mice. Am J Pathol 1994; 144(5):927-937.

69. Grondal G, Gunnarsson I, Ronnelid J, Rogberg S, Klareskog L, Lundberg I. Cytokine production, serum levels and disease activity in systemic lupus erythematosus. Clinical and Experimental Rheumatology 2000; 18(5):565-570.

70. Linkerisraeli M, Deans RJ, Wallace DJ, Prehn J, Ozerichen T, Klinenberg JR. Elevated Levels of Endogenous Il-6 in Systemic Lupus-Erythematosus - A Putative Role in Pathogenesis. J Immunology 1991; 147(1):117-123.

71. Smolen JS, Steiner G, Aringer M. Anti-cytokine therapy in systemic lupus erythematosus. Lupus 2005; 14(3):189-191.

72. Horii Y, Iwano M, Hirata E, Shiiki M, Fujii Y, Dohi K et al. Role of interleukin-6 in the progression of mesangial proliferative glomerulonephritis. Kidney Int Suppl 1993; 39:S71-S75.

73. Aringer M, Smolen JS. Cytokine expression in lupus kidneys. Lupus 2005; 14(1):13-18.

74. Wu J, Cunha FQ, Liew FY, Weiser WY. IL-10 inhibits the synthesis of migration inhibitory factor and migration inhibitory factor-mediated macrophage activation. J Immunol 1993; 151(8):4325-4332.

75. Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; 174(5):1209-1220.

76. Caux C, Massacrier C, Vanbervliet B, Barthelemy C, Liu YJ, Banchereau J. Interleukin-10 Inhibits T-Cell Alloreaction Induced by Human Dendritic Cells. Int Immunol 1994; 6(8):1177-1185.

77. Beebe AM, Cua DJ, Malefyt RD. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Cytokine & Growth Factor Reviews 2002; 13(4-5):403-412.

78. Horwitz DA, Zheng SG, Gray JD. The role of the combination of IL-2 and TGF-{beta} or IL-10 in the generation and function of CD4+ CD25+ and CD8+regulatory T cell subsets. Journal of Leukocyte Biology 2003; 74(4):471-478.

79. Baecher-Allan C, Viglietta V, Hafler DA. Human CD4+CD25+ regulatory T cells. Seminars in Immunology 2004; 16(2):89-98.

80. Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med 1994; 179:305-310.

81. Yin ZN, Bahtiyar G, Zhang N, Liu LZ, Zhu P, Robert ME et al. IL-10 regulates murine lupus. J Immunol 2002; 169(4):2148-2155.

82. Gunnarsson I, Nordmark B, Bakri AH, Grondal G, Larsson P, Forslid J et al. Development of lupus-related side-effects in patients with early RA during sulphasalazine treatment - the role of IL-10 and HLA. Rheumatol 2000; 39(8):886-893.

83. Tyrrell-Price J, Lydyard PM, Isenberg DA. The effect of interleukin-10 and of interleukin-12 on the in vitro production of anti-double-stranded DNA antibodies from patients with systemic lupus erythematosus. Clin Exp Immunol 2001; 124(1):118-125.

84. Casali P, Burastero SE, Balow JE, Notkins AL. High-affinity antibodies to ssDNA are produced by CD-B cells in systemic lupus erythematosus patients. J Immunol 1989; 143(11):3476-3483.

85. Won WJ, Kearney JF. CD9 Is a Unique Marker for Marginal Zone B Cells, B1 Cells, and Plasma Cells in Mice. J Immunol 2002; 168(11):5605-5611.

86. Martin F, Kearney JF. B1 cells: similarities and differences with other B cell subsets. Current Opinion in Immunology 2001; 13(2):195-201.

87. Kraal G. Cells in the marginal zone of the spleen. International Review of Cytology 1992; 132:31-71.

88. Mebius RE, Nolte MA, Kraal G. Development and function of the splenic marginal zone. Critical Reviews in Immunology 2004; 24(6):449-464.

89. Morse HC, Kearney JF, Isaacson PG, Carroll M, Fredrickson TN, Jaffe ES. Cells of the marginal zone - origins, function and neoplasia. Leukemia Research 2001; 25(2):169-178.

90. Spencer J, Perry ME, Dunn-Walters DK. Human marginal-zone B cells. Immunology Today 1998; 19:421-426.

91. Martin F, Oliver AM, Kearney JF. Marginal Zone and B1 B Cells Unite in the Early Response against T-Independent Blood-Borne Particulate Antigens. Immunity 2001; 14(5):617-629.

92. Martin F, Kearney JF. Marginal-zone B cells. Nat Rev Immunol 2002; 2(5):323-335.

93. Oliver AM, Martin F, Kearney JF. IgM(high)CD21(high) lymphocytes enriched in the splenic marginal zone generate effector cells more rapidly than the bulk of follicular B cells. J Immunology 1999; 162(12):7198-7207.

94. Attanavanich K, Kearney JF. Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. J Immunology 2004; 172(2):803-811.

95. Lopes-Carvalho T, Kearney JF. Development and selection of marginal zone B cells. Immunological Reviews 2004; 197:192-205.

96. Dammers PM, Visser A, Popa ER, Nieuwenhuis P, Kroese FGM. Most marginal zone B cells in rat express germline encoded Ig V_H genes and are ligand selected. J Immunology 2000; 165:6456-6169.

97. Dunn-Walters DK, Isaacson PG, Spencer J. Analysis of mutations in immunoglobulin heavy chain variable region genes of microdissected marginal zone (MGZ) B cells suggests that the MGZ of human spleen is a reservoir of memory B cells. J Exp Med 1995; 182:559-566.

98. Wither JE, Roy V, Brennan LA. Activated B cells express increased levels of costimulatory molecules in young autoimmune NZB and (NZB x NZW)F-1 mice. Clinical Immunology 2000; 94(1):51-63.

99. Wither JE, Paterson AD, Vukusic B. Genetic dissection of B cell traits in New Zealand black mice. The expanded population of B cells expressing up-regulated costimulatory molecules shows linkage to Nba2. European Journal of Immunology 2000; 30(2):356-365.

100. Grimaldi CM, Michael DJ, Diamond B. Cutting Edge: Expansion and Activation of A Population of Autoreactive Marginal Zone B Cells in a Model of Estrogen-Induced Lupus. J Immunol 2001; 167(4):1886-1890.

101. Li Y, Li H, Weigert M. Autoreactive B Cells in the Marginal Zone that Express Dual Receptors. J Exp Med 2002; 195(2):181-188.

102. Zeng D, Lee MK, Tung J, Brendolan A, Strober S. Cutting edge: A role for CD1 in the pathogenesis of lupus in NZB/NZW mice. J Immunology 2000; 164(10):5000-5004.

103. Li YJ, Li H, Ni DY, Weigert M. Anti-DNA B cells in MRL/lpr mice show altered differentiation and editing pattern. J Exp Med 2002; 196(12):1543-1552.

104. Okada T, Ngo VN, Ekland EH, Forster R, Lipp M, Littman DR et al. Chemokine Requirements for B Cell Entry to Lymph Nodes and Peyer's Patches. J Exp Med 2002; 196(1):65-75.

105. Forster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A Putative Chemokine Receptor, BLR1, Directs B Cell Migration to Defined Lymphoid Organs and Specific Anatomic Compartments of the Spleen. Cell 1996; 87(6):1037-1047.

106. Reif K, Ekland EH, Ohl L, Nakano H, Lipp M, Forster R et al. Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. Nature 2002; 416(6876):94-99.

107. Lu TT, Cyster JG. Integrin-mediated long-term B cell retention in the splenic marginal zone. Sci 2002; 297(5580):409-412.

108. Lu TT, Cyster JG. Retention of B cells in the splenic marginal zone is integrin-mediated. Arthritis and Rheumatism 2002; 46(9):S416-S417.

109. Cinamon G, Matloubian M, Lesneski MJ, Xu Y, Low C, Lu T et al. Sphingosine 1-phosphate receptor 1 promotes B cell localization in the splenic marginal zone. Nature Immunology 2004; 5(7):713-720.

110. Rosen H, Goetzl EJ. SPHINGOSINE 1-PHOSPHATE AND ITS RECEPTORS: AN AUTOCRINE AND PARACRINE NETWORK. Nat Rev Immunol 2005; 5(7):560-570.

111. Karlsson MCI, Guinamard R, Bolland S, Sankala M, Steinman RM, Ravetch JV. Macrophages control the retention and trafficking of B lymphocytes in the splenic marginal zone. J Exp Med 2003; 198(2):333-340.

112. Nolte MA, Arens R, Kraus M, van Oers MHJ, Kraal G, van Lier RAW et al. B Cells Are Crucial for Both Development and Maintenance of the Splenic Marginal Zone. J Immunol 2004; 172(6):3620-3627.

113. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Sci 2003; 301(5638):1374-1377.

114. Carsetti R, Rosado MM, Wardemann H. Peripheral development of B cells in mouse and man. Immunological Reviews 2004; 197:179-191.

115. Cancro MP. Peripheral B-cell maturation: the intersection of selection and homeostasis. Immunological Reviews 2004; 197(1):89-101.

116. Loder F, Mutschler B, Ray RJ, Paige CJ, Sideras P, Torres R et al. B Cell Development in the Spleen Takes Place in Discrete Steps and Is Determined by the Quality of B Cell Receptor 杕 erived Signals. J Exp Med 1999; 190(1):75-90.

117. Allman D, Lindsley RC, DeMuth W, Rudd K, Shinton SA, Hardy RR. Resolution of three nonproliferative immature splenic B cell subsets reveals multiple selection points during peripheral B cell maturation. J Immunol 2001; 167(12):6834-6840.

118. Srivastava B, Quinn WJ, III, Hazard K, Erikson J, Allman D. Characterization of marginal zone B cell precursors. J Exp Med 2005; 202(9):1225-1234.

119. Srivastava B, Lindsley RC, Nikbakht N, Allman D. Models for peripheral B cell development and homeostasis. Seminars in Immunology 2005; 17(3):175-182.

120. Hayakawa K, Asano M, Shinton SA, Gui M, Allman D, Stewart CL et al. Positive selection of natural autoreactive B cells. Sci 1999; 285(5424):113-116.

121. Martin F, Kearney JF. Positive selection from newly formed to marginal zone B cells depends on the rate of clonal production, CD19, and btk. Immunity 2000; 12(1):39-49.

122. Cyster JG, Healy JI, Kishihara K, Mak TW, Thomas ML, Goodnow CC. Regulation of B-lymphocyte negative and positive selection by tyrosine phosphatase CD45. Nature 1996; 381(6580):325-328.

123. Levine MH, Haberman AM, Sant'Angelo DB, Hannum LG, Cancro MP, Janeway CA et al. A B-cell receptor-specific selection step governs immature to mature B cell differentiation. Proceedings of the National Academy of Sciences of the United States of America 2000; 97(6):2743-2748.

124. Aggarwal BB. Signalling pathways of the TNF superfamily: A double-edged sword. Nature Reviews Immunology 2003; 3(9):745-756.

125. Mackay F, Mackay CR. The role of BAFF in B-cell maturation, T-cell activation and autoimmunity. Trends in Immunology 2002; 23(3):113-115.

126. Scapini P, Nardelli B, Nadali G, Calzetti F, Pizzolo G, Montecucco C et al. G-CSF-stimulated Neutrophils Are a Prominent Source of Functional BLyS. J Exp Med 2003; 197(3):297-302.

127. Nardelli B, Belvedere O, Roschke V, Moore PA, Olsen HS, Migone TS et al. Synthesis and release of B-lymphocyte stimulator from myeloid cells. Blood 2001; 97(1):198-204.

128. Hase H, Kanno Y, Kojima M, Hasegawa K, Sakurai D, Kojima H et al. BAFF/BLyS can potentiate B-cell selection with the B-cell coreceptor complex. Blood 2004; 103(6):2257-2265.

129. He B, Chadburn A, Jou E, Schattner EJ, Knowles DM, Cerutti A. Lymphoma B Cells Evade Apoptosis through the TNF Family Members BAFF/BLyS and APRIL. J Immunol 2004; 172(5):3268-3279.

130. Szodoray P, Jonsson R. The BAFF/APRIL System in Systemic Autoimmune Diseases with a Special Emphasis on Sjogren's Syndrome. Scandinavian Journal of Immunology 2005; 62(5):421-428.

131. Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: A tutorial on B cell survival. Ann Rev Immunol 2003; 21:231-264.

132. Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J et al. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med 2000; 192(10):1453-1465.

133. Stohl W, Xu D, Kim KS, Koss MN, Jorgensen TN, Deocharan B et al. BAFF overexpression accelerates glomerular disease inmice with an imcomplete genetic predisposition to systemic lupus erythematosus. Arthritis and Rheumatism. In press.

134. Pers JO, DARIDON CAPU, DEVAUCHELLE VALE, JOUSSE SAND, SARAUX ALAI, JAMIN CHRI et al. BAFF Overexpression Is Associated with Autoantibody Production in Autoimmune Diseases. Ann NY Acad Sci 2005; 1050(1):34-39.

135. Mackay F, Browning JL. BAFF: A fundamental survival factor for B cells. Nat Rev Immunol 2002; 2(7):465-475.

136. Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000; 404:995-999.

137. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB et al. Reduced Competitiveness of Autoantigen-Engaged B Cells due to Increased Dependence on BAFF. Immunity 2004; 20(4):441-453.

138. Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F et al. Excess BAFF Rescues Self-Reactive B Cells from Peripheral Deletion and Allows Them to Enter Forbidden Follicular and Marginal Zone Niches. Immunity 2004; 20(6):785-798.

139. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Sci 2001; 293:2111-2114.

140. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001; 14:573-582.

141. Ng LG, Sutherland APR, Newton R, Qian F, Cachero TG, Scott ML et al. B Cell-Activating Factor Belonging to the TNF Family (BAFF)-R Is the Principal BAFF Receptor Facilitating BAFF Costimulation of Circulating T and B Cells. J Immunol 2004; 173(2):807-817.

142. Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. Blood 2004; 103(2):689-694.

143. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C et al. BCMA Is Essential for the Survival of Long-lived Bone Marrow Plasma Cells. J Exp Med 2004; 199(1):91-98.

144. von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001; 14:573-582.

145. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T et al. Activation and accumulation of B cells in TACI-deficient mice. Nat Immunol 2001; 2:638-643.

146. Seshasayee D, Valdez P, Yan M, Dixit VM, Tumas D, Grewal IS. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. Immunity 2003; 18(2):279-288.

147. Rudofsky UH, Evans BD, Balaban SL, Mottironi VD, Gabrielsen AE. Differences in Expression of Lupus Nephritis in New-Zealand Mixed H-2(Z) Homozygous Inbred Strains of Mice Derived from New-Zealand Black and New-Zealand White Mice - Origins and Initial Characterization. Lab Invest 1993; 68(4):419-426.

148. Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. Immunity 1994; 1(3):219-229.

149. Morel L, Yu Y, Blenman KR, Caldwell RA, Wakeland EK. Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. Mammalian Genome 1996; 7(5):335-339.

150. Morel L, Mohan C, Croker BP, Sobel E, Wakeland EK. Sle1 on murine chromosome 1 is a key SLE-susceptibility locus. Faseb Journal 1998; 12(4):A605.

151. Mohan C, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis - Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. J Clin Invest 1998; 101(6):1362-1372.

152. Sobel ES, Mohan C, Morel L, Schiffenbauer J, Wakeland EK. Genetic dissection of SLE pathogenesis: Adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. J Immunol 1999; 162(4):2415-2421.

153. Sobel ES, Satoh M, Chen WF, Wakeland EK, Morel L. The major murine systemic lupus erythematosus susceptibility locus Sle1 results in abnormal functions of both B and T cells. J Immunol 2002; 169(5):2694-2700.

154. Mohan C, Morel L, Yang P, Wakeland EK. Genetic dissection of systemic lupus erythematosus pathogenesis - Sle2 on murine chromosome 4 leads to B cell hyperactivity. J Immunol 1997; 159(1):454-465.

155. Xu Z, Butfiloski EJ, Sobel ES, Morel L. Mechanisms of peritoneal B-1a cells accumulation induced by murine lupus susceptibility locus Sle2. J Immunol 2004; 173(10):6050-6058.

156. Mohan C, Morel L, Kontaridis M, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis: Sle3 potentiates Sle1-induced autoimmunity. Faseb Journal 1998; 12(4):A605.

157. Mohan C, Yu Y, Morel L, Yang P, Wakeland EK. Genetic dissection of Sle pathogenesis: Sle3 on murine chromosome 7 impacts T cell activation, differentiation, and cell death. J Immunol 1999; 162(11):6492-6502.

158. Wakui M, Kim J, Butfiloski EJ, Morel L, Sobel ES. Genetic Dissection of Lupus Pathogenesis: Sle3/5 Impacts IgH CDR3 Sequences, Somatic Mutations, and Receptor Editing. J Immunol 2004; 173(12):7368-7376.

159. Morel L, Croker BP, Blenman KR, Mohan C, Huang G, Gilkeson G et al. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. Proc Natl Acad Sci U S A 2000; 97(12):6670-6675.

160. Berland R, Wortis HH. Role of NFAT in the regulation of B-1 cells. B 1 Lymphocytes in B Cell Neoplasia 2000; 252:131-140.

161. Muller G, Hopken UE, Lipp M. The impact of CCR7 and CXCR5 on lymphoid organ development and systemic immunity. Immunological Reviews 2003; 195(1):117-135.

162. Groeneveld PHP, Erich T, Kraal G. In vivo Effects of LPS on Lympholyte-B Subpopulations - Migration of Marginal Zone-Lymphocytes and Igd-Blast Formation in the Mouse Spleen. Immunobiology 1985; 170(5):402-411.

163. Erickson LD, Lin LL, Duan B, Morel L, Noelle RJ. A genetic lesion that arrests plasma cell homing to the bone marrow. Proc Natl Acad Sci U S A 2003; 100:12905-12910.

164. Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Ann Rev Immunol 2005; 23:127-159.

165. Lo CG, Lu TT, Cyster JG. Integrin-dependence of lymphocyte entry into the splenic white pulp. J Exp Med 2003; 197(3):353-361.

166. Ansel KM, Ngo VN, Hyman PL, Luther SA, Forster R, Sedgwick JD et al. A chemokine-driven positive feedback loop organizes lymphoid follicles. Nature 2000; 406(6793):309-314.

167. Spiegel S, Kolesnick R. Sphingosine 1-phosphate as a therapeutic agent. Leukemia 2002; 16(9):1596-1602.

168. Vora KA, Nichols E, Porter G, Cui Y, Keohane CA, Hajdu R et al. Sphingosine 1-phosphate receptor agonist FTY720-phosphate causes marginal zone B cell displacement. Journal of Leukocyte Biology 2005; 78(2):471-480.

169. Ishikawa S, Sato T, Abeuo M, Nagai S, Onai N, Yoneyama H et al. Possible roles of aberrant expression of B lymphocyte chemoattractant (BLC/CXCL13) in breakdown of immunological tolerance and autoantibody production in murine lupus. Journal of Leukocyte Biology 2001;83.

170. Ishikawa S, Nagai S, Sato T, Akadegawa K, Yoneyama H, Zhang YY et al. Increased circulating CD11b(+)CD11c(+) dendritic cells (DC) in aged BWF1 mice which can be matured by TNF-alpha into BLC/CXCL13-producing DC. European Journal of Immunology 2002; 32(7):1881-1887.

171. Chen Y, Pikkarainen T, Elomaa O, Soininen R, Kodama T, Kraal G et al. Defective Microarchitecture of the Spleen Marginal Zone and Impaired Response to a Thymus-Independent Type 2 Antigen in Mice Lacking Scavenger Receptors MARCO and SR-A. J Immunol 2005; 175(12):8173-8180.

172. Lanoue A, Clatworthy MR, Smith P, Green S, Townsend MJ, Jolin HE et al. SIGN-R1 Contributes to Protection against Lethal Pneumococcal Infection in Mice. J Exp Med 2004; 200(11):1383-1393.

173. Chen Y, Pikkarainen T, Elomaa O, Soininen R, Kodama T, Kraal G et al. Defective Microarchitecture of the Spleen Marginal Zone and Impaired Response to a Thymus-Independent Type 2 Antigen in Mice Lacking Scavenger Receptors MARCO and SR-A. J Immunol 2005; 175(12):8173-8180.

174. Endres R, Alimzhanov MB, Plitz T, Futterer A, Kosco-Vilbois MH, Nedospasov SA et al. Mature follicular dendritic cell networks depend on expression of lymphotoxin beta receptor by radioresistant stromal cells and of lymphotoxin beta and tumor necrosis factor by B cells. J Exp Med 1999; 189(1):159-167.

175. Rennert PD, Browning JL, Mebius R, Mackay F, Hochman PS. Surface lymphotoxin alpha/beta complex is required for the development of peripheral lymphoid organs. J Exp Med 1996; 184(5):1999-2006.

176. Alimzhanov MB, Kuprash DV, KoscoVilbois MH, Luz A, Turetskaya RL, Tarakhovsky A et al. Abnormal development of secondary lymphoid tissues in lymphotoxin beta-deficient mice. Proceedings of the National Academy of Sciences of the United States of America 1997; 94(17):9302-9307.

177. Rennert PD, James D, Mackay F, Browning JL, Hochman PS. Lymph node genesis is induced by signaling through the lymphotoxin beta receptor. Immunity 1998; 9(1):71-79.

178. Gonzalez M, Mackay F, Browning JL, Kosco-Vilbois MH, Noelle RJ. The sequential role of lymphotoxin and B cells in the development of splenic follicles. J Exp Med 1998; 187(7):997-1007.

179. Grimaldi CM, Michael DJ, Diamond B. Cutting edge: Expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. J Immunology 2001; 167(4):1886-1890.

180. Chen X, Martin F, Forbush KA, Perlmutter RM, Kearney JF. Evidence for selection of a population of multi-reactive B cells into the splenic marginal zone. Int Immunol 1997; 9(1):27-41.

181. Cyster JG, Goodnow CC. Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. Immunity 1995; 3(6):691-701.

182. Schmidt KN, Hsu CW, Griffin CT, Goodnow CC, Cyster JG. Spontaneous follicular exclusion of SHP1-deficient B cells is conditional on the presence of competitor wild-type B cells. J Exp Med 1998; 187(6):929-937.

183. Mandik-Nayak L, Seo SJ, Eaton-Bassiri A, Allman D, Hardy RR, Erikson J. Functional consequences of the developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. J Immunology 2000; 164(3):1161-1168.

184. Ekland EH, Forster R, Lipp M, Cyster JG. Requirements for Follicular Exclusion and Competitive Elimination of Autoantigen-Binding B Cells. J Immunol 2004; 172(8):4700-4708.

185. Chen Y, Cuda C, Morel L. Genetic determination of T cell help in loss of tolerance to nuclear antigens. J Immunol 2005; 174(12):7692-7702.

186. Hippen KL, Tze LE, Baness EA, Wortis HH, Behrens TW. Spontaneous loss of immunologic tolerance in anergic B cells lacking CD5. Arthritis and Rheumatism 1999; 42(9):S53.

187. Xu Z, Duan B, Croker BP, Wakeland EK, Morel L. Genetic dissection of the murine lupus susceptibility locus S*le2:* contributions to increased peritoneal B-1a cells and lupus nephritis map to different loci. J Immunol 2005; 175:936-943.

188. Spencer NFL, Daynes RA. IL-12 directly stimulates expression of IL-10 by CD5(+) B cells and IL-6 by both CD5(+) and CD5(-) B cells: Possible involvement in age-associated cytokine dysregulation. Int Immunol 1997; 9(5):745-754.

189. Cross JT, Benton HP. The roles of interleukin-6 and interleukin-10 in B cell hyperactivity in systemic lupus erythematosus. Inflammation Research 1999; 48(5):255-261.

190. Hagiwara E, Gourley MF, Lee S, Klinman DM. Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10:Interferon-gamma-secreting cells in the peripheral blood. Arthritis Rheum 1996; 39(3):379-385.

191. Deenen GJ, Kroese FGM. Kinetics of Peritoneal B-1A Cells (Cd5 B-Cells) in Young-Adult Mice. European Journal of Immunology 1993; 23(1):12-16.

192. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+ CD25+ T cell-mediated suppression by dendritic cells. Sci 2003; 299:1033-1036.

193. Ming JE, Steinman RM, Granelli-Piperno A. IL-6 enhances the generation of cytolytic T lymphocytes in the allogeneic mixed leucocyte reaction. Clin Exp Immunol 1992; 89(1):148-153.

194. Luzina IG, Atamas SP, Storrer CE, daSilva LC, Kelsoe G, Papadimitriou JC et al. Spontaneous formation of germinal centers in autoimmune mice. Journal of Leukocyte Biology 2001; 70(4):578-584.

195. Fujimoto M, Poe JC, Hasegawa M, Tedder TF. CD19 regulates intrinsic B lymphocyte signal transduction and activation through a novel mechanism of processive amplification. Immunologic Research 2000; 22(2-3):281-298.

196. Bradney AP, Fujimoto M, Poe JC, Tedder TF. CD19 regulates CD22 function in B cell development and B cell antigen receptor signal transduction. Faseb Journal 1999; 13(5):A987.

197. Chung JB, Wells AD, Adler S, Jacob A, Turka LA, Monroe JG. Incomplete activation of CD4 T cells by antigen-presenting transitional immature B cells: Implications for peripheral B and T cell responsiveness. J Immunology 2003; 171(4):1758-1767.

198. Cervenak L, Magyar A, Boja R, Laszlo G. Differential expression of GL7 activation antigen on bone marrow B cell subpopulations and peripheral B cells. Immunology Letters 2001; 78(2):89-96.

199. Tedder TF, Inaoki M, Sato S. The CD19-CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. Immunity 1997; 6(2):107-118.

200. Fujimoto M, Poe JC, Jansen PJ, Sato S, Tedder TF. CD19 amplifies B lymphocyte signal transduction by regulating Src-family protein tyrosine kinase activation. J Immunology 1999; 162(12):7088-7094.

201. 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(1):39-50.

202. Liu K, Liang C, Liang Z, Tus K, Wakeland EK. Sle1ab mediates the aberrant activation of STAT3 and Ras-ERK signaling pathways in B lymphocytes. J Immunol 2005; 174(3):1630-1637.

203. Mohan C, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis: Sle1 on murine chromosome 1 leads to loss of tolerance to H2A/H2B/DNA subnucleosomes. Arthritis and Rheumatism 1996; 39(9):1134.

204. Morel L, Blenman KR, Sobel ES, Croker BP. Functional and genetic characterization of Sle1, a major susceptibility locus in murine lupus. Arthritis and Rheumatism 2001; 44(9):S374.

205. Chen Y, Perry D, Boackle SA, Sobel ES, Molina H, Croker BP et al. Several genes contribute to the production of autoreactive B and T cells in the murine lupus susceptibility locus Sle1c. J Immunol 2005; 175(2):1080-1089.

206. Vyse TJ, Drake CG, Rozzo SJ, Roper E, Izui S, Kotzin BL. Genetic linkage of IgG autoantibody production in relation to lupus nephritis in New Zealand hybrid mice. J Clin Invest 1996; 98(8):1762-1772.

BIOGRAPHICAL SKETCH

Biyan Duan was born in P. R. China on Sep. 23, 1971. He graduated from Tianjin Medical University and received his Bachelor of Medicine in 1995. Then he became a clinical resident of Tianjin No.3 Hospital, at the Department of Pediatrics for 3 years. In Sep. 1998 he started study for his master's degree in clinical immunology at Tianjin Medical University. In fall, 2000, he was accepted by the Interdisciplinary Program in Biomedical Sciences at the University of Florida. He did his dissertation research under the supervision of Dr. Laurence Morel and received his PhD in May 2006.