HAPLOTYPE-SPECIFIC EFFECTS OF THE SLAM/CD2 FAMILY ON THE IMMUNE RESPONSE

APPROVED BY SUPERVISORY COMMITTEE

Edward K. Wakeland, Ph.D.

Michael Bennett, M.D.

Chandra Mohan, M.D., Ph.D.

Christopher Lu, M.D.

Nancy Olsen, M.D.

DEDICATION

I would like to thank the numerous people who have made my experience in graduate school both rewarding and fulfilling. I have been very fortunate to work under the mentorship of Dr. Edward K. Wakeland who help foster the skills in becoming a competent thinker and technically sound scientist. I also wish to deeply thank the administrative staff of the Immunology graduate program as well as the MSTP program. Without their tremendous help, my career would have undoubtedly come to an alarming halt a long, long time ago. I wish to acknowledge the members of my Graduate Committee for their time and guidance. Finally, I wish to thank the many friends in school as well as family who have supported me this entire time. What at times seemed like an eternity in graduate school now appears as just a brief moment in my life, but I will always remember and cherish the memories of our times together.

HAPLOTYPE-SPECIFIC EFFECTS OF THE SLAM/CD2 FAMILY ON THE IMMUNE RESPONSE

by

CHARLES MINH NGUYEN

DISSERTATION

Presented to the Faculty of the Graduate School of Biomedical Sciences

The University of Texas Southwestern Medical Center at Dallas

In Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

The University of Texas Southwestern Medical Center at Dallas

Dallas, Texas

June, 2007

HAPLOTYPE-SPECIFIC EFFECTS OF THE SLAM/CD2 FAMILY
ON THE IMMUNE RESPONSE

CHARLES MINH NGUYEN, PH.D.

The University of Texas Southwestern Medical Center at Dallas, 2007

EDWARD K. WAKELAND, Ph.D.

The Sle1b susceptibility interval mediates a breach in tolerance to nuclear antigens in the NZM2410 model of systemic lupus erythematosus (SLE). Congenic B6 mice carrying the *Sle1b* locus produce anti-nuclear autoantibodies (ANAs) but do not develop lupus nephritis as seen in the parental NZM2410 strain. Fine mapping of the *Sle1b* locus placed it within a 900kb interval between 171.3 and 172.2Mb on chromosome 1. A Bacterial Artificial Chromosome (BACs) contig that spanned the interval was constructed, and a tiling pathway comprised of six BACs was sequenced. Sequence analysis revealed a dense region of 24 expressed genes. Expression studies determined numerous polymorphisms between B6 and the B6.*Sle1b* congenic and identified a cluster of

genes known as the Slam/CD2 family as the primary candidates for *Sle1b*. These immunoregulatory receptors play a role in intercellular interactions and regulate function in several immune cell lineages. Of the seven family members within the locus, *Ly108* appears to be the strongest candidate as B6.*Sle1b* shows a differential expression in isoforms. *Ly108-1* is highly expressed, while *Ly108-2* is expressed at much lower level in the congenic when compared to B6. When lymphocytes are stimulated, *Ly108-2* is strongly up-regulated in B6, but not B6.*Sle1b.* Sequence analysis of the extra-cellular immunoglobulin domains of the Slam/CD2 family revealed two stable haplotypes in a panel of 33 common inbred strains of mice. The first haplotype is only found in B6 and other C57-related strains. The more common second haplotype is found in *Sle1b* and other autoimmune strains such as MRL, NOD, and NZB, as well as non-autoimmune strains such as 129Sv and Balb/c. The presence of this haplotype on B6 mediates autoimmunity as B6 congenics carrying the *Sle1b* locus from 129Sv also produce ANAs. Signaling studies on both B6.*Sle1b* and B6.*129* reveal an altered pattern of calcium mobilization upon stimulation in T cells. In addition, CD4 T cells from B6.*Sle1b* demonstrate a reduction in IL-4 expression and secretion upon activation, suggesting that haplotype 2 of the Slam/CD2 family alters the immune response in T cells. Studies to understand the mechanisms by which this haplotype mediates autoimmunity are in progress.

TABLE OF CONTENTS

Publications

Nguyen C[1], Wandstrat AE[1], Limaye N[1], Chan AY, Subramanian S, Tian X-H, Yim Y-S, Pertsemlidis A, Gardner, Jr. HR, Morel L, Wakeland EK. (2004) Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* 21 (6): 769-80. ([1]These authors contributed equally to this study)

Kumar KR, Li L, Yan M, Bhaskarabhatla M, Mobley AB, **Nguyen C**, Mooney JM, Schatzle JD, Wakeland EK, Mohan C. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. Science. 2006; 312 (5780): 1665-9.

Nguyen C, Limaye N, Wakeland EK (2002) Susceptibility genes in the pathogenesis of murine lupus. *Arthritis Res* 4 (Suppl 3): S255-63.

List of Figures

List of Tables

Chapter 1: Introduction

SLE is a complex autoimmune disease of unknown etiology. It is estimated to affect about 1 in 2000 people, and the clinical presentation is highly variable and can be fatal, with a 10-year mortality rate of 28%. There is a strong gender bias, with a female: male ratio of about 9:1 between the ages of 15 and 50 years. Ethnicity also influences the incidence of the disease: African-Americans and Hispanics are approximately two to four times more likely to contract the disease than Caucasians (1,2).

SLE is primarily characterized by the production of autoantibodies directed against nuclear antigens such as double-stranded DNA and chromatin. These antinuclear autoantibodies (ANAs) cause end-organ damage by a variety of mechanisms, notably via immune-complex-mediated inflammation, which can result in glomerulonephritis (GN), arthritis, rashes, serositis, and vasculitis. At the molecular level, defects in both immune complex clearance and in B-cell and T-cell tolerance and function have been implicated in the pathogenesis of this disease (3).

There is a large body of evidence supporting a genetic basis for susceptibility to SLE. Estimates of the concordance rates in monozygotic twins range between 25% and 69%, while the rate is only 1–2% in dizygotic twins. In addition, 10–12% of SLE patients have first-degree or second-degree relatives with the disease (4). Stochastic processes and environmental influences clearly also play a significant role in disease development and exacerbation. These can

include stress, bacterial and viral infections, sun exposure, exogenous hormones and certain drugs (2).

SLE caused by single gene defect

SLE rarely occurs as a result of a single gene mutation. However, deficiencies in the early components of the complement cascade can cause the disease: 95% of C1q-deficient and C4-deficient patients develop lupus, as do 10% of patients with C2 deficiency (5,6). In animal models, disruption of Fas and Fas ligand (FasL) also has major effects in disease susceptibility (7,8). SLE more commonly occurs as a complex, polygenic disease, with MHC and non-MHC genes interacting with each other as well as environmental triggers to create the varied clinical manifestations (9,10).

Given that single gene deficiencies account for a very small percentage of SLE cases, susceptibility to lupus is mediated by multiple genetic and environmental factors. As with any multifactorial disease, this complicates the identification of disease genes. There is a low degree of penetrance associated with individual susceptibility loci, and different combinations of genes may be associated with disease development in different families. In addition, the lack of any strong selection against the susceptibility loci makes them fairly common in the general population. This makes their detection by linkage analysis difficult in human populations, which have a high degree of genetic variability. Nonetheless, numerous SLE susceptibility intervals have been identified in the human genome (11-13).

Animal Models of Lupus

A useful alternative strategy when dealing with genetically complex diseases like SLE is the elucidation of disease mechanisms in suitable animal models. These studies often yield valuable insights that can then be applied to human studies (14,15).

There are numerous synthetic murine models and spontaneous murine models of lupus. The synthetic models include transgenics and targeted gene disruptions in which candidate disease genes that are involved in a variety of lymphocytic interactions, apoptosis, or antigen clearance are silenced or overexpressed (16-18). As might be expected, disrupting or enhancing pathways involved in normal immune surveillance and reactivity often results in autoimmunity.

In addition to synthetic mouse models, there are also classic spontaneous models, including the (NZB × NZW)F1 (or NZB/W) mouse and the congenic recombinant NZM2410 strain derived from this cross, the MRL/*lpr* mouse, and the BXSB/*yaa* mouse. NZB/W mice develop systemic autoimmunity with ANA production and immune-complex-mediated GN, much like that seen in humans. There is also a strong female gender bias in disease susceptibility, which is not seen in the NZM2410 strain. It is thought that this may be due to the presence of very strong susceptibility alleles in the NZM2410 strain, which cause a phenotype severe enough to mask the effects of sex hormones (19). The *lpr* mutation of the Fas gene, which is involved in apoptosis, is a strongly potentiating factor for

autoimmunity. In combination with a susceptible genome, it causes systemic disease in MRL/*lpr* mice. In the case of BXSB mice, the Y-linked autoimmune accelerator gene causes severe disease in males when expressed in the susceptible BXSB genome.

Numerous linkage analyses have been carried out in each of these strains to identify the chromosomal regions responsible for mediating susceptibility to various component phenotypes such as ANA production, splenomegaly, and GN (20). In each study, three or more loci were linked to disease susceptibility. This holds true in the case of the (SWR × NZB)F1 strain. Mice of this strain also develop disease very similar to that seen in NZB/W mice, and linkage analysis has recently been carried out to understand the contribution of the SWR genome to disease susceptibility (21).

More than 50 loci have been found to affect susceptibility to lupus or one of its component phenotypes (22,23), pointing to the complexity and the polygenic nature of this disease. Loci on chromosomes 1, 4, 7, and 17 have been identified in multiple studies, which indicate that genes in these regions may be important in immune regulation and function, and may play a role in mediating disease in a manner not specific to strain. Many mapped loci co-localize with regions linked to other autoimmune diseases like insulin-dependent diabetes, experimental autoimmune encephalomyelitis, and experimental induced arthritis in various murine models (24). This makes it probable that certain loci affect autoimmune susceptibility in general by modulating processes such as immune

reactivity or apoptosis, while other genes play a role in determining the specific target organ and antigens involved in disease pathogenesis.

Linkage analysis followed by congenic dissection is a powerful strategy that is used to narrow identified regions showing linkage to susceptibility, down to a point where physical mapping and candidate gene analysis can be meaningfully carried out. In congenic dissection, the various genomic regions are moved individually onto a resistant genome or *vice versa*, allowing one to see the effects of each individual locus. Using this technique, significant advances have been made towards identifying genes that may be involved in the loss of tolerance to nuclear antigens in the spontaneous models of murine lupus.

Linkage analysis of the NZM2410 strain

Linkage analysis of susceptibility to GN and ANA production in the NZM2410 strain by Morel and Wakeland identified three prominent loci (25), and congenic strains were then made by moving each locus onto the lupus-resistant C57Bl/6J (B6) background (26). The B6.*Sle1* congenic carries the *Sle1* interval, found on chromosome 1. The congenic is associated with the production of autoantibodies against H2A/H2B/DNA sub-nucleosomes and with the elevated expression of the activation markers B7.2 on B cells and CD69 on $CD4^+$ T cells (27). B6.*Sle2* harbors the *Sle2* interval on chromosome 4 and shows a reduction in the threshold for activation of B cells, leading to the production of polyclonal IgM and increased numbers of peritoneal and splenic B1 cells when present on the B6 genetic background (28). B6.*Sle3*, whose congenic interval lies on

chromosome 7, possesses an affected T-cell compartment, as well as the production of polyclonal IgG by B cells. These B6.*Sle3* congenic mice also have an expansion of $CD4^+$ T cells, along with a decrease in activation-induced cell death of these cells following stimulation with anti-CD3. The T cells exhibit a stronger proliferative response *in vitro* to T-dependent antigen stimulation, but not to T-independent antigen stimulation (29).

Significantly, neither the *Sle2* nor the *Sle3* interval is sufficient to cause the production of autoantibodies and kidney disease on the lupus-resistant B6 background. When combined with *Sle1* as a bi-congenic model, however, they cause renal lesions and proteinuria with varying penetrance (30). This demonstrates the need for both an initiating factor and the amplification of the immune response for disease pathogenesis to occur. The *Sle1* locus thus appears to be an initiating factor in murine lupus that causes systemic autoimmunity, resulting in GN when it is combined with either the *Sle2* or *Sle3* loci in bi-congenic strains of mice, or with the BXSB-derived *yaa* gene (31).

Given the multiple loci that were identified in the NZM2410 strain, the susceptibility to lupus is highly complex. But to further complicate the issue, it was recently discovered that there existed epistatic modifiers or genes that suppress autoimmunity. Most of the *SLE* intervals were derived from the NZW strain, which does not develop significant autoimmune phenotypes seen in the NZM2410 strain (32).

Linkage analysis then confirmed that there existed four suppressive loci, labeled '*Sles*' (SLE suppressor), in the NZW genome (32). The *Sles1* locus, which is a specific suppressor of *Sle1*, can in fact completely suppress the entire autoimmune cascade that is caused by *Sle1* on a B6/NZW heterozygous background. This includes a powerful humoral autoimmune response, and a high penetrance of fatal lupus nephritis (>75%). *Sles1* is located on chromosome 17, in the complement region of the murine MHC. B6 mice congenic for both the *Sle1* and the *Sles1* intervals are phenotypically indistinguishable from B6 mice. Heterozygosity at the H2 locus has previously been linked to lupus susceptibility in mouse models as well as human lupus (16).

Sles2 is located on a region on chromosome 4 that was previously shown to contain NZB-derived susceptibility loci, and is linked to suppression of autoantibody production (32). *Sles3* on chromosome 3 is located near Il2, a region that has been linked to diabetes and experimental autoimmune encephalomyelitis susceptibility in murine models (32). It is linked with humoral autoimmunity and, more weakly, nephritis suppression. *Sles4* on chromosome 9 is linked with protection from nephritis but not humoral autoimmunity, and its effect is entirely male specific (32). These four suppressive loci, along with the *Sle* loci, help illustrate the complexity of SLE and the importance of epistatic interactions among susceptibility genes in leading to autoimmunity.

The major mouse model that our lab studies is the B6.*Sle1b* subcongenic line. B6.*Sle1b* was derived from *Sle1* when it was discovered through fine

mapping that the *Sle1* locus actually consisted of a cluster of functionally-related loci (33). The highest penetrance of anti-chromatin autoantibody production as well as earliest age of onset of the phenotype was mediated by the *Sle1b* locus (34). *Sle1b* is associated with the loss of tolerance to H2A/H2B/DNA subnucleosome, which is characteristic of the entire *Sle1* interval (35). *Sle1b* mice show elevated levels of both total IgM and IgG and elevated surface expression of activation markers on B and T cells (36).

Because *Sle1b* was the most potent locus within the *Sle1* interval, one of the major projects of our lab was to positionally clone the gene(s) responsible for the *Sle1b* phenotype. From sequencing the entire *Sle1b* interval, we discovered a gene-dense region, making analysis for candidate genes of *Sle1b* cumbersome. However, what caught our attention was the presence of a cluster of genes with immunological relevance known as the Slam/CD2 family.

Slam/CD2 Family of Immuno-receptors

The Slam/CD2 family of receptors is comprised of seven members: CD244 (2b4), CD229 (Ly9), Cs1 (19a24, CRACC), Cd48 (Bcm-1), Cd150 (Slam), Ly108 (NTB-a), and Cd84 (37). These cell-surface receptors are characterized by a two immunoglobulin (Ig)-like extracellular domains, a single transmembrane domain, and an intercellular segment that contain tyrosine-based motifs TxYxxV/I, known as immuno-tyrosine switch motifs (ITSM) (38,39). CD48 is the only exception as it lacks an intracellular domain and is a GPI-linked receptor. These members are located on human chromosome 1q23, which is a region that

has been associated with SLE susceptibility in numerous human linkage studies (12,13,40). In the mouse, the family lies in a syntenic region on mouse chromosome 1 (41). Many of these members have been reported to have multiple isoforms as a result of alternative splicing and have differential functions (42).

The Slam/CD2 family of molecules is widely expressed in immune-related tissues, and their expression levels can be influenced by antigen-receptor stimulation as well as cytokines (43). They are known to interact with each other in either homotypic or heterotypic fashion, such as Cd150/Cd150 interaction and CD244/Cd48, respectively (44). Members of this family have been shown to deliver both activating and inhibitory signals during cell-cell interactions among T and B cells, monocytes, and NK cells (45-47). Phosphorylation of the ITSM motifs in their cytoplasmic tails allows for the recruitment of downstream signaling molecules, such as SAP and Eat-2, and leads to the modulation of a variety of immune functions, which include T cell activation, cytokine secretion, as well as NK cell cytotoxicity (48,49). The family's ability to influence immune cell functions have been shown to heavily depend upon numerous factors that include their expression levels, functional avidity for their ligand pair, and the availability of downstream signal transduction molecules (50-52).

CD244

CD244 was first identified in the attempt to identify receptors involved in non-MHC restricted killing by NK cells (53 54). CD244 (2b4) is expressed

predominantly on NK cells, as well as CD8 T cells, monocytes, and basophils.(53 55). Unlike most of the other members of Slam/CD2 family, CD244 has been shown to interact in a heterotypic manner with another family member, Cd48 (56 57). Two isoforms of CD244 have been identified in the mouse: CD244-L and CD244-S (58). Both isoforms share the same extracellular domains and differ only in their cytoplasmic domains. The long isoform contains four ITSM motifs while the short has only one such motif (59). Schatzle et al. demonstrated that CD244-L is inhibitory in terms of cytotoxic function in NK cells while CD244-S is activating when expressed in RNK-16 cell line. In CD8 T cells, data involving anti-CD244 mAb has shown that CD244 plays a role in CD8 proliferation (60). Data from CD244-deficient mice reveal an enhanced cell-mediated cytotoxicity and interferon-gamma secretion (61). Interestingly, retroviral-mediated expression of CD244-L, but not CD244-S, in the CD244-deficient NK cells restored the phenotype, suggesting that CD244 in the mouse acts primarily in an inhibitory manner (62). Thus the data supporting its role as an activating receptor using the anti-CD244 mAb may actually reflect the blocking of CD244 with its ligand pair, Cd48 (63).

CD229

CD229 was first characterized from lymphoid cell lines and was discovered to belong to the Slam/CD2 family (64). The molecule is expressed on T cells and mature B cells (65). It has been recently demonstrated that Ly9 interacts with itself and colocalizes to the immune synapse of B and T cells upon antigen stimulation (66). Structurally, CD229 is unique amongst the other

Slam/CD2 family members because its extracellular segment consists of four Ig-variable-like domains (67). CD229 has possible isoforms based on our own Northern analysis data in mice (68) and human lymphoid tissue (69). Currently, no reports have elucidated the role of CD229 in the immune system.

Cs1

Cs1 was first identified by three independent groups (70-72). It is expressed on nearly all NK cells, activated CD8 T cells, B cells, and mature dendritic cells (73). Cs1 is another member of the family who is a self-ligand (71). It mediates cytotoxicity in NK cells in a Sap-independent manner and requires activation of extracellular signal-regulated kinases-1/2 (ERK 1/2) (74). In CD8 T cells on the other hand, Cs1 does not mediate cytotoxicity, thus it has been suggested that it may act in a costimulatory fashion (75). Similar to CD244, two isoforms of Cs1 have been described in the mouse. The longer isoform has an ITSM motif, while the shorter isoform does not have any motifs (76). When the individual isoforms were transfected into the rat NK cell line RNK-16, it was demonstrated that the long isoform can mediate cytotoxicity while the short isoform had no effect (77). This argues that Cs1-L may play an activating role (78).

Cd48

Cd48 was first identified as the molecule that was recognized by the monoclonal Ab 5-8A10 that could inhibit anti-CD3 induced T cell activation (79). It

is expressed on B and T cells, NK cells, and monocytes (80). It is the only member of the Slam/CD2 family that lacks an intercellular signaling domain. Instead, it is linked to the cell surface by a glycosylphosphatidylinositol (GPI) anchor (81). It was initially reported to be able to bind to CD2 with low affinity (82), however later it was discovered that Cd48 bound to CD244 with a higher affinity (83). Cd48 functions in a costimulatory manner, potentiating CD4 and CD8 T cell activation and proliferation (84,85). In support of this, splenocytes from CD48-deficient mice show a diminished response in CD4 T cells to Concanavalin -A and anti-CD3 stimulation (86). No change in LPS response was observed; however, there was a dramatic defect in response to phytohemagglutinin (PHA) (87).

Cd150

Cd150 was first identified and characterized on human B cells as IPO-3 (88). It is expressed on resting/activated B cells and activated T cells, as well as $CD45RO^{high}$ memory T cells, T-cell clones, and thymocytes (89,90). In addition, studies in mice have demonstrated that Cd150 is expressed on highly polarized Th1 and Th2 populations, but is only maintained on Th1 clones, and not Th2 (91). Recently, Mavaddat et al. demonstrated that Cd150 interacts in a homophilic manner but in a low affinity manner (92). Activated human T cells have been reported to express a membrane form, a soluble form that is secreted, a cytoplasmic form, and a variant membrane form with a truncated cytoplasmic tail (93). In the mouse, two isoforms of Cd150 have been reported with mSlam1 having three ITSM motifs and mSlam2 having only one (94).

In human studies, cross-linking Cd150 on activated peripheral T cells or T cell clones led to a costimulatory effect on proliferation and IFN-gamma production (95). Other groups have also reported this Th1-biasing response upon Cd150 activation in CD4 T cells (96-98). Data from the Cd150 knockout mice suggests that Cd150 does indeed play a role in balancing IL-4 and IFN-gamma production. When CD150-deficient CD4 T cells are subjected to TCR stimulus, there is a decrease in IL-4 production and slightly higher IFN-gamma production when compared to wild-type littermates (99).

Cd84

Cd84 was first identified as an antigen that was recognized by three different monoclonal mAbs directed against CD84 from a human B cell line (100), and the same group later cloned the murine homolog (101). It is expressed on B and T cells, monocytes, platelets, and granulocytes (102). Two possible isoforms have been described (103), but structural and signaling properties regarding the cytoplasmic domains were not reported. It has been demonstrated that CD84 is another homophilic molecule, and in humans, ligation of CD84 enhances anti-CD3-induced IFN-gamma production (104) and anti-CD3-induced proliferation of T cells (105).

Ly108

Ly108 was first identified fortuitously from erythrocyte RNA while attempting to study p53-regulated genes (106). It is expressed on T, B, and NK cells in both human and mouse (107-109). It is up-regulated upon activation of

human B and T cells (110). Ly108 has at least two alternatively spliced isoforms in the mouse, termed Ly108-1 and Ly108-2 (111). These two isoforms share the same extracellular and transmembrane domains and differ only in their cytoplasmic tails. Ly108-1 has two ITSM motifs, while Ly108-2 has three (112-113). Valdez et al. demonstrated that engaging Ly108 has a costimulatory effect on anti-CD3 stimulated CD4 T cells leading to an increase in proliferation and IFN-gamma secretion (114). No isoform-specific function has been established.

SAP adaptor

Slam-Associated Protein (SAP) is an SH2-domain containing protein encoded by the Sh2d1a gene that acts as a signaling adaptor downstream of the Slam/CD2 family members (115). Sap is expressed mostly in NK and T cells (116) and binds to the ITSM motifs of the Slam/CD2 family in a phosphorylation-dependent manner (117,118). The role of this molecule in the regulation of the immune system became evident when it was discovered that mutations and/or deficiencies of Sap led to X-linked lymphoproliferative disorder (XLP) (119,120)

Studies involving Sap knockout mice have provided a clue to the function of Sap and its relation to the Slam/CD2 family. Sap appears to play a significant role in Th2 responses, as Sap deficient mice exhibit a dramatically impaired IL-4 response upon T cell activation (121,122). Furthermore, when these mice are challenged with pathogens such as Toxoplasma gondii or Leishmania major, they exhibit an exaggerated IFN-gamma response (121,122). Additional characterization of this model has suggested that the defective IL-4 response is independent of IFN-gamma because when Sap knockouts were bred to IFN-

gamma knockouts, the IL-4 phenotype persisted (123). Interestingly, the T cells had the capacity to secrete IL-4 when polarized under Th2-promoting conditions, which indicated that the T cells were capable of responding to Th2 cytokines and can still differentiate into the appropriate Th subsets (124).

The Slam/CD2 family wide expression pattern across lymphoid tissues suggests a possible redundancy in functionality. This is also supported by the data that engaging the receptors, as in the case of Cd150, Ly108, and Cd84, leads to a Th1 biased response. In the data I will present, CD4 T cells from our *Sle1b* mouse model also show a Th1-biased response when stimulated with anti-CD3 and anti-CD28 antibodies. The significance of this will be discussed, and the role of this family of receptors as a whole in establishing a particular cytokine environment that is conducive to autoimmunity will be suggested.

Cytokines and SLE

Cytokines have long been considered potential players in the pathogenesis of autoimmunity as the destruction of tissues in diseases such as SLE and diabetes is influenced by proinflammatory cytokines (125). However, the roles that cytokines play is not entirely absolute as many of them have multiple functions in modulating the immune system. Furthermore, the data from mouse models studying individual cytokines have often lead to ambiguous conclusions as the induction of tolerance by cytokines is more complicated than previously thought (126).

Interleukin-2 (IL-2)

IL-2 is an important cytokine regulating T cell tolerance (127). T cells from NZB/W mice were first shown to have a deficient IL-2 response when compared to non-autoimmune strains such as C57Bl/6 and DBA/2 (128). When stimulated with ConA, both splenic and lymph node T cells show a reduced capacity to produce IL-2 as the mice aged (129). They also showed that MRL/*lpr* exhibits a more pronounced reduction in response than that seen in B/W mice, which they say correlates with the early autoimmune phenotype seen in MRL/*lpr* mice. Furthermore, B6/*lpr* also showed decreased IL-2 response, suggesting that the IL-2 defect is associated with the *lpr* gene. How this IL-2 defect plays a role in disease pathogenesis is still not clear. One group has demonstrated that adding anti-IL2R monoclonal antibody, which blocks the alpha subunit of the IL-2 receptor, ameliorates lupus nephritis in B/W mice (130). On the contrary, another group reported that adding exogenous low and high doses of human recombinant IL-2 does not affect the autoimmune phenotype in B/W mice (131). This was also shown to be the case in MRL/*lpr* (132). In addition to SLE, IL-2 polymorphisms has been implicated in mediating susceptibility to type 1 diabetes in the NOD mouse model (133); however, this locus was then later demonstrated to be necessary but not sufficient in the development of disease in the NOD mouse (134). While IL-2 and its role in autoimmunity remains inconclusive, it is clear that IL-2 does play a role in tolerance as IL-2 deficiency has been shown to lead to an autoimmune phenotype (135).

Interferon-gamma (IFN-gamma)

Interferon-gamma is a proinflammatory cytokine that is secreted by Th1, CD8 cytotoxic T cells, and NK cells (136). It has been shown to be highly expressed in lupus mice, such as MRL and BSXB mice (137). Although contrary data has been shown by another group where it was reported that no clear Th1 or Th2 expression pattern existed (138). In the B/W model, two different groups have demonstrated that disease is exacerbated with the administration of exogenous IFN-gamma. Moreover, the addition of anti-IFN-gamma monoclonal antibody or soluble IFN-gamma receptor improved the autoimmune phenotype (139,140).

The role of IFN-gamma in B/W mice is further supported by data involving IFN-gamma knockout mice that show a dramatic reduction in lupus nephritis (141). What is interesting is that IFN-gamma deficiency on the MRL/*lpr* background leads to a suppression of disease despite the mice having high levels of ANAs and glomerular immune complex deposits (142). This suggests that IFN-gamma plays more of an important role in end organ disease pathogenesis, rather than pathways leading to a breach in tolerance. However, the notion that IFN-gamma simply acts as a pro-inflammatory cytokine in autoimmunity cannot be accepted because IFN-gamma deficiency can lead to susceptibility to experimental allergic encephalomyelitis (EAE) in Balb/c mice (143). In fact, it has been demonstrated that IFN-gamma can mediate effector T cell apoptosis (144). Thus, IFN-gamma can mediate both inflammation in the pathogenesis of autoimmune disease as well as promote apoptosis to regulate immune responses.

Interleukin-6 145

IL-6 is a pleiotropic cytokine involved in immune regulation and inflammation. It plays an important role in the final maturation stages of B cells developing into antibody-secreting cells (146). In 1989, it was reported that IL-6 transgenic mice developed massive plasmacytosis with autoantibody production and glomerulonephritis that resembled SLE (147). One of the first reports that suggested IL-6 having a role in murine lupus came out the following year when it was discovered that adding exogenous IL-6 to B cells from old B/W mice augmented IgG anti-DNA antibody production (148). Another group later confirmed that the administration of recombinant IL-6 to B/W mice exacerbated the autoimmune phenotype (149). One group has reported no difference in the serum levels of IL-6 in B/W mice when compared to non-autoimmune strains; however, they did detect higher levels of IL-6 in MRL/lpr mice (150). Another group demonstrated by RT-PCR that splenocytes from B/W mice did have increased level of IL-6 expression (151). Furthermore, Dr. K. Liu in our lab has observed that B cells and monocytes from Sle1ab recombinant mice produce higher levels of IL-6 as demonstrated by intracellular FACS analysis (152). Not surprising then, it has been previously shown that blocking the IL-6 receptor by antibody suppresses the development of lupus nephritis in the B/W mice (153). However, the role of IL-6 in lupus is still not entirely clear because treating B/W mice with neutralizing anti-IL-6 antibody suppressed the development of glomerulonephritis (154) while another group reported no effect (155).

Interleukin-4 (IL-4)

IL-4 is an immunoregulatory cytokine that is involved in regulating B cells and promoting the development of Th2 cells (156). While one group has found no difference in IL-4 expression in lupus mice (157), others have reported either an increase (158) or decrease (159-161) in the levels of expression. To make matters more complicated, it has been reported that IL-4 transgenic expression in B cells prevented lupus nephritis in (NZW x B6.yaa)F1 (162). However, another group reported that the when IL-4 stimulated splenocytes are transferred to B/W mice of the same age, the production of IgG anti-dsDNA antibody was enhanced and that the treatment with neutralizing anti-IL-4 ameliorated the disease (163). Finally, IL-4 knockout on the MRL/*lpr* background lead to reduced end organ disease despite producing IgG2a and IgG2b autoantibodies (164), demonstrating again that pathways mediating end-organ damage may be independent of pathways mediating loss in tolerance. The conflicting reports on IL-4 does not allow for a clear conclusion as to the mechanisms by which it mediates autoimmunity.

Interleukin-10 (IL-10)

IL-10 is another pleioptropic cytokine that plays an important immunoregulatory role in the immune system. Because IL-10 inhibits Th1 responses and mediates B cell proliferation, differentiation, and antibody secretion, it has been hypothesized that this cytokine plays a potential role in the pathogenesis of SLE. IL-10 can also inhibit the activation of monocytes, dendritic cells, and macrophages that can lead to a decrease in the production of

inflammatory cytokines (165). IL-10 may play a role in inducing tolerance as it can downregulate MHC class II expression as well as intercellular adhesion molecules on APCS (166). Polymorphisms in the promoter of IL-10 have been associated with human SLE (167). In murine models of lupus, IL-10 has been reported to be increased in expression in lymph nodes and splenic tissue in BXSB male mice and MRL/lpr (168,169). Also, the continuous administration of anti-IL-10 to B/W mice at an early age does indeed delay the onset of ANA production and lupus nephritis and significantly improved survival rates (170). Although one would hypothesize that IL-10 influences B cell activation and function and thus could mediate B cell hyperactivity in lupus mice, the data from the mouse model may not accurately reflect human SLE because IL-10 does not activate mouse splenic B cells (171). This is a reminder that cytokine studies in murine lupus models should carefully be interpreted if conclusions made were to be applied to human SLE.

Tumor Necrosis Factor alpha (TNFa)

Tumor necrosis factor alpha (TNFa) is a multifunctional cytokine that plays both a proinflammatory and immunoregulatory role. It is secreted by macrophages and NK cells and has been implicated in autoimmunity, but the data between human and mice is not consistent. While high sera levels of TNFa have been demonstrated in SLE and RA patients (172), findings in lupus mice are inconsistent. A polymorphism in the TNFa gene in the B/W mice has been correlated with low TNFa expression levels (173). When young B/W mice are placed on recombinant TNFa therapy, the onset of kidney disease was delayed;

however, this effect was not observed once the disease had progressed (174). On the contrary, elevated TNFa levels have also been reported to be involved in lupus mice (175-177). In the MRL/*lpr* model, it has been shown that tubular epithelial cells produce TNFa along with colony-stimulating factor 1 (CSF-1) to recruit macrophages to the kidney, leading to inflammation and renal disease (178). Furthermore, TNFa has been shown to be highly expressed in kidney tissues in B/W mice (179). When 4 months-old B/W mice were then injected with low doses, but not high doses, of TNFa, the renal injury was accelerated (180). This is perplexing because Jacob et al. administered to B/W mice TNFa at a dosage ten times higher than the largest dose from the Brennan group and observed a delay in the development of lupus nephritis (181). This protective effect is not unique to lupus murine models as the administration of TNFa to NOD mice provides protection from autoreactive islet-specific T cells (182,183). Despite being a proinflammatory cytokine, how TNFa contributes to the pathogenesis as well as protection from autoimmunity in mouse models is still not fully understood.

Interleukin-17 (IL-17)

IL-17 is a proinflammatory cytokine that is produced by activated T cells that is suspected to be involved in inflammatory diseases (184). It has been demonstrated to be elevated in the serum of SLE and Systemic multiple sclerosis (SSc) patients, as well as the synovial fluid from RA patients, and has thus been suggested to contribute to the inflammatory process seen in autoimmune and degenerative diseases (185,186). While there are no currently published findings

on IL-17 involvement in lupus mouse models, most of the work on IL-17 pertains to rheumatoid arthritis and pulmonary diseases. Studies have demonstrated its role in the induction of proinflammatory cytokines in monocytes along with neutrophil recruitment and macrophage activation (187).

Interleukin-21 (IL-21)

IL-21 is a key cytokine that plays a role in the transition between innate and adaptive immune responses. It is secreted by activated T cells and plays a supportive role in B and T cell proliferation as well as NK cytotoxic function (188). Data from the IL-21R knockouts suggests that IL-21 also mediates B cell function with the help of IL-4 (189). When IL-21 expression was examined in the spleens from BXSB/yaa mice, it was demonstrated that IL-21 is greatly elevated and thus suggested a possible role in the pathogenesis of lupus (190).

Th1/Th2 Paradigm

The large body of work on T cell cytokines and their influence on the immune system have been organized into two distinct models based on cytokine secretion and function: the Th1 and Th2 lineages (191). Th1 cells are derived from naïve T-cell precursors following their interaction with APCs in the presence of IL-12. Th1 cells go on to secrete IL-2 and IFN-gamma to further augment the Th1 response. Th1 cells function to protect against intracellular pathogens, activate phagocytes, and promote delayed-type hypersensitivity responses. Th2 cells are generated in the presence of IL-4, and subsequent to activation, they produce IL-4, IL-5, IL-10, and IL-13. Th2 cells protect against extracellular

pathogens, activate eosinophils, and promote antibody-mediated immune responses. (192,193).

The fine balance between these two functionally different T helper subsets and their cytokine profiles is considered to have a significant impact in many diseases (194-197). Given the inflammatory cytokine profile of Th1 cells, it has been argued that diseases such as diabetes and experimental autoimmune encephalomyelitis (EAE) are Th1-mediated, while Th2 act to counter Th1 effects and promote tolerance (198). However, not all diseases conveniently fall within this Th1/Th2 paradigm because SLE is a humoral Th2-mediated autoimmune disease where Th1 cytokines have also been implicated (199). Clearly, IFN-gamma has been demonstrated to be highly elevated in lupus-prone strains of mice (200), and IFN-gamma knockout mice suggest that the inflammatory cytokine is required for disease pathogenesis (201). Furthermore, studies demonstrating the reduction of mortality in B/W and MRL/*lpr* mice with anti-IL4 implicates the important role of Th2 cytokines in lupus pathogenesis (202). It is difficult then to use the Th1/Th2 paradigm in describing lupus, especially given that the model has been recently revised. The Th1 and Th2 classifications have been extended to other cell types, such as CD8, NK, dendritic, macrophages, and even B cells (199,203).

In the upcoming chapters, we describe our attempts in finding the *Sle1b* gene and how it might mediate the autoimmune phenotype in our B6.*Sle1b* congenic line. We discuss the identification and characterization of the genes within the *Sle1b* interval and focus upon the Slam/CD2 gene cluster that lies

within it as the strongest candidates. We demonstrate expression pattern differences of this family between B6 and the B6.*Sle1b* congenic as well as a difference in isoform expression for *Ly108* and *CD229*. Dr. Nisha Limaye from our lab has already demonstrated two major haplotypes of the Slam/CD2 family. In conjunction to that finding, we present calcium flux data that indicates altered signaling events in T cells from mice that carry haplotype 2. Finally, we demonstrate that *Sle1b* CD4 T cells exhibit a reduced IL-4 cytokine response under primary and secondary stimulation. We propose that the polymorphisms of the Slam/CD2 family influence early TCR events and lead to an altered cytokine profile in the B6.*Sle1b* mice.

Chapter 2: Methods

Mice

The mice used in the studies were kept under specific pathogen-free (SPF) conditions at the University of Texas Southwestern Medical Center. The introgression of the SLAM/CD2 interval from 129/SvJ onto C57BL/6 was performed using marker assisted selection protocols 204, and required five backcross generations onto C57BL/6. The B6.*129c1* congenic carries a 129-derived congenic interval from *D1MIT148* to *D1MIT115* (roughly four Mb) and includes *Sle1b*, but excludes *Sle1a, Sle1d,* and *Sle1c.* The B6.*Castc1* congenic strain has a congenic interval similar in size to B6.*Sle1.* B10.Br, FVB, C3H mice were kindly provided by Dr. Michael Bennett.

BAC Contig Construction

The BAC contig that spans the *Sle1b* interval was constructed by screening the C57Bl/6J-derived BAC library created by de Jong and colleagues (205) and the CJ7/129Sv-derived CalTech library (206) with probes designed from D1Mit148, D1Mit146, D1Mit113, and D1Mit149 markers as well as genes already known to lie within the interval. Several markers were also used from published YAC and BAC contigs to help screen the libraries (207). In addition, oligo primers designed from BAC-end-sequences (BES) as well as the ESTs and gene probes were used to PCR screen the positive BACs to help confirm and situate their position and orientation within the contig.

BAC DNA Extraction and Insert Sequencing

BAC DNA was prepared following the Wellcome Trust Centre for Human Genetics and Institute of Molecular Medicine protocol. (http://www.molbiol.ox.ac.uk/~jmejia/more_protocols.html). For sequencing libraries, BAC DNA was sheared in a nebulizer (Glas-Col, Inc.; Terre Haute, IN) at 150-psi nitrogen for 2.5 minutes. Fragment ends were mended by incubating with 0.2mM dNTPs, 40 units T4 DNA polymerase for 15 min. at RT, and addition of 25 units Klenow for 1-hour at RT. DNA was purified by phenol-chloroform extraction, and ethanol precipitation, and run on a 1% agarose gel. 1-3kb fragments were extracted using the GenecleanII kit (Qbiogene; Carlsbad, CA) and ligated into the pUC18 vector using the Rapid Ligation Kit (Roche Boehringer Mannheim; Indianapolis, IN). DH5α TM competent cells (Invitrogen; Carlsbad, CA) were transformed, and plated onto LB plates with 100ug/ml ampicillin, 50ug/ml X-gal, and 1mM IPTG. Plasmid DNA was extracted using an Autogen 740 (AutoGen; Holliston, MA) and sequenced using M13 primers on the Beckman CEQ 2000XL (Beckman Coulter; Fullerton, CA). Sequence was assembled using phred, phrap, and consed (http://www.phrap.org).

Cell Preparation

Splenocytes were harvested from mice into RPMI-10 complete media (10% fetal calf serum, 1% penicillin/streptomycin, 1% glutamine, 25mM HEPES, and 10mM beta-mercaptoethanol). Red blood cells were lysed with ACK lysing buffer (0.1mM EDTA, 0.15M NH_4Cl, 10mM $KHCO_3$ pH 7.2) to yield single cell

splenocyte suspension. Splenic cells were then used directly in stimulation assays or purified via positive selection for $B220^+$ or $CD4^+$ T cells using Dynabeads mouse pan B ($B220^+$) or $CD4^+$ magnetic beads respectively (Dynal; Brown Deer, WI). For cytokine analysis, $CD4^+$ T cells were negatively isolated using a cocktail of biotinylated antibodies that consisted of anti-HSA (M1/69), B220 (RA3-6B2), CD8 (53-6.7), NK1.1 (PK136), CD49b (DX5), and Cd11b (M1/70) (BD Pharmingen; San Diego, CA), and streptavidin-magnetic beads (BD Pharmingen) in conjunction with the Imagnet direct magnet (BD Pharmingen) as outlined in manufacturer's protocol.

RNA Isolation and cDNA Preparation

For Northern analysis, total RNA was isolated from splenic tissues using Trizol (Invitrogen; Carlsbad, CA), according to manufacturer's instruction. For quantitative real-time PCR, total RNA was isolated from splenocytes, B cells, and T cells with the Qiagen Rneasy Mini Kit (Qiagen; Valencia, CA) and genomic DNA was removed with treatment with DNase I, according to Qiagen protocol. cDNA was synthesized from 1 ug total RNA with the Taqman Reverse Transcriptase Kit, according to manufacturer's instructions (Applied Biosystems; Foster City, CA).

Quantitative real-time PCR

Quantitative real-time PCR was performed on 10 ng cDNA using the 2X SYBR Green PCR Master Mix (Applied Biosystems) in 20ul reactions. The GeneAmp

5700 Sequence Detection System (Applied Biosystems) was used for the initial expression studies, and it was later updated to the 7300 Real-time PCR System (Applied Biosystems). The run condition was modified into a 3-step cycle: 95^{o}C for 20sec, 55^{o}C for 20sec, and 72^{o}C for 30sec for 40 cycles. Primer sequences used in the studies are listed in Table 1. For *GATA-3*, a Taqman probe and primer pair combination was kindly provided by Applied Biosystems. Using the 2x Taqman PCR Master Mix (Applied Biosystems), the run condition was set at the manufacturer's instructions: 2-step cycle of 95^{o}C for 15sec, 60^{o}C for 1min for 40 cycles. For each primer set, standard curves were generated using serial dilutions of cDNA. To control for loading, G*apdh, Actin,* or *B-2 microglobulin* was used as reference genes for normalization. Data is either presented as the mean fold difference of B6.*Sle1b* relative to B6 or as normalized to reference gene. A Student's t-test or paired t-test was used to determine significance.

Northern RNA Analysis

5ug of total splenic RNA was resolved on a 1% denaturing formaldehyde gel and transferred to Hybond-N+ nylon membrane (Amersham Biosciences; Piscataway, NJ) with the Turboblotter system (Schleicher & Schuell Biosciences; Keene, NH), according to manufacturer's directions. Oligo primers (listed in Table 1) were used to generate hybridization probes via PCR amplification from C57Bl/6 splenic cDNA. PCR oligos were radioactively labeled with the Rediprime II kit (Amersham), and purified with Quick Spin G-50 Sephadex Columns (Roche). ULTRAhyb solution (Ambion; Austin, TX) was used for hybridization according to

manufacturer's directions. Membranes were washed with 2X SSC/ 0.1% SDS, followed by 0.1X SSC/0.1% SDS, and exposed with Phosphor Screen and PhosphoImager Storm system (Amersham). Tissue blots were mouse poly A+ Northern blots available from OriGene (Rockville, MD)

Calcium Flux Analysis

Splenic B and T cells from 1-2 month old B6 and B6.*Sle1b* mice were enriched by negative selection using Dynabeads mouse pan T (Thy1.2) and B (B220) magnetic beads (Dynal), respectively. 10^6 cells were labeled with 4uM Fluo-3 (Molecular Probes; Eugene, OR) and 200 ug/ml Pluronic F-127 (Molecular Probes) at 37°C for 30 minutes and then washed twice with HBSS buffer. Experimental runs were performed on the FACSScan (Becton Dickinson; Franklin Lakes, NJ). Samples were run for 45 seconds to establish baseline reading. At the 45 sec mark, an antibody cocktail (1.7ug/ml biotin TCR β chain H57-597, 1.7ug/ml biotin CD4 (GK1.5), and 1.7ug/ml biotin CD8a (53-6.7) (BD Pharmingen; San Diego, CA)) was added. Data was acquired for 1 min and was followed by cross-linking with 20ug/ml streptavidin (Roche). After 5 min, 2uM ionomycin (Calbiochem; San Diego, CA) was added to achieve maximal response, followed by 2mM $MnCl_2$ (Sigma-Aldrich; St. Louis, MO) to achieve minimal response. Analysis was performed on FlowJo version 4.1 (Tree Star; Ashland, OR). Calcium concentrations were determined as described (208). Mean calcium concentrations were determined at the baseline, peak response,

and post response. Paired Student's t-Tests were used to determine significance.

CFSE and BrdU Analysis

2-3 x 10^7 splenocytes were labeled with 2.5uM carboxy-fluorescein diacetate (CFSE) (Molecular Probes) in RPMI-10 media for 15 min at 37^{o}C. Cells were washed twice with media and were plated out at 2.5 x10^6 cells/ml in triplicates in 96-well round bottom plates in the presence of either F(ab')$_2$ anti-IgM (Jackson ImmunoResearch; West Grove, PA) or anti-CD3e (145-2C11) (BD Pharmingen**)**. Cells were harvested at 24hr, 48hr, and 72hr time points and Fc-blocked with 2.4G2 (American Type Culture Collection; Rockville, MD). Cells were then stained on ice with CD19-PE (1D3) to assess for B cell proliferation or CD4-PE (H129.19) and CD8-PerCPCy5.5 (53-6.7) (BD Pharmingen) for T cell proliferation. Flow cytometric data on the stained cells were acquired on the FACSScan (Becton Dickinson) and analyzed using FlowJo (Tree Star). Data is presented as CD19-gated or CD4 and CD8-gated CFSE histograms. BrdU assays were performed with the FITC BrdU Flow Kit (BD Pharmingen). 0.8mg/ml BrdU was introduced into the drinking water at for 9 days, followed by a change to normal water. Mice were sacrificed two weeks later, and splenocytes were harvested and analyzed according to manufacturer's directions.

In-Vitro Stimulation Assays for RNA Analysis

Splenocytes were isolated and plated in triplicates in DMEM-10 or RPMI-10 in 96-well round bottom plates at $1x10^5$ cells per well. $F(ab')_2$ anti-IgM (Jackson ImmunoResearch), anti-CD40 (209), anti-CD3e (145-2C11), anti-CD28 (37.51) (BD Pharmingen), phorbol myristate acetate (PMA) (Sigma; St. Louis, MO) and ionomycin (Calbiochem) were added to media. Cells were harvested at various time points for RNA isolation with RNEasy Mini kit (Qiagen).

Cytokine Assays

CD4 T cells were negatively purified as described above and plated at $1x10^6$/ml in 96 round bottom plates pre-coated with 1ug/ml anti-CD3e with or without 10ug/ml anti-CD28 (BD Pharmingen). Supernatant and cells were harvested at 24hr, 48hr, and 72hr for cytokine ELISAs and RNA isolation for real-time quantitative PCR. Supernatants were diluted at 1:2 (IL-4) or 1:25 (Interferon gamma) and assessed for cytokine levels with the CytoSet ELISA kit (Biosource International; Camarillo, CA) as outlined in manufacturer's directions.

Gene Name	Direction	Sequence (5' → 3')	Product Size (bp)
IL-4	For	ACAGGAGAAGGGACGCCAT	55
	Rev	GAAGCCCTACAGACGAGCTCA	
IFN-gamma	For	AGCAACAGCAAGGCGAAAA	55
	Rev	CGCTTCCTGAGGCTGGAT	
IL-10	For	GGTTGCCAAGCCTTATCGGA	55
	Rev	ACCTGCTCCACTGCCTTGCT	
IL-17	For	GCTCCAGAAGGCCCTCAGA	55
	Rev	AGCTTTCCCTCCGCATTGA	
IL-21	For	CCTGGAGTGGTATCATCGCTTT	55
	Rev	TGATTGTGACACTTTTCTGGGAAT	
TNFa	For	CATCTTCTCAAAATTCGAGTGACAA	55
	Rev	TGGGAGTAGACAAGGTACAACCC	
T-bet	For	CCTGTTGTGGTCCAAGTTCAAC	55
	Rev	CACAAACATCCTGTAATGGCTTGT	
B2M	For	GACCCTGGTCTTTCTGGTGCTT	55
	Rev	TCAGTATGTTCGGCTTCCCATT	
Actin	For	GCTTCTTTGCAGCTCCTTCGT	60
	Rev	CTTCTGACCCATTCCCACCA	
Cs1 probe	For	GGAGAGAAAGACCAGGCCTTAACA	55
	Rev	CTATGAGCACAGCCTCATTCATCA	
Slam probe	For	ACAAAAGTGTCCGCATCCGCATCCTC	60
	Rev	TGATTTCTGTACTTGGGCATAA	
Cd48 probe	For	TGTGCTTCATAAAACAGGGATG	53
	Rev	TGAATGATGAGTGTTGTGACCA	
Cd84 probe	For	CAGGGCACTTATAAAGGACGAA	55
	Rev	TCATCTTCTCAGAAAGCTGCAC	
Cd229 probe	For	CCAGTGTCACCAAGAGTCCTGT	57
	Rev	CATCCATAGACCCATCCACTTT	
Ly108 probe	For	ACTGCCCTCGTTATCAACCTAA	55
	Rev	CATACACAGTGTTCCCTGGAGA	
Ly108-1 probe	For	ATCCCCAAACACTTCAGACTCTAC	55
	Rev	CTGCACTGGGAAGGTCTTTTATAG	
Ly108-2 probe	For	TTGAGGAAATTAAACAATGCTGTC	55
	Rev	ATTCCACTTCTCTTAGGTCCATTG	
Gapdh probe	For	TGCTGAGTATGTCGTGGAGTCT	55
	Rev	TGCTGTAGCCGTATTCATTGTC	
Actin probe	For	AGCCATGTACGTAGCCATCC	60
	Rev	ACTCATCGTACTCCTGCTTGCT	

Table 1: Primers used for Real-time Quantitative PCR and Northern blotting analysis. An annealing temperature of 55º was used for all reactions

Gene Name	Direction	Sequence (5' → 3')	Product Size (bp)
B4galt3	For	TTCGACTATTCTCATCCCCACGAT	55
	Rev	CTTTCTGGACAGTAGGGCAATGCT	
Ppox	For	CAGATGGTGCGATCTTTGAACTTG	55
	Rev	GACAGGCAAGACTTCAGACTCCAA	
Usp23	For	GAGTGGGAGCCAAGATACCATTTC	55
	Rev	CTGGGAGGCAAAGGTCGTAACAT	
Ucf1	For	TCCGGTATGTGGAGAACAACAAGA	55
	Rev	AGGAAATCGTGGATGTACCAGCAT	
Rpl27	For	GCGATCCAAGATCAAGTCCTTTGT	55
	Rev	GTCCCTGAACACATCCTTGTTGAC	
Dedd	For	TGTGATATCAGGCTCCGAGTTC	55
	Rev	GGGTCCTGCTTATTGGAGAAGA	
Nit1	For	CATCAACACCAAACAAGCAAGAGA	55
	Rev	GGAGTAATGTCTCGGCAGGGTTT	
Pfdn2	For	GAGGGCAACAAGGAGCAGATAC	55
	Rev	ATGAGACGAATGTTGTGCTTTTC	
Usf1	For	ATGTACAGGGTGATCCAGGTGTCA	55
	Rev	GGATCACTGCCTGGGTCATAGACT	
Jam	For	GATCAGTGTCCCCTCCTCTGTCA	55
	Rev	ATATCCCGTCCTTGAACCAGGAAT	
Itln	For	AACAGGTGGGGCAATTCTTTCTTT	58
	Rev	GTGCGCAGGAAATAGAGACCATCT	
CD244	For	TCTGAAGAAGTGGTTGGTGTCTCA	55
	Rev	CATCATTATACCAATTCAGGATCTCAA	
CD229	For	TCCTGCACCTTCACCCTAATCTG	55
	Rev	GGGTGTGGCTTCCATCGTATGTAT	
Cs1	For	AGGAATCCAGTCAGCAACAGTTT	55
	Rev	AAAAATAGTCAGCAAGACAGCAAA	
Clpx	For	AACACCAGCATACTTTGCCTCAAAA	55
	Rev	TCCCAGAGGCTGATTTCTTACTGC	
Cd48	For	CTTGAAGAAAAACAATGGTGCACTT	55
	Rev	TTCACGCAGCACTCTCATGTAGT	
Cd150	For	CAAAACCCAGGAGAACGAGA	55
	Rev	CGGTGCAGTTGTAGATGCTG	
Cd84	For	GTCCAGCAGCCATGTACAGACACT	55
	Rev	GCCAACATCGGAATGAGAATAAGC	
Ly108	For	TCACAAGTCACTGCCCTCGTTATC	55
	Rev	CAGGGAGTAGGACTGGGTGATGTT	
Ltap	For	TGGATCCTGGAGAAGTATTACCAT	55
	Rev	AATACACCTTGAAGCCAGACACTT	
Nhlh1	For	TGGAAGCCTTCAACCTAGCCTTC	55
	Rev	ATGGCCAGGCGTAGGATCTCAAT	
Nic	For	CTGCTCAACGCCACTCATCAGATT	55
	Rev	GGTCAACACCCACTTCAGGTCTTC	
Copa	For	GCCTGTGAGAAAACCCCACAGAT	55
	Rev	CGGTAAGATGCAGCACAAATGTCA	
Pxf	For	TGCTAATGGCGAACAGTGTCTGAT	55
	Rev	CCTCAGTTCCCAGTGACTCTGTTG	
Gapdh	For	TGCACCACCAACTGCTTAG	55
	Rev	GGATGCAGGGATGATGTTC	
Ly108-1 RT	For	GACCACACTCATGCCCTGAC	55
	Rev	GTATTCAGCCTAGGAGAAATGG	
Ly108-2 RT	For	CCGGCTATAACCAACCCATT	55
	Rev	AAGCCAGAGCTGTGGTGACA	

Chapter 3: Association of Polymorphisms of the Slam/CD2 Family with Murine Lupus

Genetic predisposition is a central element in susceptibility to many common autoimmune diseases, including systemic lupus erythematosus (SLE) (210-212). We have used congenic dissection to characterize the susceptibility genes in the lupus-prone NZM2410 mouse (27,213,214). These analyses identified *Sle1*, on murine chromosome 1, as causal for a loss in immune tolerance ~~that leads~~ leading to anti-nuclear autoantibody (ANA) production, on the B6 background. Interestingly, *Sle1* is syntenic with genomic intervals that are associated with susceptibility to SLE in human linkage studies (12,40).

Fine mapping of *Sle1* revealed a cluster of four loci (designated *Sle1a*-*Sle1d*) within the B6.*Sle1* congenic interval, each contributing ~~some~~ a portion of the phenotypes originally associated with "*Sle1*" (215). *Sle1b* is the most potent member of this cluster, mediating gender-biased and highly penetrant ANA production in the B6.*Sle1b* congenic strain. *Sle1b* leads to fatal lupus nephritis when combined with the autoimmune-accelerating mutations *lpr* or *yaa*, illustrating the importance of this locus to lupus pathogenesis (216). Analyses of *Sle1* expression in mixed bone-marrow chimeras indicate that genes in the *Sle1* cluster are cell intrinsic, mediating activation phenotypes in both B and T cell lineages developed from B6.*Sle1*-derived bone marrow stem cells (217; 218). These results would suggest that all of the susceptibility alleles in the *Sle1* cluster are expressed in at least the lymphocytic lineages, although we have not

repeated this experiment with bone marrow derived from the ~~that vary at~~ individual *Sle1*-cluster loci.

Here we describe the genomic characterization of the *Sle1b* interval, which identifies a highly polymorphic seven-member cluster of SLAM/CD2-family genes in the middle of the *Sle1b* critical interval. Analyses of this family reveal extensive sequence and expression-level differences between B6 and B6.*Sle1b* and associate a subset of SLAM/CD2-family members with autoimmunity. Given the well-established role of this family in the modulation of cellular activation and signaling in the immune system, they are ideal candidates for mediating the *Sle1b* phenotype (for reviews see 219-222; 223, 224).

Mapping and sequencing the *Sle1b* congenic interval

Under the direction of Dr. Amy Wandstrat, we constructed a Bacterial Artificial Clone (BAC) contig, or a set of overlapping BACs, that spanned the *Sle1b* interval. Using probes designed from D1MIT markers and genes already known to lie within the interval, we screened by hybridization both the C57Bl/6J-derived BAC library created by de Jong and colleagues (225) and the CJ7/129Sv-derived CalTech library (226) to identify positive clones. Several markers were also used from published YAC and BAC contigs to help screen the libraries (227). In addition, oligo primers designed from BAC-end-sequences (BES) as well as the ESTs and gene probes were used to PCR screen the positive BACs to help confirm their position and orientation within the region (Figure 1). Using polymorphic markers derived from the ends of the contig, PCR

analysis positioned the telomeric end of the *Sle1b* within a 10kb interval lying between *ApoA2* and *B4galt3*, and the centromeric end inside a 15kb interval with the *Pxf* locus (Figure 2). Thus, the *Sle1b* interval was contained within seven overlapping BAC clones (BAC 41: 194d6, BAC 47: 48o11, BAC 25: 171k8, BAC 40: 145f9, 77a8, 438k9, and BAC 95: 462j8). (Figure 2)

The BACs that spanned the *Sle1b* interval were then subjected to sequencing. More than 8700 sequence reads were collected and assembled, and when combined with sequences available in the public genomic databases (www.genome.ou.edu), provided between 9 to 19-fold coverage of the entire region. The resulting sequences were subjected to various gene-prediction software that included PANORAMA (http://atlas.swmed.edu), GenescanII (http://genes.mit.edu/GENSCAN.html) and HMMgene (http://www.cbs.dtu.dk/services/HMMgene) .

Figure 2 depicts the genetic map of the *Sle1b* interval. Public databases place the genomic location of the *Sle1b* interval between 171.3Mb and 172.2Mb along mouse chromosome 1. The region is approximately 900kb in length and contains 24 expressed genes and two pseudogenes. RT-PCR analysis revealed that 19 of the genes are expressed in splenic tissue. Of great interest, seven of these genes, *CD244, CD229, Cs1, Cd48, Cd150, Ly108, and Cd84,* are members of the SLAM/CD2 gene superfamily (228). These members are cell-surface receptors that have been implicated in regulatory immune functions and are known to interact with each other in both homotypic and heterotypic fashion.

More importantly, out of the 19 candidate genes within the *Sle1b* interval, they are the only ones that have an obvious immunological role (229,230).

Extensive polymorphisms distinguish the SLAM/CD2 family genes in B6 and B6.*Sle1b*

In assessing the candidacy of the genes mediating the *Sle1b* phenotype, two conditions had to be fulfilled. First, the *Sle1b* gene must be expressed in the spleen, and second, *Sle1b* must exhibit a functional polymorphism between B6 and B6.*Sle1b.* Real-time quantitative PCR (RT-PCR) and Northern blot analysis determined that 19 of the genes within the interval are expressed in splenocytes. Sixteen of these are expressed in B and T cells as shown in Figures 3. Of these 16 genes analyzed, only the members of the Slam/CD2 family show expression differences greater than two-fold and are statistically significant between B6 and B6.*Sle1b*. These criteria make them our primary candidates. The two-fold cut-off was set due to the detection limitation of the Applied Biosystems GeneAmp 5700 Sequence Detection System (personal communication with ABI representatives). Four members of the Slam/CD2 family vary in their expression in splenic $B220^+$ B cells and/or $CD4^+$ T cells between B6 and B6.*Sle1b*. (Figure 3a, 3b). *Cd84* and *Ly108* are up-regulated in B cells from young B6.*Sle1b* mice which met statistical significance. *CD229* and *Cd150,* despite exhibiting a trend for higher expression levels in B cells from B6.*Sle1b* mice, did not meet significance however (Figure 3a). On the other hand, *Cs1, Cd48,* and *Ly108* are down-regulated in B6.*Sle1b* T cells. *Cd150* also shows a decrease in expression

but is not statistically significant (Figure 3b). These expression differences were detected in young female mice (<3 months-old), well prior to the development of ANA or any other autoimmune manifestations.

Northern blot analysis of the Slam/CD2 family was also performed. We analyzed total RNA from splenic tissues 2 months-old female B6 and B6.*Sle1b* mice. While all the members, except for CD48, show multiple bands indicative of possibly different isoforms, only *Ly108* and *CD229* show a difference in expression pattern between the two strains of mice (Figure 4). *Ly108* exhibited three prominent bands at 1.5, 2.6, and 4.7 kb, which is consistent with the findings reported in the original Ly108 cloning paper (231). What was striking about this was that B6.*Sle1b* showed a more prominent band at 4.7 and 2.6 kb, whereas B6 strongly expressed the 1.5 kb band. *CD229* also exhibited a pronounced pattern difference where B6.*Sle1b* expressed a 3.2 kb band that is barely detectable in B6 (Figure 4). As for *Cd84*, *Cs1*, *Cd48*, and *Cd150*, there were no obvious differences in the expression patterns between the two strains (Figure 4). *Ly108* and *CD229* were also assessed for tissue distribution on the poly-A+ tissue Northern blot (232) (Figure 5). The tissue blots demonstrate that both genes are most strongly expressed in the thymus, followed by the spleen. Detectable bands were also noted in the liver and testis for *CD229*.

Expression of isoforms of Ly108 is different in B6 and B6.*Sle1b*

Because Northern blot analysis revealed a possible difference in isoform usage between B6 and B6.*Sle1b*, Ly108 became a strong candidate for the

Sle1b gene. We refer to the long and short isoform based on message length *Ly108-1* and *Ly108-2*, respectively. The exon/intron structure is depicted in Figure 6a. Upon finishing our sequencing of our *Sle1b* BAC contig, we discovered that the terminal exons differed between the two isoforms (Figure 6a). Both isoforms share the first seven exons, but each would splice to a unique exon and 3' UTR. *Ly108-1* splices to exon 8, while *Ly108-2* splices to exon 9 (Figure 6a). Interestingly, the longer isoform encodes for a shorter protein, whereas the shorter isoform encodes for a longer protein. Ly108-1 protein is 331 amino acids in length and has two tyrosine-based signaling motifs. Ly108-2 protein is 351 amino acids in length and shares the same two tyrosine motifs that Ly108-1 has but has two additional tyrosines, one of which conforms to the ITSM motifs (Figure 6b).

The next step was to design real-time oligos that could distinguish the two isoforms. RT-PCR was performed on B and CD4 T cell RNA and confirmed our Northern blot findings. In B and T cells, *Ly108-1* shows an increase in the level of expression for B6.*Sle1b* mice, whereas *Ly108-2* is down-regulated (Figure 6c). This strongly correlated with the observed increase in the 2.6 kb band and decrease in the 1.5 kb band. To further confirm that the 2.6 kb band was indeed related to *Ly108-1*, an oligo probe designed from exon 8 was hybridized to confirm *Ly108-1* (Figure 6d). When a probe designed from exon 9 was used, the 1.5 kb band was indeed *Ly108-2*. What was interesting was that the *Ly108-2* probe also hybridized to the 3 kb band (Figure 6d). Although we are uncertain whether it represents another isoform or unspliced transcript, the band is evident

in the original Northern blot and does not differ between the two strains. Peck and Ruley also observed high molecular weight bands in their Northern analyses, and they attribute it to partially unspliced RNA transcript or additional splice variants through the use of alternative polyadenylated signals (233).

Many of the Slam/CD2 family members are up-regulated upon cell activation (234,235). This led us to examine the effects of antigen-stimulation on *Ly108* isoform expression in our mice. Total splenocytes from young B6 and B6.*Sle1b* females were cultured in the presence of increasing concentrations of LPS for 48hrs. What was observed was that the ratio of *Ly108-2* to *Ly108-1* was up-regulated in B6, but not in B6.*Sle1b* (Figure 7a). Statistical significance was not assessed as the experiment was only performed twice. Instead, the experiment was repeated with three mice in each group. To assess *Ly108-2* expression differences in B cells, anti-IgM at 10ug/ml was used to stimulate the cells for 72hrs. 0.2ug/ml of anti-CD3e was used for 48hrs to detect T cell changes. Basal level of *Ly108-2* was approximately 2-fold higher in resting splenocytes from B6 than from B6.*Sle1b* (Figure 7b). Upon anti-IgM or anti-CD3e stimulation, approximately a 7-fold increase in *Ly108-2* expression was observed in B6 but not B6.*Sle1b* (Figure 7c, 7d). *Ly108-1* was not examined.

Dr. Nisha Limaye in our lab had previously characterized a panel of inbred laboratory strains of mice to determine whether the SLAM/CD2-family gene cluster in B6.*Sle1b* is a unique feature of the genome of the NZM2410 strain. Her sequence analysis of the extracellular immunoglobulin regions of the SLAM/CD2 family revealed that the majority of these inbred strains of mice carry

alleles that are indistinguishable from those of B6.*Sle1b.* The autoimmune-associated haplotype of B6.*Sle1b,* termed SLAM/CD2 haplotype 2, is the most common version in laboratory strains, being detected in every autoimmune-prone strain analyzed as well as many non-autoimmune strains such as 129/SvJ (Figure 8a). This then posed the question whether *Ly108* and *CD229* expression correlated with the two haplotypes. We were interested in knowing whether or not the expression differences of these two genes were a unique feature of the *Sle1b* interval, so we compared the expression patterns of *Ly108* and *CD229* amongst mice belonging to the two haplotypes. Isoform usage for both genes did mostly correlate with the haplotype (Figure 8b), with one exception. For *CD229*, the expression pattern in the B6.Castc1 congenic resembles B6. B6 and B10.Br showed similar banding patterns where *Ly108-2* is the predominant band, whereas B6.*Sle1b*, B6.Castc1, NZW, 129Sv/J, Balb/c, FVB, and C3H exhibited similar patterns demonstrating the up-regulation of *Ly108-1*. Real-time quantitative RT-PCR of the individual isoforms confirmed the Northern findings (Figure 8c).

This strongly suggests that the expression patterns from *Sle1b* and its connection to an autoimmune phenotype is somehow epistatically influenced by background genes. If inbred strains of mice such as Balb/c and 129Sv/J share the same expression patterns and B6.*Sle1b* but are not autoimmune, this would argue that either there are suppressive genes in their genome or permissive genes in the B6 genome that allows the *Sle1b* interval, when moved onto the B6 background, to cause autoimmunity. As Dr. Limaye demonstrated in her

dissertation, when the *Sle1b* locus from 129Sv/J or CAST/ei are introgressed onto B6, these mice do indeed exhibit a loss in tolerance and produce highly penetrant autoantibodies (Figure 9).

Ly108-1 Transgenic Exhibited no Qualitative Effects on Lymphocytes

To gain insight into the role of Ly108 in autoimmunity, we generated isoform-specific Ly108 transgenic mice. Our strategy would allow us to address two questions. First, would the overexpression of Ly108-1 in the B6 mouse lead to an autoimmune phenotype? Second, would the overexpression of Ly108-2 in the B6.*Sle1b* mouse mitigate its phenotype? Dr. Limaye designed the two constructs as shown in Figure 10a. A full-length cDNA encoding either isoforms 1 or 2 was cloned into the pCAGGs vector, generously provided by Dr. Toru Miyazaki, driven by a β-actin promoter. One founder line for Ly108-2 on the B6 background was established and was subsequently bred onto B6.*Sle1b*. Unfortunately, the expression level of *Ly108-2* in the spleen was indistinguishable from non-transgenic littermate, and no expression in the liver was detected (Figure 10b). Real-time PCR confirmed that the overall expression of *Ly108-2* in the spleens from transgenic mice were no different from B6.*Sle1b* (Figure 10c). Interestingly, real-time analysis did detect *Ly108-2* expression in the liver from the Ly108-2 transgenics (Figure 10c).

One founder that expressed high levels of *Ly108-1* was crossed onto B6. The Northern blot revealed that the level of *Ly108-1* in the spleen was higher in the transgenic line than the non-transgenic littermate, but surprisingly, the level of

expression appeared similar to that of B6.*Sle1b* (Figure 10b). Furthermore, a very high level of *Ly108-1* was detected in the liver, but not in the negative littermate (Figure 10b). Real-time studies in both hepatic and splenic tissues confirmed the Northern findings (Figure 10d).

To ascertain the effects of the overexpression of the long isoform on lymphocyte development, the Ly108-1 transgenic was then characterized by FACS analysis, and it was determined the transgenic had normal lymphocyte percentages (Figure 10e). Both male and female Ly108-1 transgenics were aged and bled at five and twelve months of age, and unfortunately, these mice failed to produce autoantibodies detected by ELISA (Figure 10f). The impact of overexpressing of Ly108-1 on cellular proliferation was also examined by CFSE labeling. However, no differences were detected in CD19 B cells, CD4 T cells (Figure 10g), and CD8 T cells (not shown) between the Ly108-1 transgenic and B6 mice. Finally, using an anti-Ly108 monoclonal antibody created by our collaborator Dr. John Schatzle, we demonstrated no differences in the levels of surface expression in splenocytes and thymocytes (Figure 10h-i). Although the mAb against Ly108 is unable to distinguish the difference between the isoforms, the lack of increased surface expression suggested that the Ly108-1 transgene in spite of having an increased expression in the spleen and liver, did not seem to affect splenic or thymic expression. Thus, the data regarding Ly108 overexpression and its possible role in mediating the *Sle1b* phenotype remains inconclusive.

The work presented here together with Dr. Limaye's sequence analysis reveal extensive variations between B6 and B6.*Sle1b* within the *Sle1b* region, predominantly in the SLAM/CD2-family cluster. Numerous structural and expression polymorphisms distinguish the seven genes in the SLAM/CD2 cluster as Dr. Limaye's data and our real-time quantitative RT-PCR data suggest. Many of these structural mutations are located in the extracellular domains that are believed to be essential for the function of these molecules. In addition, four of these genes vary in their levels of expression in B and T lymphocytes between B6 and B6.*Sle1b*. In contrast, the non-SLAM/CD2 genes within the *Sle1b* interval showed less sequence diversity and exhibited no changes in their levels of expression between the two strains of mice (Figure 3a, 3b). *Ly108* is the most promising candidate because of the differential isoform expression in B6.*Sle1b*. *CD229* also showed a difference in expression patterns by Northern blot analysis, but this expression difference was not noted in the B6.*Castc1* congenic mouse that is also autoimmune (Figure 8b). As Dr. Limaye has already shown, the B6.*Castc1* mouse does produce anti-nuclear autoantibodies, which argues that *CD229* isoform usage may not be related to the autoimmune phenotype. However, the notion that the entire haplotype of the Slam/CD2 family instead of one single member mediating the *Sle1b* phenotype cannot be ruled out.

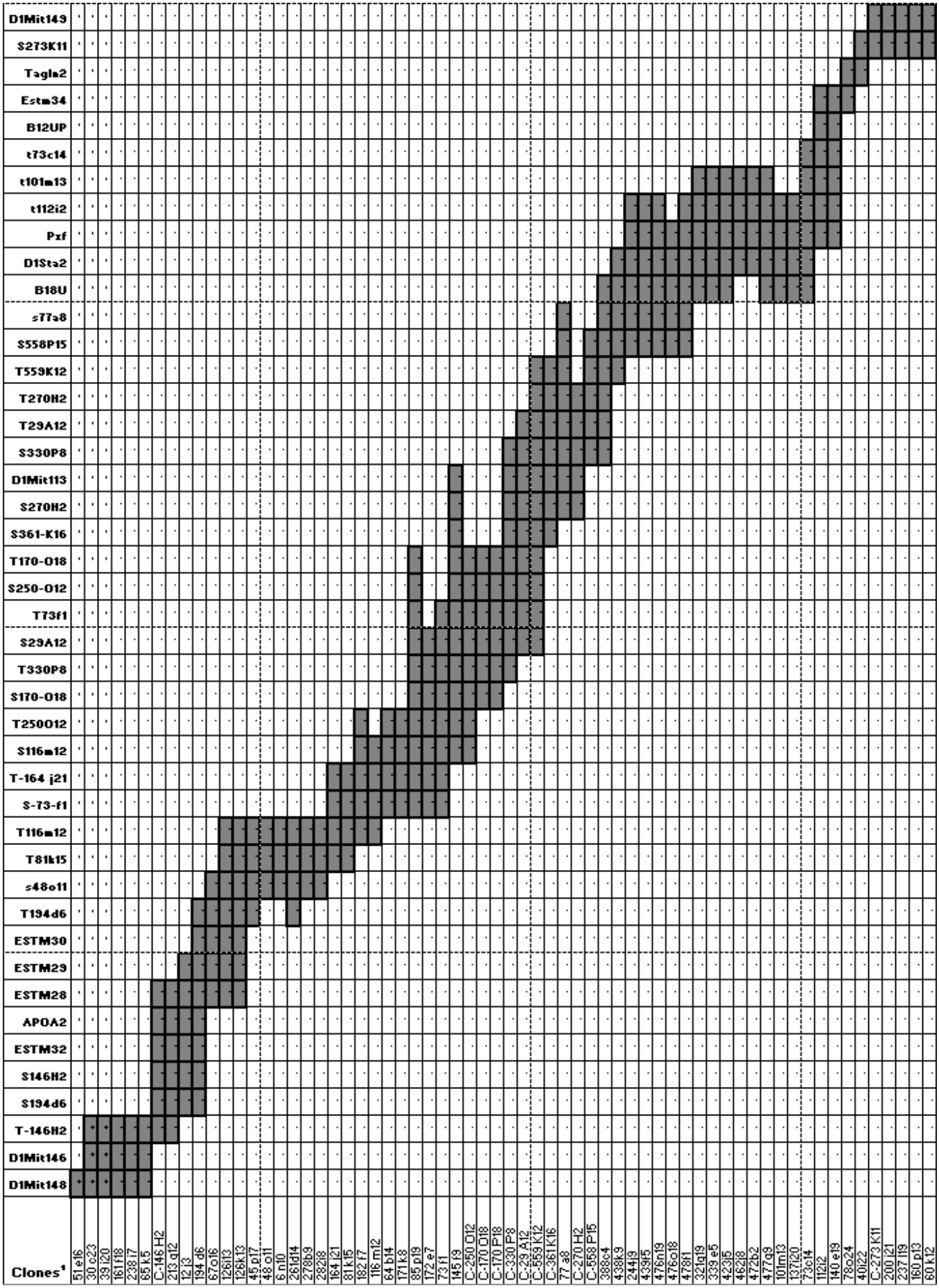

D1Mit149
S273K11
Tagla2
Estm34
B12UP
t73c14
t101m13
t112i2
Pxf
D1Sta2
B18U
s77a8
S558P15
T559K12
T270H2
T29A12
S330P8
D1Mit113
S270H2
S361-K16
T170-O18
S250-O12
T73f1
S29A12
T330P8
S170-O18
T250O12
S116m12
T-164 j21
S-73-f1
T116m12
T81k15
s48o11
T194d6
ESTM30
ESTM29
ESTM28
APOA2
ESTM32
S146H2
S194d6
T-146H2
D1Mit146
D1Mit148
Clones[1]
51e16
30c23
39i20
161f18
238i7
65k5
C-146 H2
213q12
12i3
194 d6
67o16
126l13
126k13
45p17
48 o11
6 n10
261d14
278b9
282i8
164 j21
81k15
182 f7
116 m12
64 b14
171k8
85 p19
172 e7
73 f1
145 f9
C-250 O12
C-170 O18
C-170 P18
C-330 P8
C-29 A12
C-553 K12
C-361 K16
77 a8
C-270 H2
C-558 P15
388c4
438k9
244i9
439f5
476n19
476o18
478f1
321q19
339 e5
423i5
462i8
472b2
477q9
101m13
137i20
73c14
112i2
140 e19
78o24
40i22
C-273 K11
200 i21
237 l19
160 p13
60 k12

Figure 1. Completed BAC contig spanning the *Sle1b* congenic interval

Polymorphic markers, including microsatellite markers, known genes, as well as ESTs, that were used to orient the BAC clones are listed from left to right, and the BAC clones are listed from top to bottom. The entire BAC contig is comprised of 100 BAC clones that were taken from both the RPCI-23 and CalTech library. Highlighted boxes represent the markers for that an individual clone was positive for by PCR. The B6 allelic version of the *D1Mit148* and *D1Mit149* markers at the proximal and distal ends, respectively, define the minimal *Sle1b* interval. *D1Mit113* is the original peak linkage marker identified for *Sle1b*.

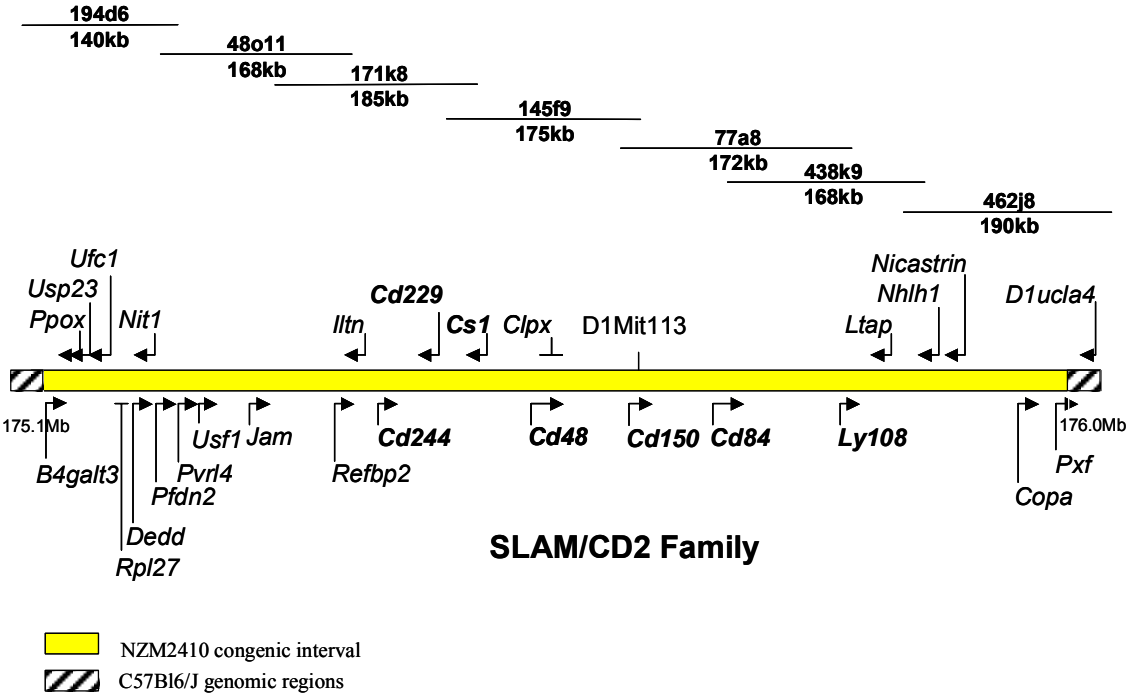
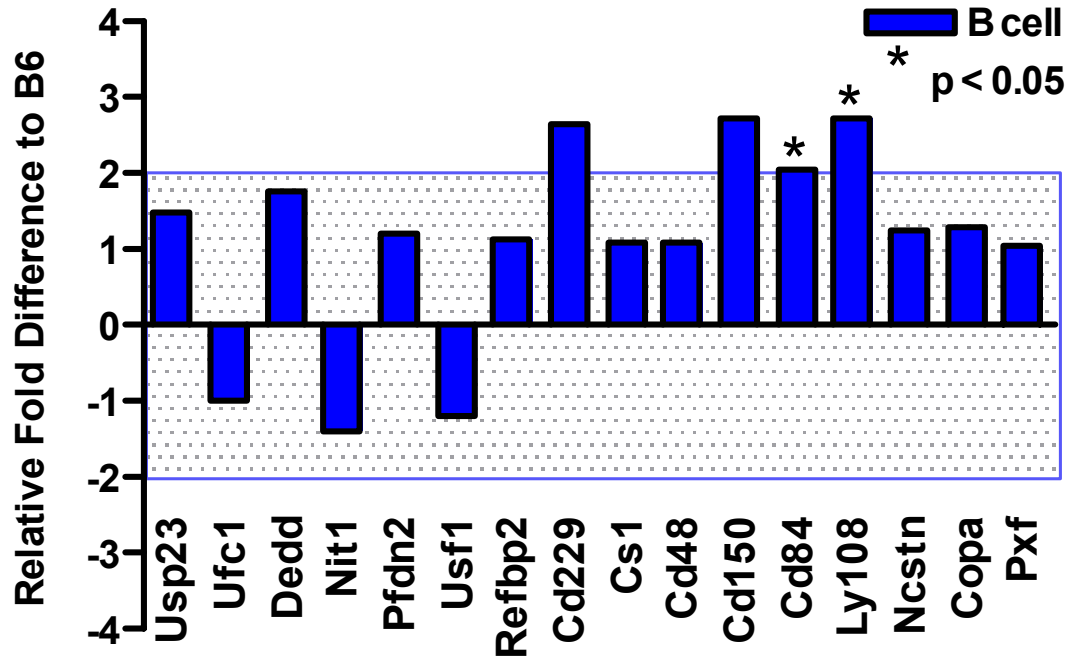


Figure 2. The genetic map of the Sle1b interval. The numbered lines above the map represent the tiling pathway comprised of B6-derived BACs. The gray bar represents the *Sle1b* region as defined by congenic breakpoint mapping. The hatched bar is the area outside of *Sle1b* and is the C57Bl/67 genome. The arrows denote transcriptional direction. Genes bolded belong to the Slam/CD2 family.


B cell
p < 0.05
Relative Fold Difference to B6
4
3
2
1
0
-1
-2
-3
-4
*
*
*
Usp23
Ufc1
Dedd
Nit1
Pfdn2
Usf1
Refbp2
Cd229
Cs1
Cd48
Cd150
Cd84
Ly108
Ncstn
Copa
Pxf

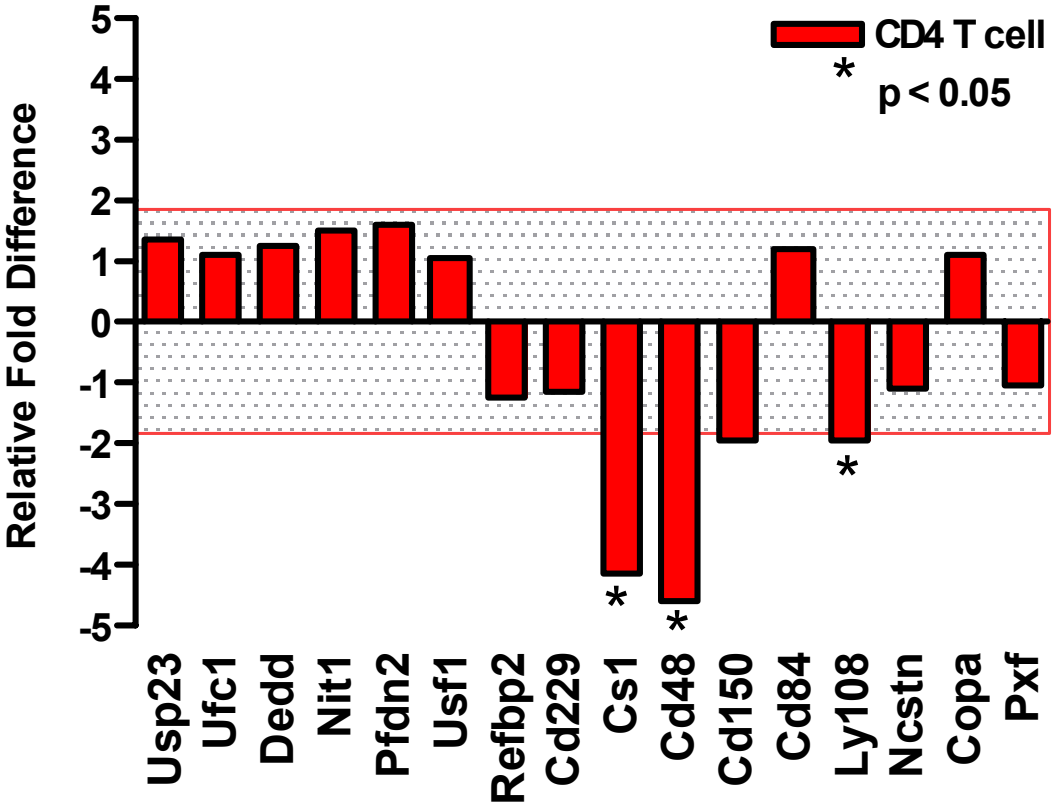
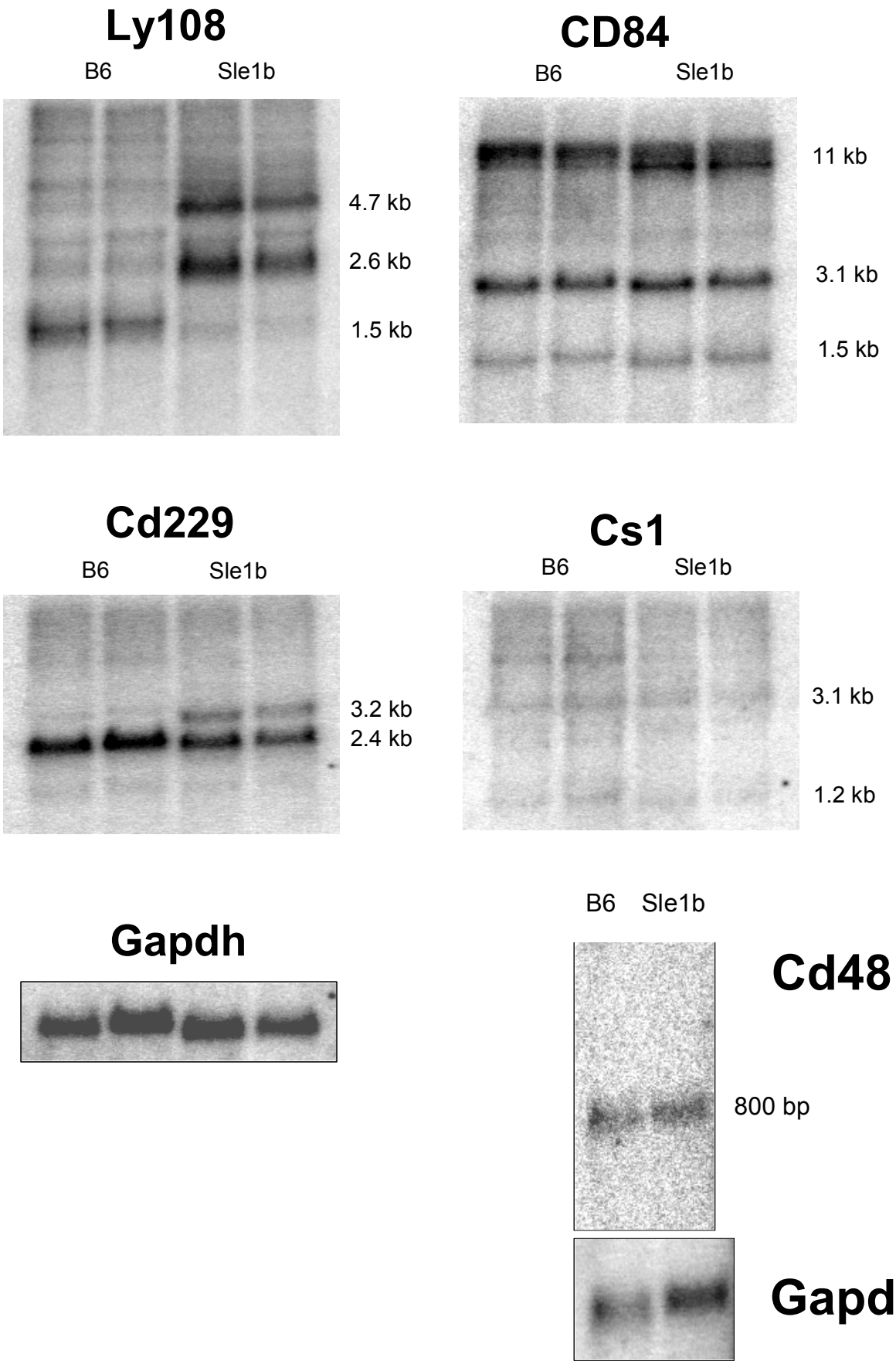

CD4 T cell
p < 0.05
Relative Fold Difference
5
4
3
2
1
0
-1
-2
-3
-4
-5
*
*
*
Usp23
Ufc1
Dedd
Nit1
Pfdn2
Usf1
Refbp2
Cd229
Cs1
Cd48
Cd150
Cd84
Ly108
Ncstn
Copa
Pxf

Figure 3. Real-time quantitative PCR expression data of candidate genes. **A.** B220 B cells and **B.** CD4 T cells from in 6-8 weeks old B6 and B6.*Sle1b* females (n=5-7) were assessed for gene expression. Data is represented as a relative fold difference to B6. The box represents a 2-fold limit of detection that was used as a cut-off for identifying candidates.


Ly108
B6
Sle1b
4.7 kb
2.6 kb
1.5 kb
CD84
B6
Sle1b
11 kb
3.1 kb
1.5 kb
Cd229
B6
Sle1b
3.2 kb
2.4 kb
Cs1
B6
Sle1b
3.1 kb
1.2 kb
Gapdh
B6
Sle1b
Cd48
800 bp
Gapdh

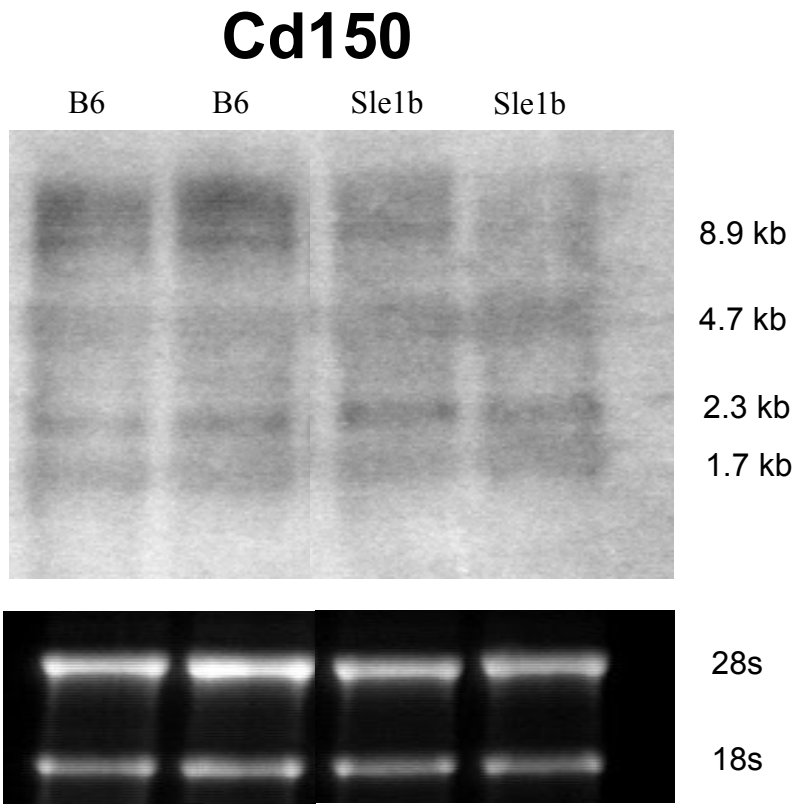


Figure 4. Northern blot analysis of SLAM/CD2 family members. Total spleen RNA from 2-3 months-old B6 and Sle1b females was resolved on a 1% denaturing formaldehyde gel. Hybridization probes were designed from cDNA sequences and are listed in Table 1.

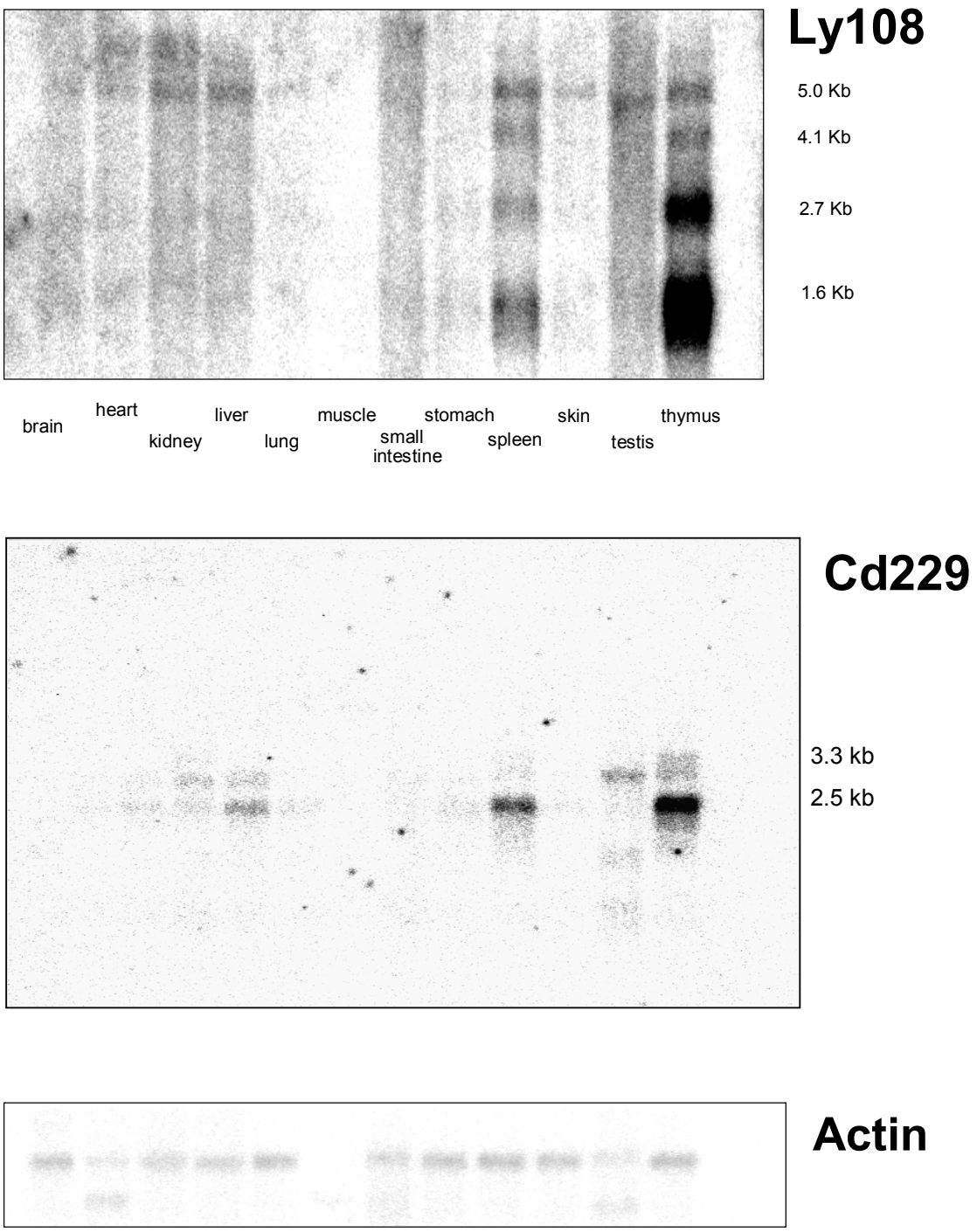


Figure 5. Tissue distribution of Ly108 and Cd229. A commercial tissue blot was used to assess expression distribution. *Ly108* and *Cd229* are strongly expressed in the thymus and the spleen.

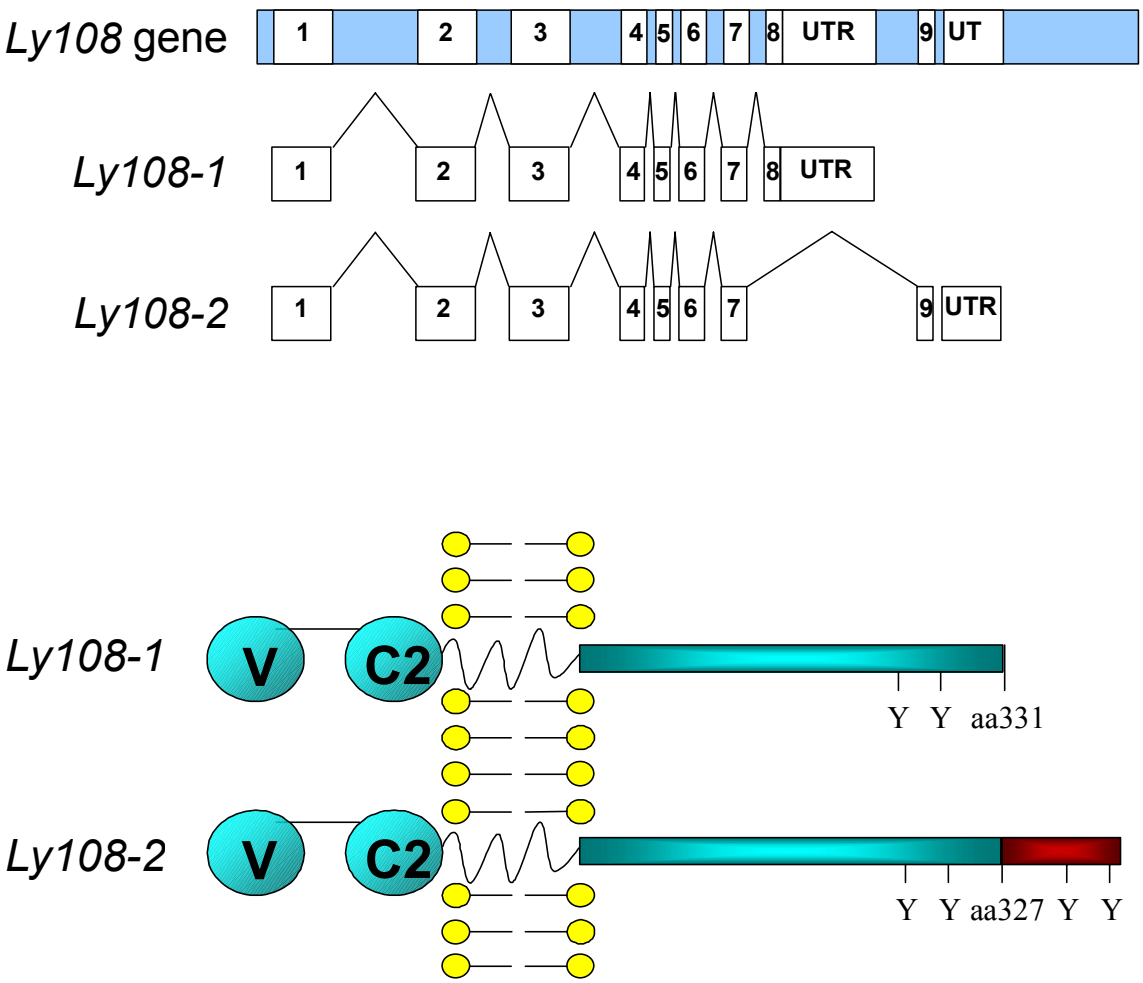


Figure 6A,6B. Ly108 splice variants. A. Exonic information of the Ly108 isoforms. Numbered boxes represent exon, and shaded boxes represent introns. *Ly108-1* and *Ly108-2* isoforms share the first seven exons and differ in the use of terminal exons. **B.** Protein structure of the individual isoforms. Both isoforms are the same until amino acid 327, where the isoforms diverge. "Y" represents tyrosine amino acids.

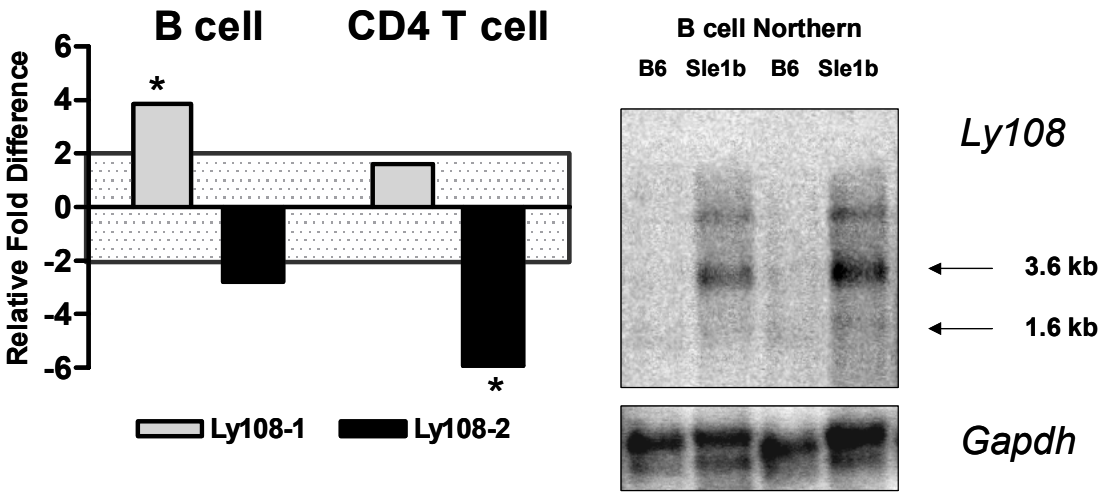
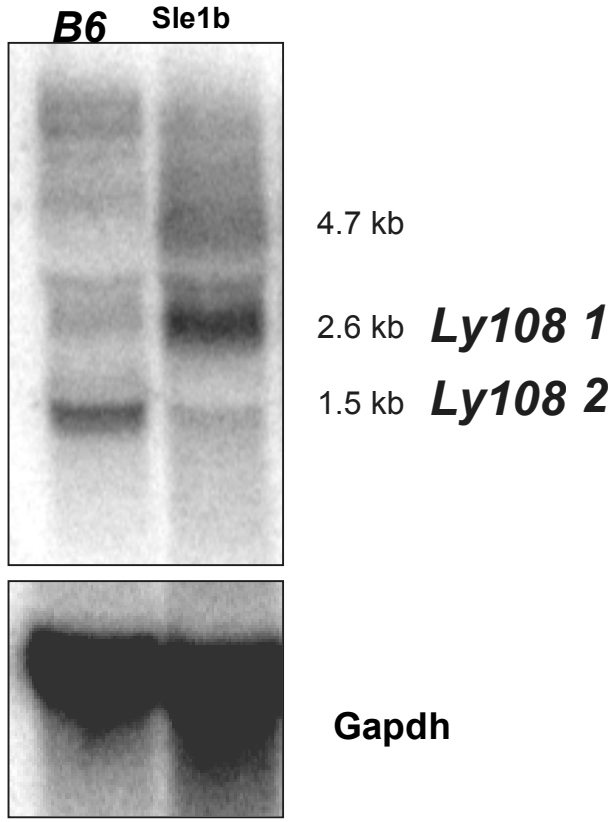


Figure 6C. Ly108 splice variants. Real-time quantitative RT-PCR was performed on B and CD4 T cell RNA to assess for isoform expression. B6 was set to 1, and B6.*Sle1b* was plotted as a fold difference to B6. The hatched box represents the 2-fold difference that was used as a minimal cut-off. Total B cell RNA was resolved on a 1% denaturing formaldehyde gel and probed with Ly108.


B6
Sle1b
4.7 kb
2.6 kb
Ly108 1
1.5 kb
Ly108 2
Gapdh

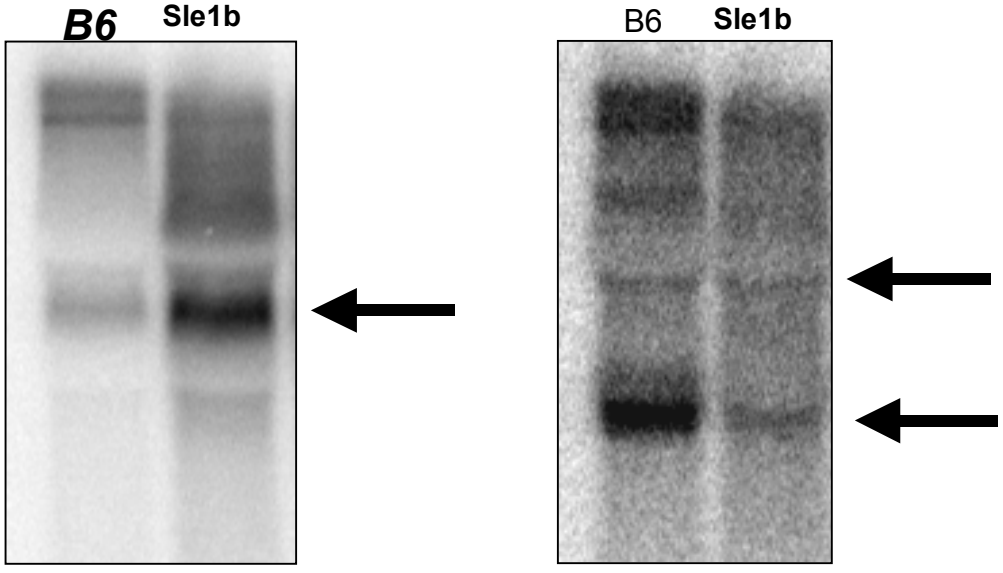

B6
Sle1b
B6
Sle1b
Ly108-1
probe
Ly108-2
probe

Figure 6D. Ly108 splice variants. Northern blot was first performed with the original *Ly108* probe that detects both isoforms. The membrane was stripped and rehybridized with isoform-specific probes. Arrows denote strong bands that are evident in original blot.

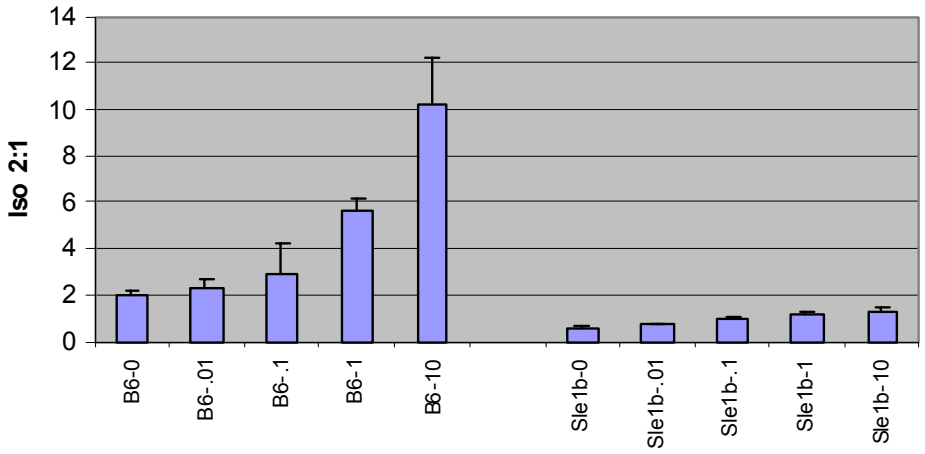


Figure 7A. Ly108-2 : Ly108-1 ratio is augmented in B6 splenocytes upon increased doses of LPS. LPS from 0 to 10ug/ml was used to stimulate for 48 hrs. Real-time RT-PCR was performed to assess for isoform expression. Data is plotted as a ratio of the two isoforms.

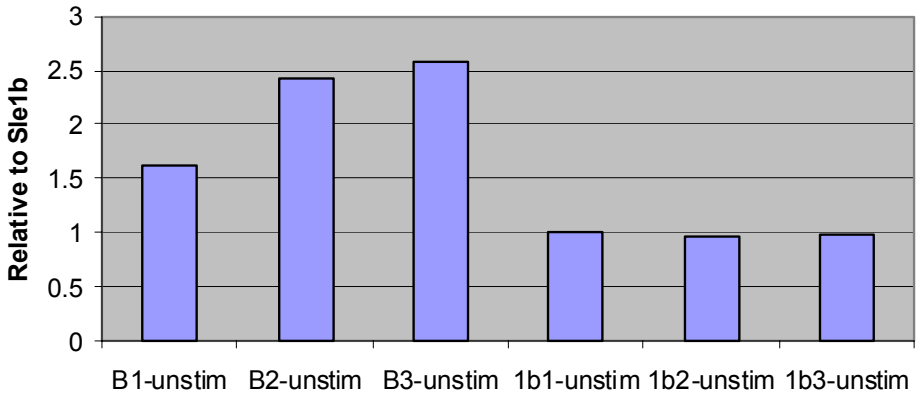


Figure 7B. Basal levels of Ly108-2 in unstimulated splenocytes from B6 and B6.Sle1b. Real-time RT-PCR was performed. *Sle1b* was set to 1, and B6 data was plotted as a fold difference to B6

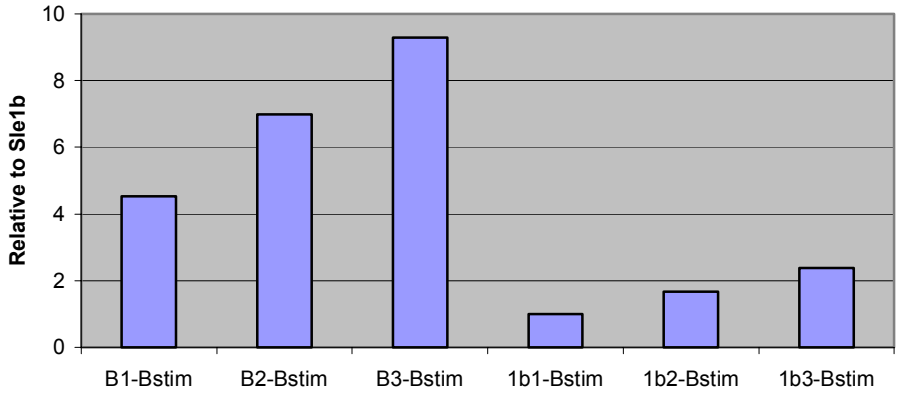


Figure 7C. Levels of Ly108-2 is up-regulated in B6 splenocytes activated with anti-IgM. Splenocytes were activated with 10 ug/ml anti-IgM for 72 hrs. Data is presented as a fold difference relative to *Sle1b*.

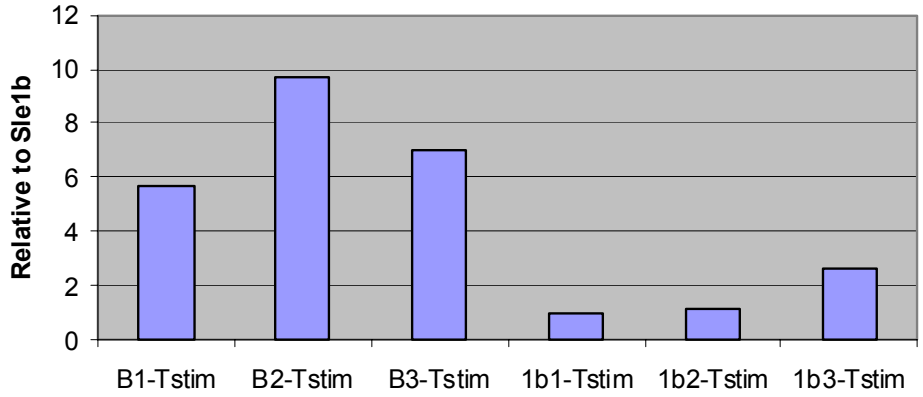


Figure 7D. Levels of Ly108-2 is up-regulated in B6 splenocytes activated with anti-CD3. Splenocytes were activated with 0.2 ug/ml anti-CD3 for 48 hrs. Data is presented as a fold difference relative to *Sle1b*.

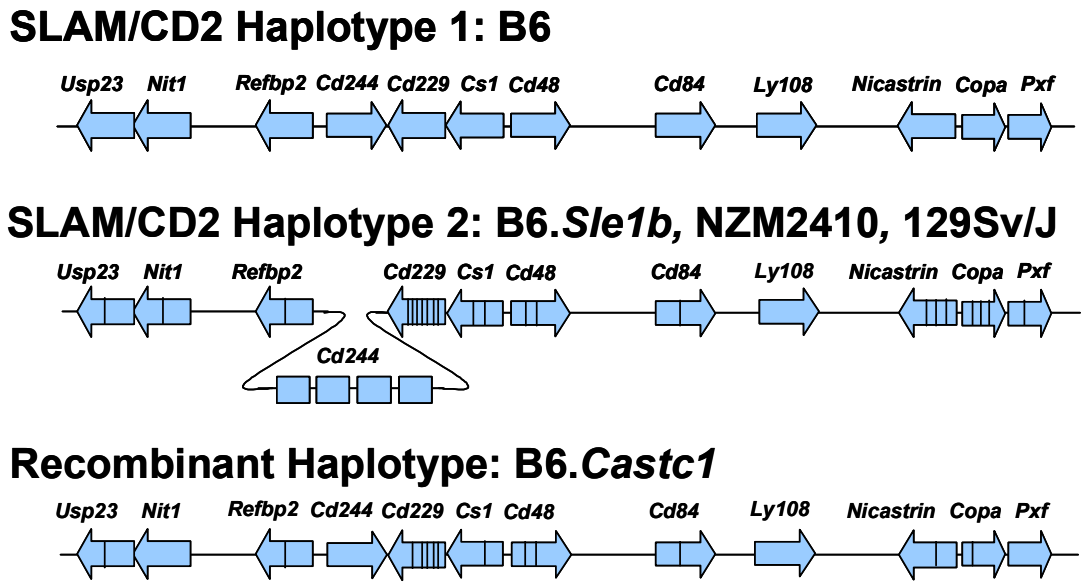


Figure 8A. Haplotype analysis of the Sle1b interval. Sequence analysis of the Slam/CD2 family demonstrated the presence of two major haplotypes. The first haplotype is shared by B6 and other B6-like strains of mice. The second haplotype is the more common of the two when a panel of inbred strains of mice were examined. Hatched marks represent SNPs that distinguish between the two haplotypes. *Cd244* shows a gene expansion in the haplotype 2. B6.*Castc1* is unique in that it is characterized by a recombinant haplotype because it has parts of haplotype 1 and haplotype 2.

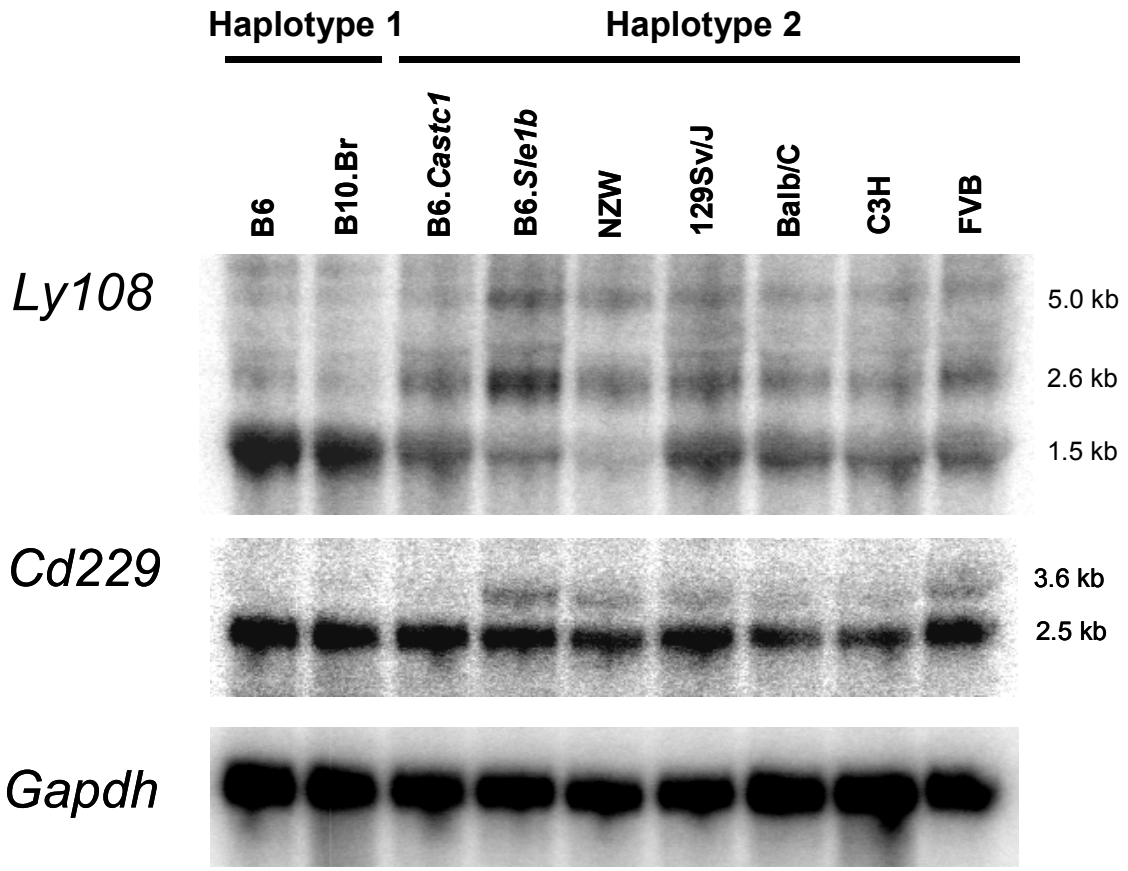


Figure 8B. Ly108 and Cd229 expression correlates with haplotype. Total splenic RNA was harvested from mice that represent the two Slam/CD2 haplotypes and was resolved on a 1% denaturing formaldehyde gel. The expression patterns for both genes correlates with the haplotype with one exception. The expression of *Cd229* in B6.*Castc1* resembles B6. B6.*Castc1* has been demonstrated to produce ANAs. This suggests that *Cd229* may not be a mediator in the loss of tolerance in this congenic line.

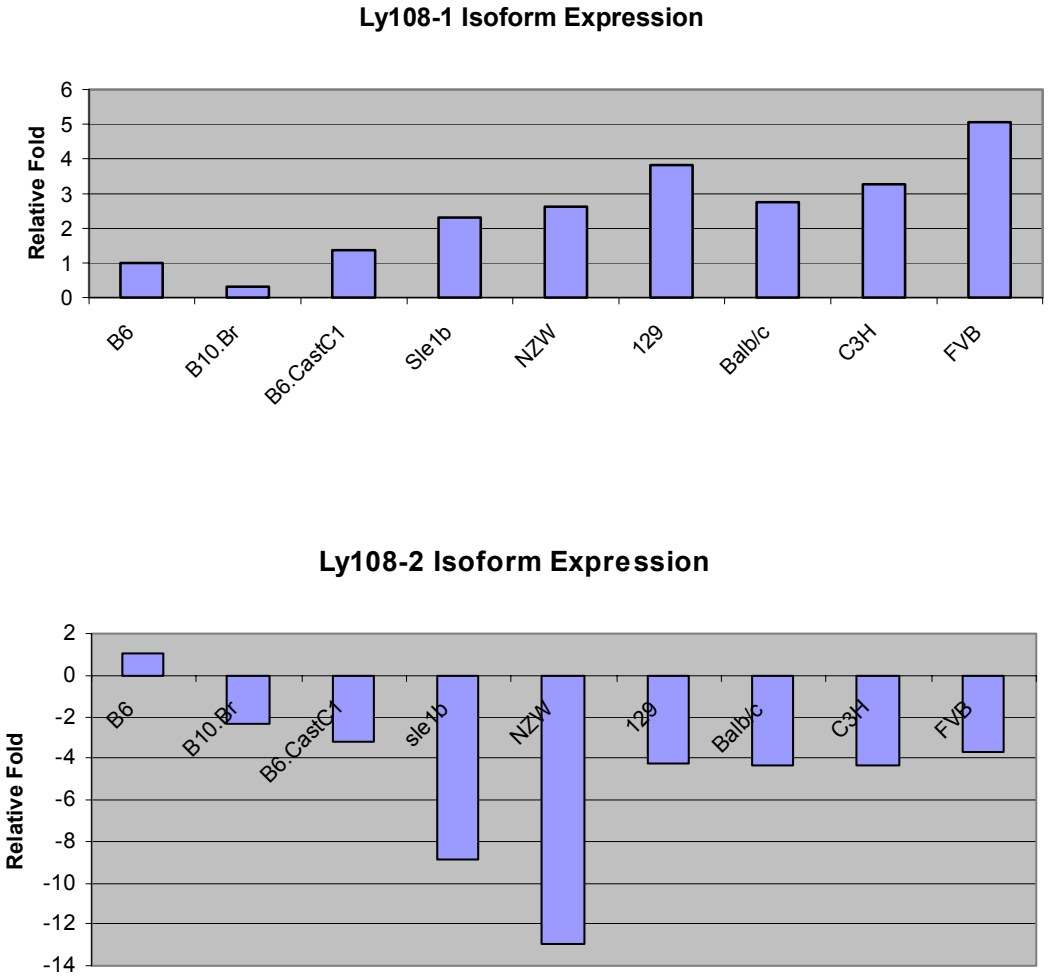


Figure 8C. Real-time quantititatve RT-PCR confirms the haplotype expression of Ly108. cDNA derived from the RNA samples used in the haplotype Northern were assessed for *Ly108* isoform expression. Data is presented as a relative fold difference to B6.

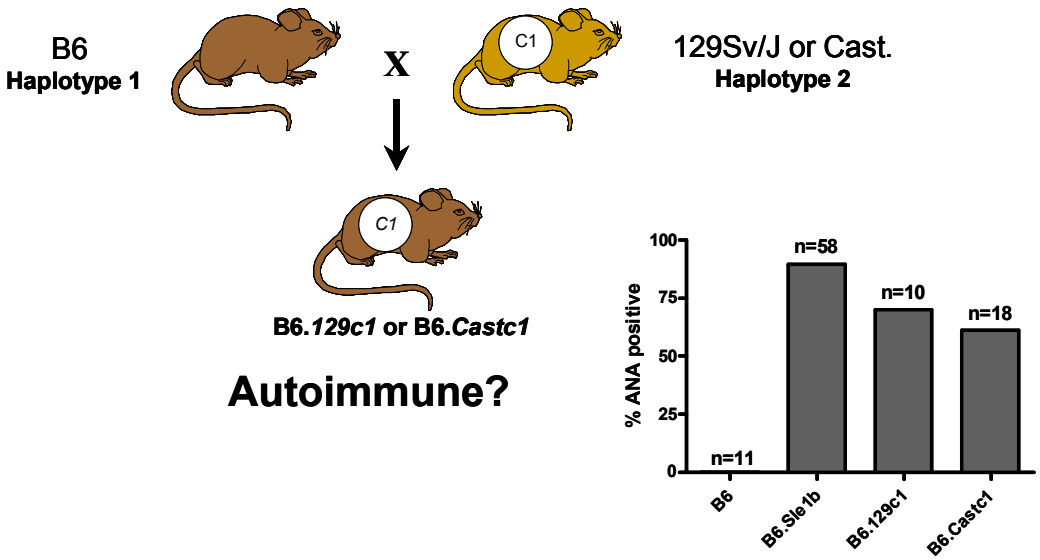


Figure 9. Moving the Sle1b interval from 129Sv/J or CAST/ei onto B6 leads to autoimmunity. Penetrance of the production of ANAs is shown in the chart. Sera from 9 months-old female mice were assayed at a 1:800 dilution for anti-total histone/dsDNA IgG by ELISA.

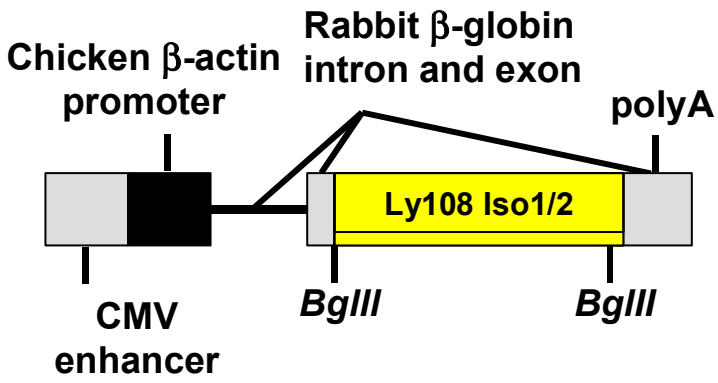


Figure 10A. Construct of Ly108 transgene. cDNA of *Ly108* isoforms were cloned into the pCAGGs vector and driven under the β actin promoter.

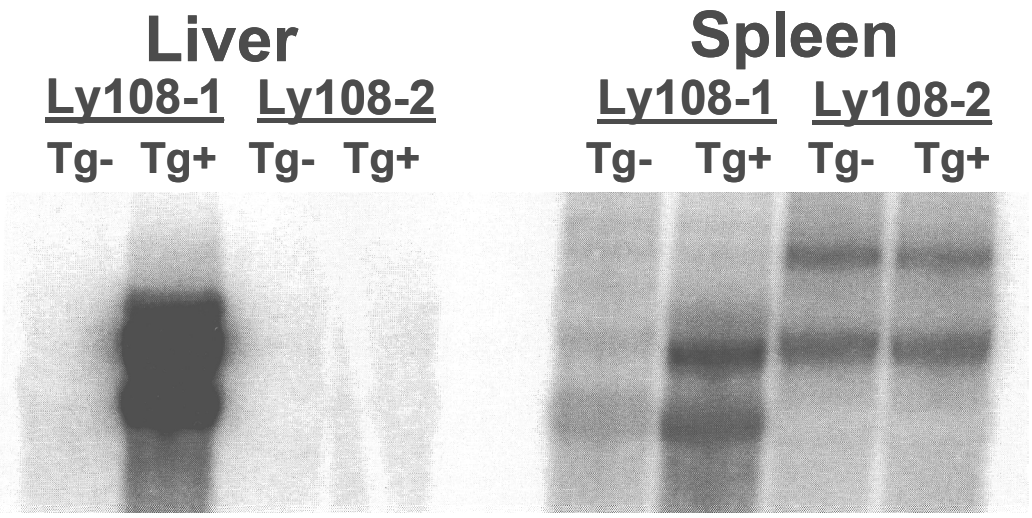


Figure 10B. Northern blot analysis of the Ly108 transgenics. Total RNA from liver and spleen were harvested from 3 months-old transgenics and negative littermates. *Ly108-1* transgenics are the B6 background. *Ly108-2* transgenics are on the B6.*Sle1b* background.

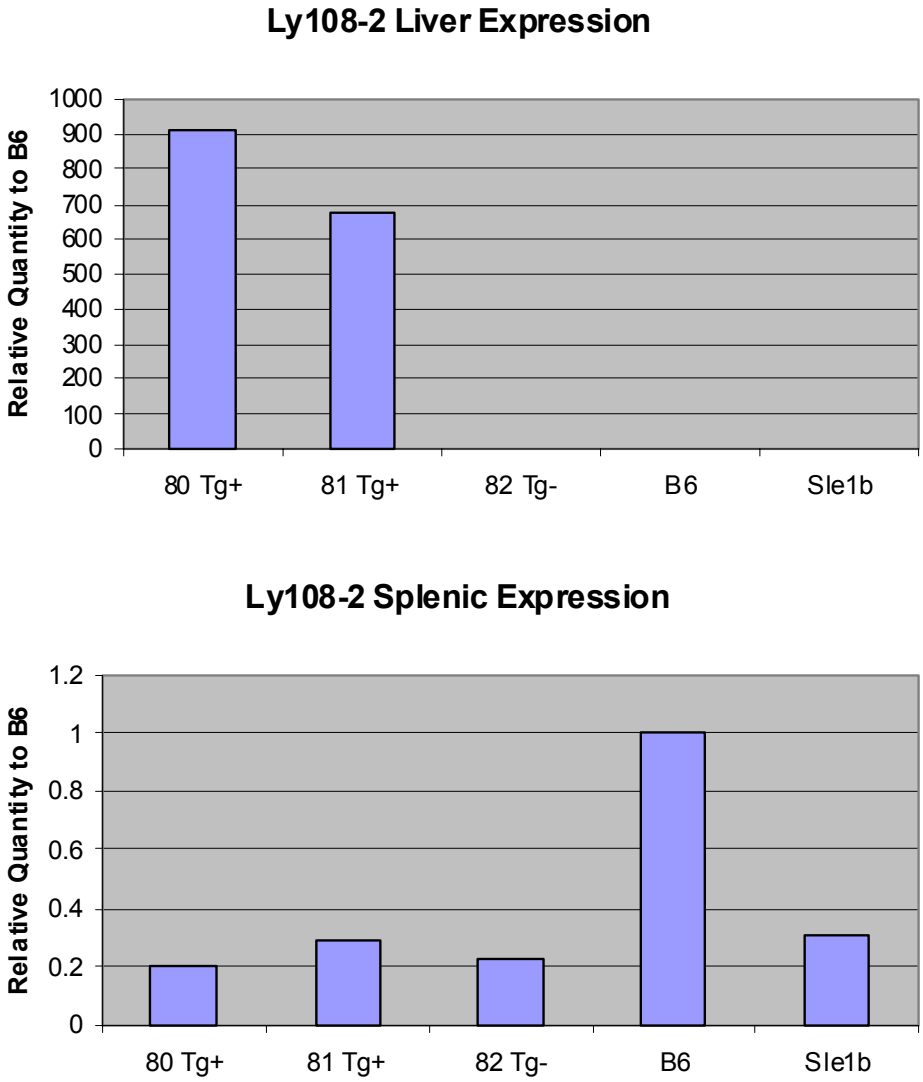


Figure 10C. Real-time quantitative RT-PCR data of Ly108-2 transgene in hepatic and splenic tissues. Mouse #80 and #81 are transgenic for *Ly108-2* and are on the B6.*Sle1b* background. Mouse #82 is a negative littermate. Expression levels for the transgenic are similar to B6.*Sle1b.*

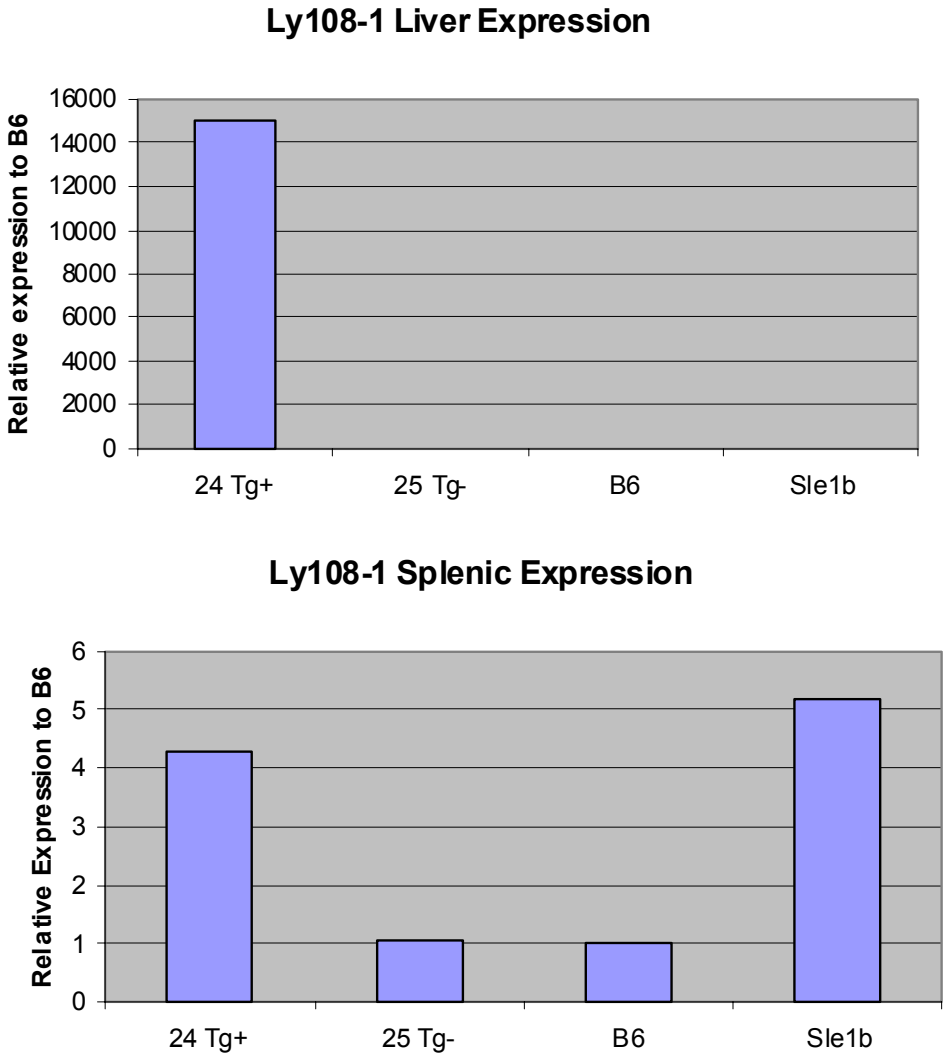


Figure 10D. Real-time quantitative RT-PCR data of Ly108-1 transgene in hepatic and splenic tissues. Mouse #24 is a positive transgenic and shows very high levels of *Ly108-1* expression in the liver. The transgenic line is on a B6 background, and the levels of expression in the spleen is higher than B6, but not that of B6.*Sle1b* .

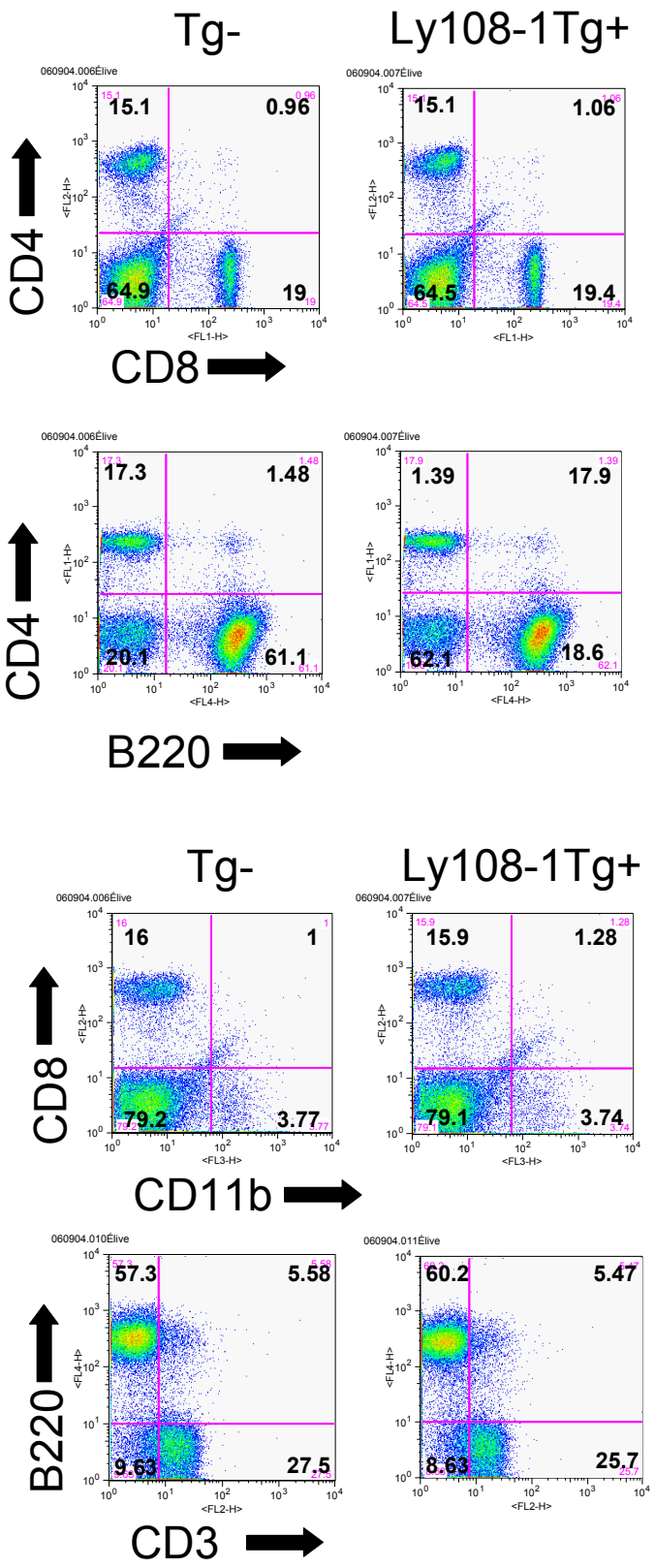


Figure 10E. Normal lymphocytic development in Ly108-1 transgenic mice.

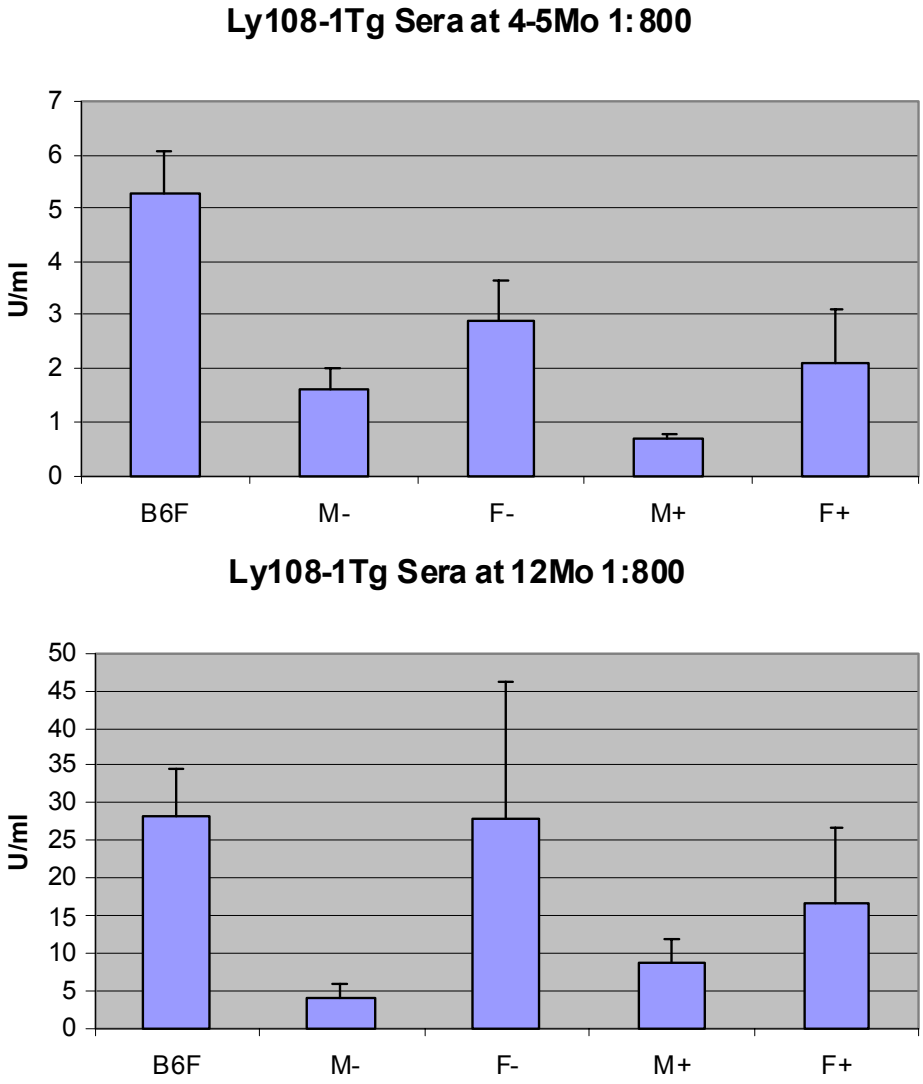


Figure 10F. ANA analysis of Ly108-1 transgenic. Sera at 1:800 dilution from male and female transgenics that were 4 or 12 months-old mice were assayed for anti-total histone/dsDNA IgG antibodies and compared to female B6 of similar age. The Ly108-1 transgenics do not exhibit autoimmunity. Interestingly, there appears to be a gender bias in the levels of antibody.

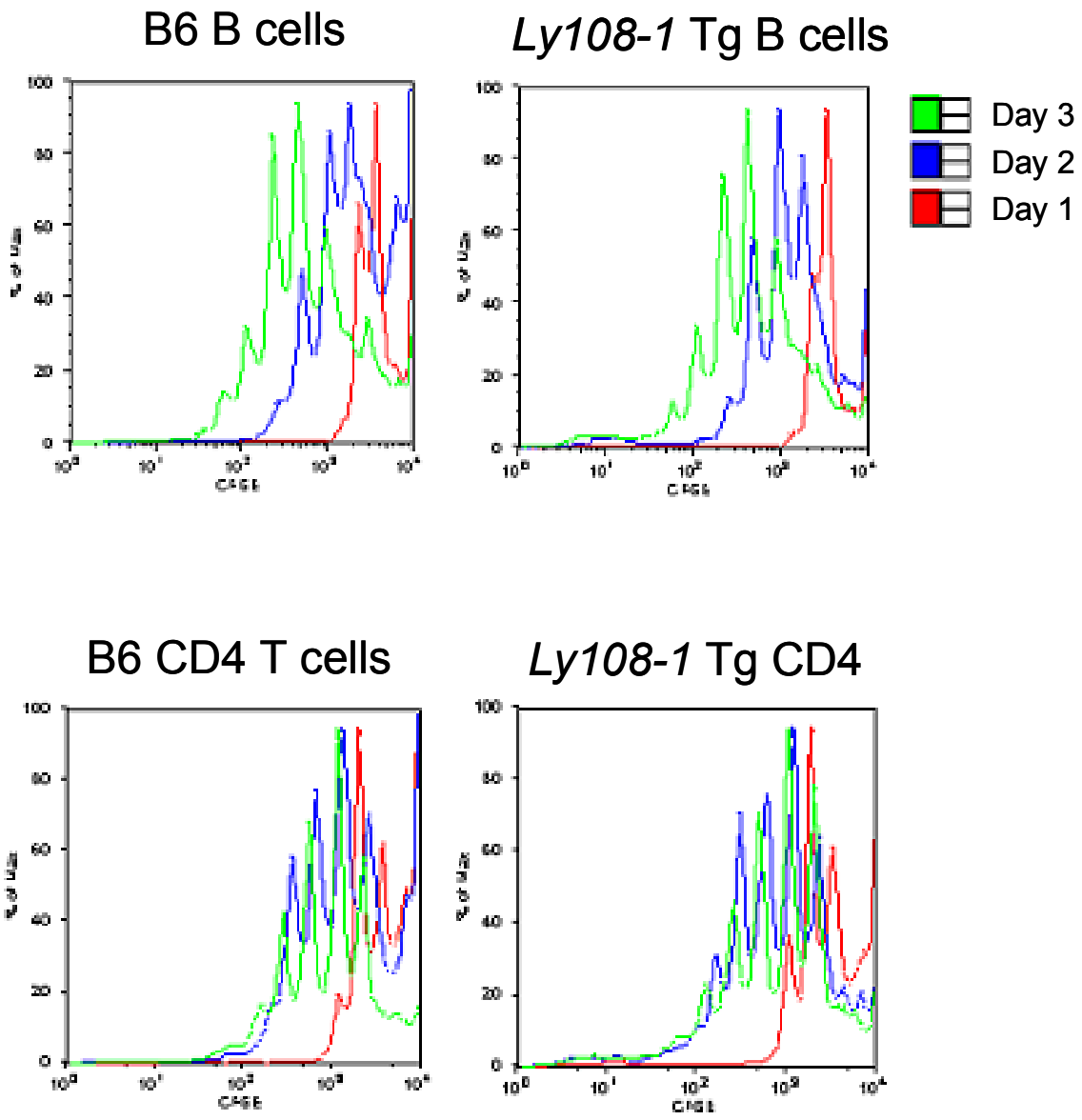


Figure 10G. CFSE analysis of Ly108-1 transgenics. No difference was detected in the proliferative capacity in B and T cell compartments of Ly108-1 transgenic mice. Splenocytes from B6 and *Ly108-1* transgenics were stimulated with 10 ug/ml anti-IgM or 0.2 ug/ml anti-CD3.

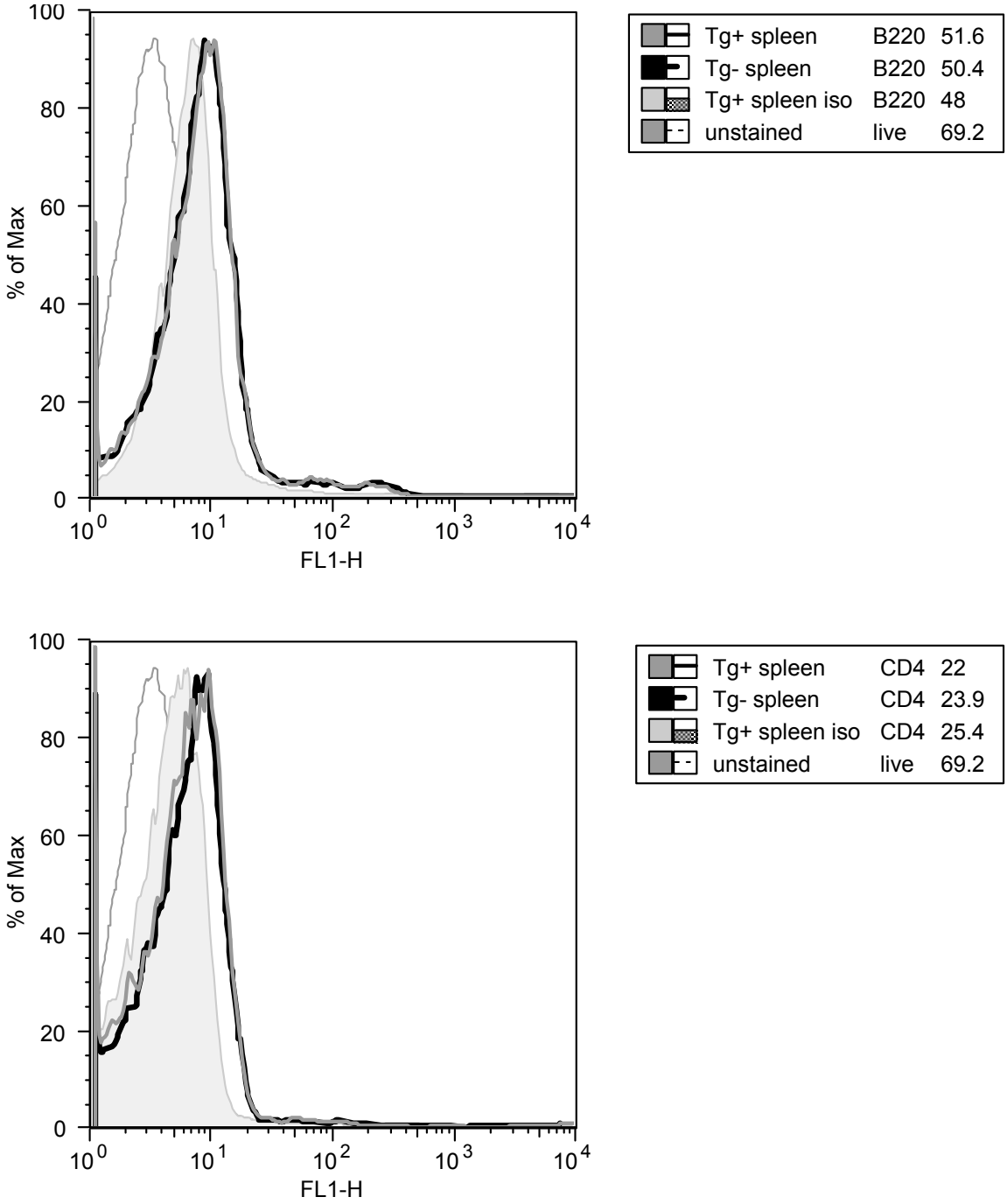


Figure 10H. Surface staining of Ly108 in *Ly108-1* transgenic mice in splenic B220 B and CD4 T cell populations.

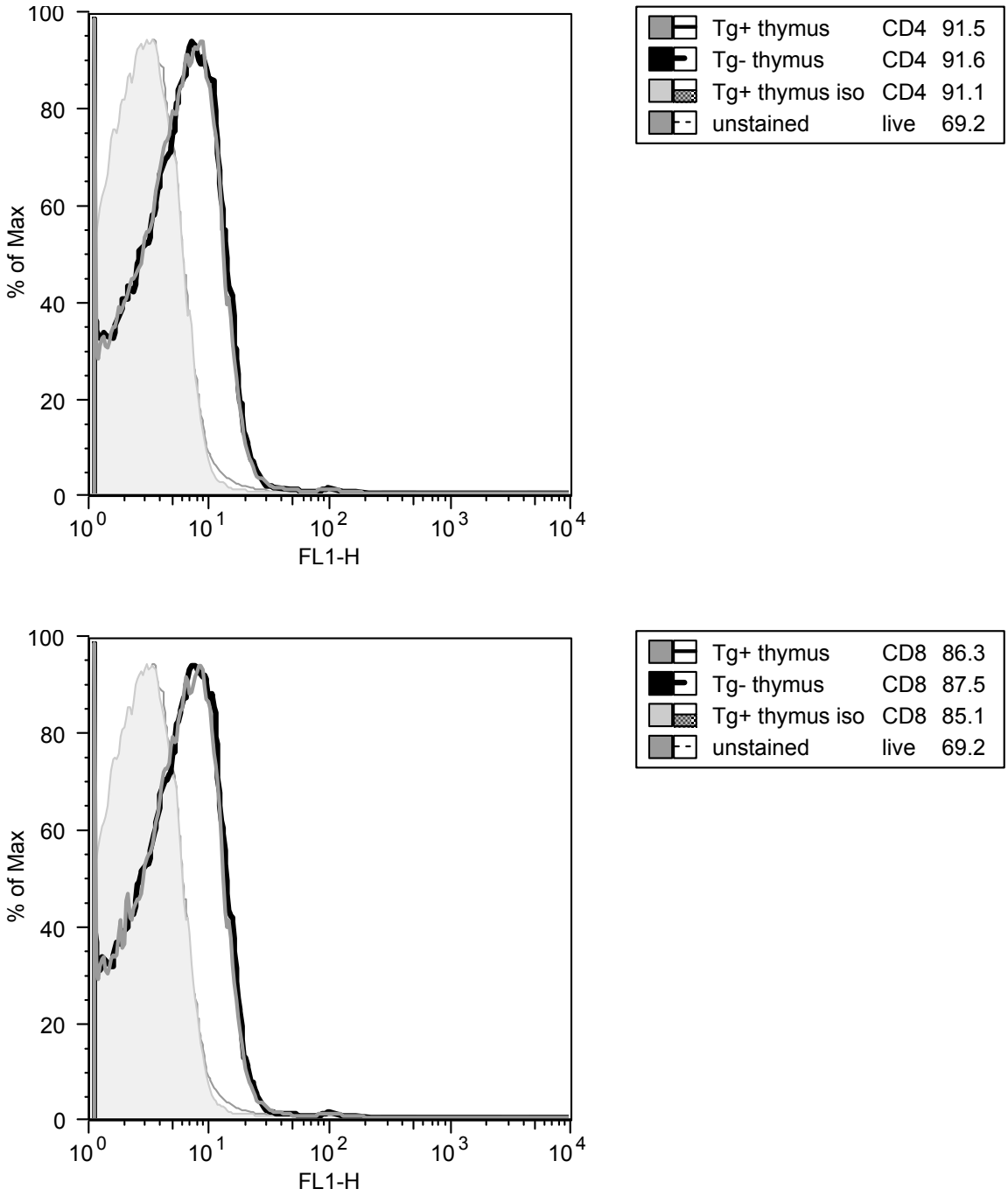


Figure 10l. Surface staining of Ly108 in *Ly108-1* transgenic mice on thymic CD4 and CD8 T cell populations.

Chapter 4. Functional Polymorphisms Associated with SLAM Haplotype 2 on the C57Bl/6 Background

Introduction

The B6.*Sle1b* congenic mouse carries a 900kb interval on chromosome 1 that is derived from the NZM2410 lupus mouse. *Sle1b* mediates a loss of tolerance to chromatin, and B6.*Sle1b* produces high titers of anti-nuclear autoantibodies but exhibits no evidence of lupus nephritis (236). The *Sle1b* locus is a 900 kb interval that is located on the telomeric end of mouse chromosome 1. In order to find the causative gene that is responsible for the *Sle1b* autoimmune phenotype, our lab has constructed a BAC contig that spans the interval. Sequence analysis has revealed a gene-dense region consisting of 26 genes. We also discovered a cluster of genes known as the Slam/CD2 family that lies within the interval. Extensive sequence analyses and expression studies has demonstrated this cluster as the strongest candidates for mediating the *Sle1b* phenotype.

Although we have not identified the causative gene for *Sle1b*, we have undertaken several studies to better understand the possible mechanisms by which this family of receptors leads to the loss of tolerance in the B6.*Sle1b* model. In this chapter, we explore the proliferative capacity of *Sle1b* lymphocytes as we hypothesize that the Slam/CD2 family leads to a hyperactive phenotype. We also discuss our findings in calcium mobilization in *Sle1b* T cells as well as an altered cytokine production upon T cell activation. How these

aberrant responses contribute to a loss of tolerance remains unclear, but it does provide us some insight as to how this gene family contributes to autoimmunity.

B6.*Sle1b* does not exhibit a hyperproliferative response in the B and T cell compartment upon antigen stimulation

Studies have shown that hyperproliferation of B and T cells are a major contributor to the pathogenesis of murine lupus (237,238). Dr. Limaye has previously demonstrated that the B6.*Sle1b* mouse exhibits splenomegaly even at an early age less than 3 months-old, well prior to the production of ANAs (Figure 11a). This enlargement of the spleen is maintained as the mice age and become seropositive. However, FACS analysis of the splenic B and T cell populations performed by S. Subramanian in the lab revealed no significant differences in the overall percentages (Figure 11b). We hypothesized that *Sle1b* may impact proliferative responses to stimulus, so proliferation was assessed by CFSE and BrdU labeling. When splenocytes from 2 months-old female B6 and B6.*Sle1b* were compared three days after the addition of B or T cell stimuli, the data indicates that there is no difference in both the proliferative capacity and kinetics of B6.*Sle1b* lymphocytes (Figure 12a-c). Because there were no gross differences, we performed cell cycle analysis with BrdU labeling and reached similar conclusions as there were no detectable differences in the G0/G1, G2, and S phase between B6 and B6.*Sle1b* (Figure 13). Interestingly enough, when 12 months-old female B6 and B6.*Sle1b* mice were examined, the *Sle1b* mice showed a remarkable decrease in proliferative response by CFSE (Figure 14).

Hypoproliferative responses have been observed in human SLE where chronic activation of autoreactive T cells leads to an "exhausted phenotype" (239,240). Such a description may be apt for the T and B cells in B6.*Sle1b* because they start to produce ANAs by the early age of 5-6 months (241).

B6.*Sle1b* T cells but not B cells exhibit an altered calcium response profile

After concluding that the proliferative capacity was similar between the two strains of mice, we decided to assess for any differences in the signaling patterns induced by antigen receptor stimulation. Altered signaling events have been shown to be abnormal, particularly calcium flux in B and T cells from SLE patients (242,243). Figure 15 outlines the strategy that was used to examine calcium mobilization. Splenocytes from young female B6 and B6.*Sle1b* mice were enriched for B or T cells via magnetic beads and subsequently labeled with Fluo-3. When B cells were stimulated with $F(ab')_2$ anti-IgM, no differences in calcium signaling was observed between the two strains of mice (Figure 16). To address the possibility of suboptimal cross-linking of the BCR, biotinylated $F(ab')_2$ anti-IgM was also used followed by the addition of streptavidin; however, no conclusive results were gained. T cells were stimulated with a cocktail containing anti-TCRβ, anti-CD4, and anti-CD8 antibodies. A subtle yet consistent delay in the calcium response was observed (Figure 17). When mean fluorescence was expressed as calcium concentration as a function of time, it became apparent that T cells from B6.*Sle1b* mice showed a slower return to baseline than B6 (Figure 17).

This led us to ask if this phenotype is a unique property to the B6.*Sle1b* congenic. Is the altered calcium flux a manifestation of moving the NZW-derived *Sle1b* interval onto the B6 background, and if so, would moving genomic intervals from other mice that share the same haplotype onto B6 yield a similar effect? As mentioned in Chapter 3, Dr. Limaye's work demonstrated that by moving the *Sle1b* locus from either 129Sv/J or CAST/Ei mice onto B6, the resultant B6.129c1 and B6.Castc1 congenic mice break tolerance to chromatin and produce high titers of ANAs (Figure 9). We then postulated that these congenics would also exhibit a prolonged calcium signaling response. T cells from NZW were first examined to address whether or not the parental strain from which the *Sle1b* interval is derived shows this response, and they were found to behave similarly to *Sle1b* (Figure 18). Remarkably, the T cells from B6.129c1 and B6.Castc1 congenics also displayed the altered calcium flux when stimulated with the antibody cocktail (Figure 19).

The Slam haplotype 2 on the B6 background leads to an altered cytokine profile

Clearly, cytokine milieu plays an important role in autoimmunity. Numerous studies have characterized the NZM2410 strain, and Mohan et al. has characterized cytokine production the B6.*Sle1* mouse (27,244). . However, the findings are not consistent. In an attempt to find a mechanism by which the B6.*Sle1b* mouse becomes autoimmune, cytokine profiling was undertaken. The strategy for the cytokine experiments is shown in Figure 20. To measure

responses to primary stimulation, CD4 T cells purified by negative selection were cultured with anti-CD3ε in the presence or absence of costimulatory anti-CD28 antibody. A fraction of the cells were allowed to rest in media alone with IL-2 for seven days, and then subjected to secondary stimulation to assess memory response. Cells were stimulated for 24, 48, and 72 hours, and harvested. RNA was isolated and measured for cytokine transcript levels by real-time PCR, and the supernatants were collected for cytokine ELISA analysis.

Multiple studies involving knockouts have implicated SAP with IL-4 and IFN-gamma production (245,246). Thus, IL-4 was first measured in primary stimulated CD4 T cells from B6 and B6.*Sle1b*. B6.*Sle1b* mice appeared to have a diminished IL-4 response, tending towards a lower expression level of IL-4 message and protein (Figure 21a-b). This trend was also observed when T cells received secondary stimulation (Figure 21c-d). What is unusual is that T cells from both B6 and B6.*Sle1b* mice that were initially stimulated with anti-CD3e alone were able to respond when subjected to secondary stimulus. In the absence of costimulation, these cells were expected to anergize (247,248) (Figure 21c-d). It is possible that the cultured T cells were able to provide costimulation to one another when plated in round-bottom wells. When IFN-gamma was measured by real-time PCR, we were surprised to find that primary-stimulated T cells from B6.*Sle1b* exhibited a lowered response (Figure 22a). ELISA data on IFN-gamma levels in the supernatants on the other hand have not been consistent as it is barely detectable, even at 72 hrs (Figure 22b). However, when CD4 T cells under secondary stimulation were examined, interferon-

gamma transcript was higher in B6.*Sle1b* than in B6 (Figure 22c), but again, no differences were detected by ELISA (Figure 22d).

To further explore the diminished IL-4 response in the *Sle1b* CD4 T cells, transcriptional factors that regulate T helper cell development were examined. T-bet and GATA-3 are considered the primary regulators of Th1 and Th2 development, respectively (249). When we examined CD4 T cells under primary stimulation, *Sle1b* T cells exhibited a trend for increased expression of T-bet when given anti-CD3 and anti-CD28 (Figure 23a). Furthermore, this trend was also noted in *Sle1b* T cells that were subjected to anti-CD3 secondary stimulation (Figure 23b). In spite of the increased levels of T-bet messages in *Sle1b*, no consistent difference was observed in GATA-3 levels in CD4 T cells under primary and secondary stimulation (Figure 23c, 23d) at 24 hrs. These findings do not appear to correlate with the IL-4 and IFN-gamma data; however, this may be due to the kinetics of expression for these transcription factors as it may be possible that GATA-3 expression returns to baseline at 24 hrs.

IL-10 is an immunoregulatory cytokine that has been associated with SLE. Polymorphisms in the IL-10 promoter region have been linked to lupus (250-252). When IL-10 was assayed in the primary stimulated T cells, no consistent differences were noted by both real-time PCR and ELISA (Figure 24a-b). Under secondary stimulation, there were also no significant differences seen in message or protein levels (Figure 24c-d).

TNF-a is a proinflammatory cytokine that has been associated with SLE and has been shown to be elevated in SLE patients (172,253). Primary

stimulation of CD4 T cells from B6.*Sle1b* showed no consistent difference in the level of expression from B6 T cells (Figure 25a). When T cells from B6.*Sle1b* mice received secondary stimulation, there appeared to be a significant increase in message (Figure 25b), but the ELISA failed to show a consistent difference at the protein level (Figure 25c). IL-17 is another pro-inflammatory cytokine that has been shown to be up-regulated in SLE patients (185). IL-17 showed no difference in expression in CD4 T cells that received primary stimulation (Figure 26a). However, under secondary stimulation, T cells from B6.*Sle1b* exhibited a significant reduction in message (Figure 26b). This is puzzling because we expected that *Sle1b* T cells would secrete proinflammatory cytokines.

IL-21 plays a role in B cell differentiation in to plasma cells and has been demonstrated to be elevated in the lupus-prone BXSB mice (254). IL-21 showed no consistent trend in primary stimulated T cells from B6.*Sle1b* (Figure 27a). Interestingly enough, when CD4 T cells were subjected to secondary stimulation with CD3 first, there was a trend for increased IL-21 expression in *Sle1b* T cells (Figure 27b). Conversely, when T cells from *Sle1b* were subjected to CD3 and CD28, there was a trend for decreased expression of IL-21 (Figure 27b). Neither result was statistical significance, however.

Activating CD4 T cells from B6.*Sle1b* leads to an altered expression of the Slam/CD2 family members

We finally assessed the effects of TCR stimulation on the levels of expression of the Slam/CD2 family members on purified CD4 T cells. Numerous

reports have shown that many of the members are up-regulated on T cell activation (255,256). We have already demonstrated that CD4 T cells *ex vivo* from B6.*Sle1b* mice show a difference in the level of RNA expression for a subset of the Slam/CD2 family members (Figure 3b). The question we wanted to address was what effect would TCR stimulation have on the expression of these genes. It is conceivable that the differences seen in the level of expression *ex vivo* are a result of pre-activated T cell population. However, we hypothesize that the *Sle1b* CD4 T cells would maintain these differences in expression since we believe that this phenotype is intrinsic to the T cell compartment.

Consistent with our findings *ex vivo*, *Cd48* was found to have a trend for lower expression in *Sle1b* T cells under resting conditions (Figure 28a). However, when anti-CD3 was given, *Cd48* showed a significant increase in *Sle1b* T cells. When costimulatory anti-CD28 was provided, there was also a trend for higher levels of *Cd48* expression (Figure 28a). Under secondary stimulation, *Sle1b* T cells showed a trend for decreased expression (Figure 28b).

Cd84 showed a significantly higher level of expression in *Sle1b* T cells in both non-stimulated and anti-CD3 primary stimulated conditions (Figure 29a). When the secondary response on *Cd84* expression was examined, *Sle1b* T cells also showed a significant increase under non-stimulatory conditions and a trend for increased expression with anti-CD3 treatment (Figure 29b).

Cs1 was found to have a lower level of expression in *Sle1b* T cells in both resting and anti-CD3/CD28 treated in the primary response assay (Figure 30a). This confirmed our previous finding that *Cs1* was down-regulated in *ex vivo*

Sle1b T cells (Figure 3b). When the secondary response was examined, *Cs1* showed a significant decrease in the level of expression with anti-CD3/CD28 treatment (Figure 30b).

CD229 showed an increased level of expression in *Sle1b* T cells for both primary and secondary response studies (Figure 31a-b). Primary stimulation with anti-CD3 and anti-CD28 led to a significant increase in *CD229* in *Sle1b* T cells.

Cd150 did not show any expression differences under non-stimulated conditions during the primary response. However, upon anti-CD3/CD28 treatment, we observed a significant increase in the level of *Cd150* expression in *Sle1b* T cells (Figure 32a). Furthermore, under secondary stimulation, there was also a significant increase in the message level in the *Sle1b* T cells (Figurer 32b).

It has previously been demonstrated that Ly108 is up-regulated upon T cell activation (257). We have also shown that *Sle1b* splenocytes do not up-regulate *Ly108-2* message upon stimulation to the extent of B6 (Figure 7). These experiments were repeated again in splenocytes with three B6 and three B6.*Sle1b* mice. When the ratio of the Ly108 isoforms 2:1 was compared between B6 and B6.*Sle1b*, B6 displayed a higher ratio of 2:1 upon activation (Figure 33a). We then asked if purified CD4 T cells would behave in a similar manner. We performed real-time analysis and calculated the isoform 2 to isoform 1 ratio. The data showed that the ratio increased in both B6 and *Sle1b* under primary and secondary conditions; however, the ratio in *Sle1b* T cells never reached that of B6 (Figure 33b-c). The differences in the isoform ratio in

CD4 T cells between the two strains is not as dramatic as the splenocyte data, but this is most likely due to the changes of expression in other cell types.

In summary, we still do have an answer as to how the Slam/CD2 haplotype mediates autoimmunity. We have not been able detect any significant differences in its capacity to proliferate in B and T cells. This is discouraging since the splenomegaly phenotype cannot be accounted for in these mice. We have demonstrated an altered calcium flux in the T cell compartment from B6.*Sle1b* as well as two other congenics, B6.129c1 and B6.Castc1, that carry the same Slam/CD2 haplotype as B6.*Sle1b*, but how this contributes to the autoantibody production is still unclear. In addition, a reduced IL-4 response as well as the up-regulation of certain Slam/CD2 family members appear to be the most consistent phenotype of *Sle1b* CD4 T cells that have undergone primary and secondary stimulation (summarized in Tables 2a-b). Given the role of IL-4 in maintaining peripheral tolerance (258), it is tempting to speculate that the skewed cytokine response in B6.*Sle1b* can promote an unnatural environment that is conducive to autoreactivity. However, the observed cytokine profile may have nothing to do with the loss of tolerance and may play more of a role in end-organ disease pathogenesis, which requires additional susceptibility genes as B6.*Sle1b* do not develop kidney disease.]

Figure 11A.

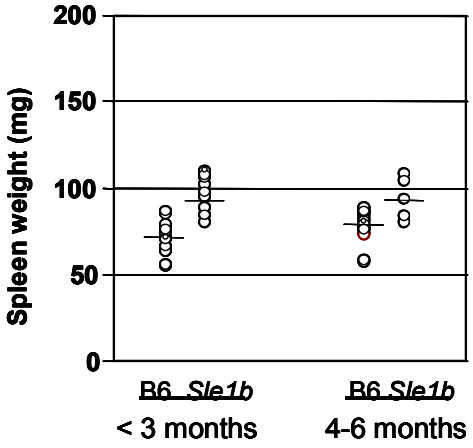


Figure 11B.

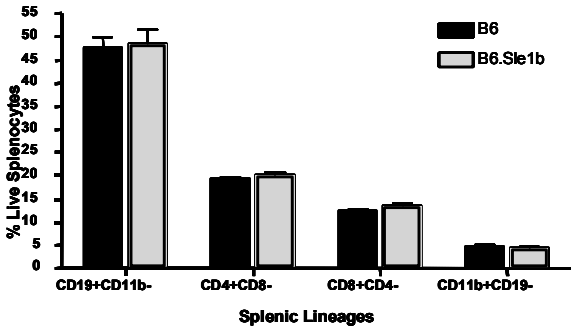


Figure 11. B6.Sle1b show signs of splenomegaly. **A.** Spleen weights of female B6 and B6.*Sle1b* mice are plotted as a function of age. **B**. FACS anslysis of CD19, CD4, CD8, and CD11b subsets show normal percentages in B6.*Sle1b* mice.

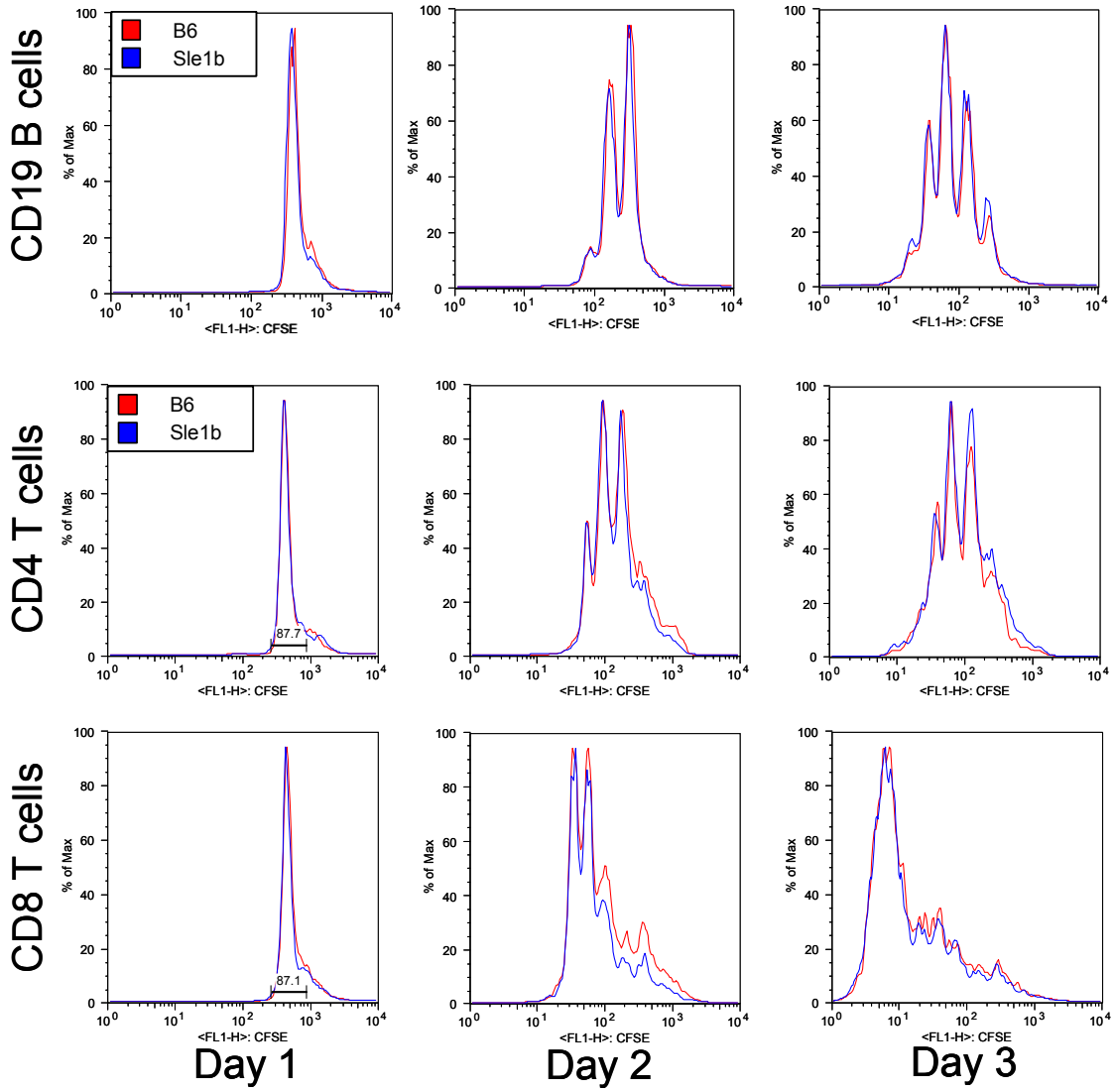


Figure 12. CFSE analysis of Sle1b lymphocytes. **A.** CD19 B cells, **B.** CD4 T cells, and **C.** CD8 T cells from B6.*Sle1b* mice do not show a difference in proliferative capacity in CD19 B cells, CD4 T cells, and CD8 T cells

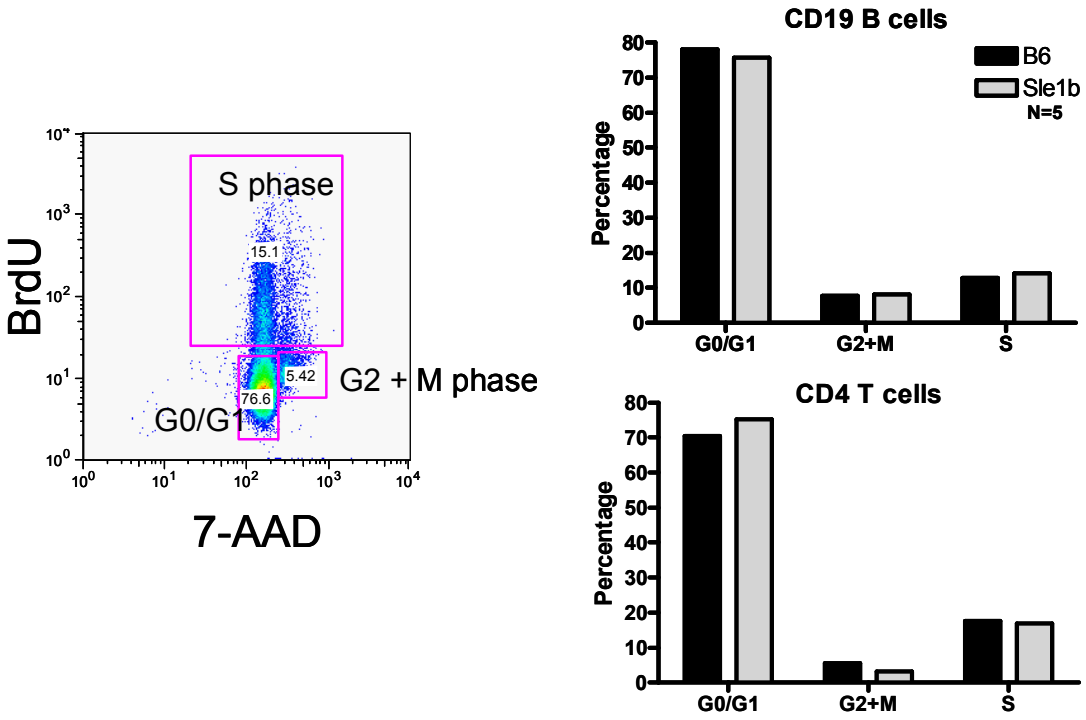


Figure 13. BrdU analysis reveals no difference in cell cycling. BrdU was added to drinking water for 9 days, after which, normal water was given. Mice were sacrificed 2 weeks later and spleens were harvested for FACS analysis. Cells were gated on CD19 or CD4, and data is presented as percentage of cells positive for BrdU.

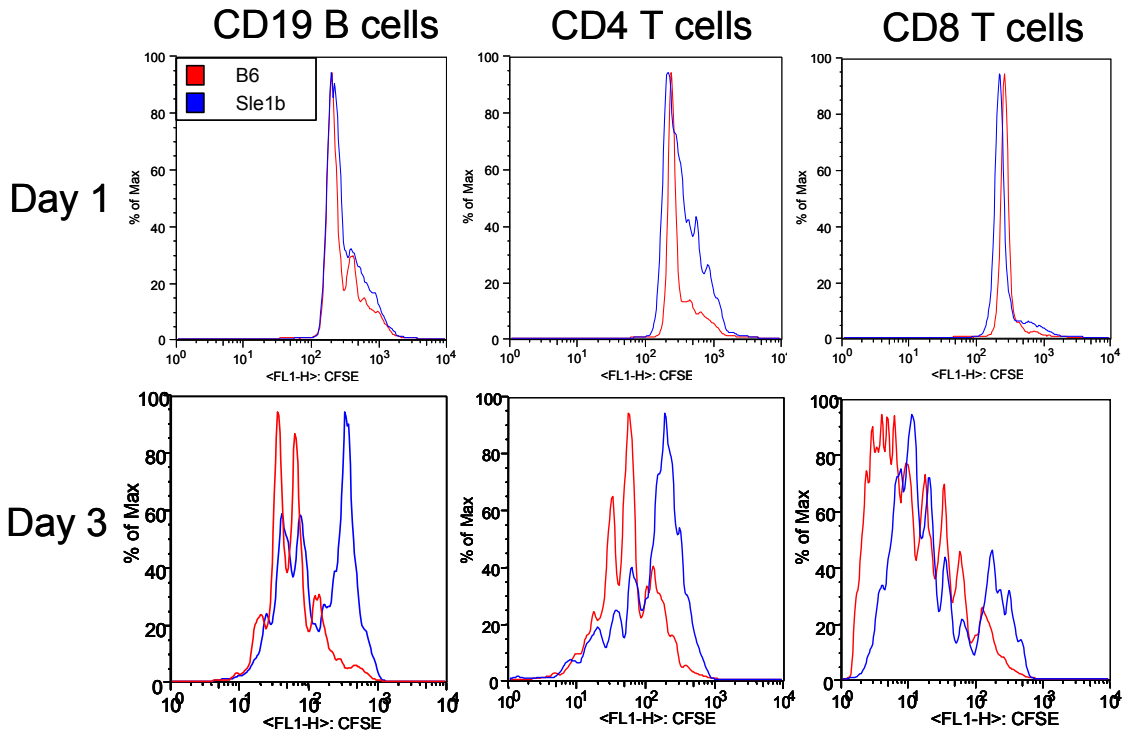


Figure 14. CFSE analysis in older mice. B6.*Sle1b* mice exhibit a diminished capacity to proliferate at 12 months of age in CD19 B cells and CD4 and CD8 T cells.

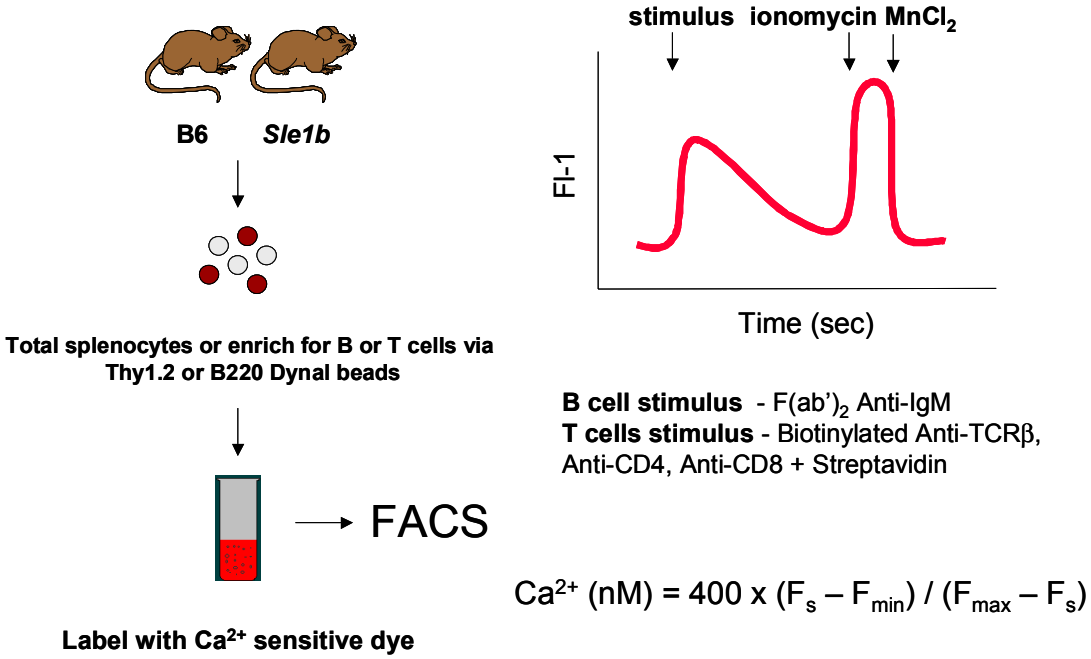


Figure 15. Strategy for calcium flux assay. Enriched B and T cells are located with Fluo-3 and stimulated with anti-IgM or biotinylated cocktail of anti-TCRβ, anti-CD4, and anti-CD8, respectively. Ionomycin was added to achieve a maximal calcium response, while $MnCl_2$ was added to determine minimal response. Calcium concentrations were then calculated with the listed equation.

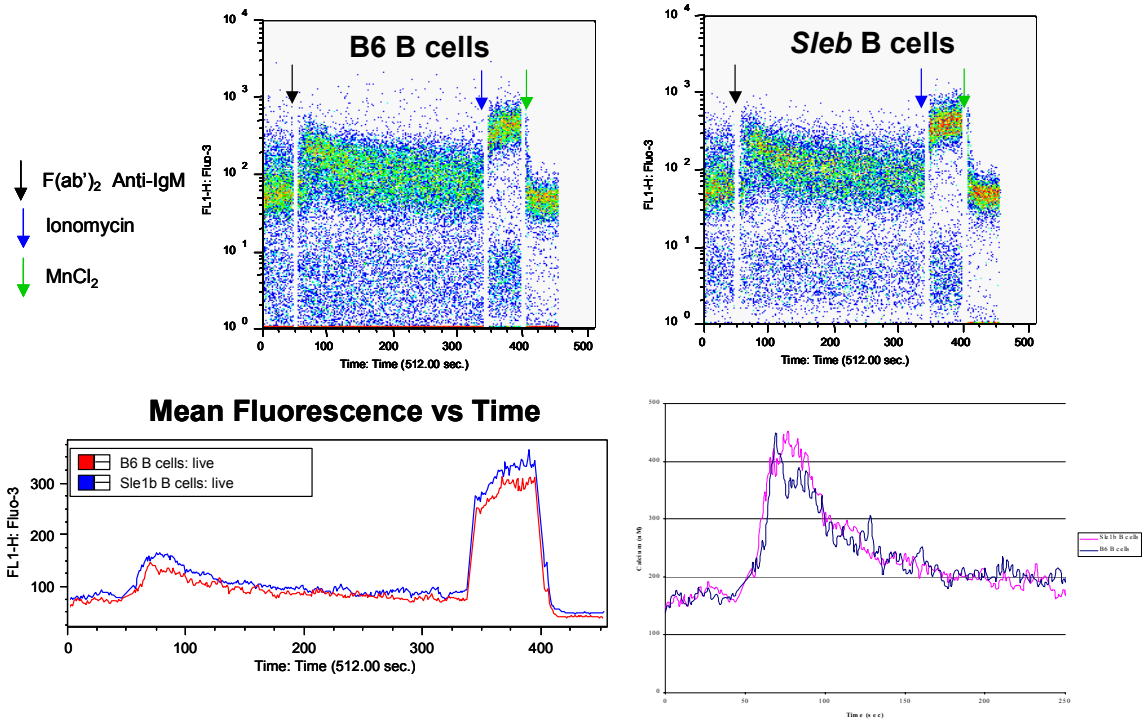


Figure 16. Calcium flux in B6.Sle1b B cells. B cells from B6.*Sle1b* show a similar calcium response to B6. Raw data plotted as a function of time is presented on top. Arrows denote when reagents were added. Mean fluorescence was determined and used to calculate calcium concentrations.

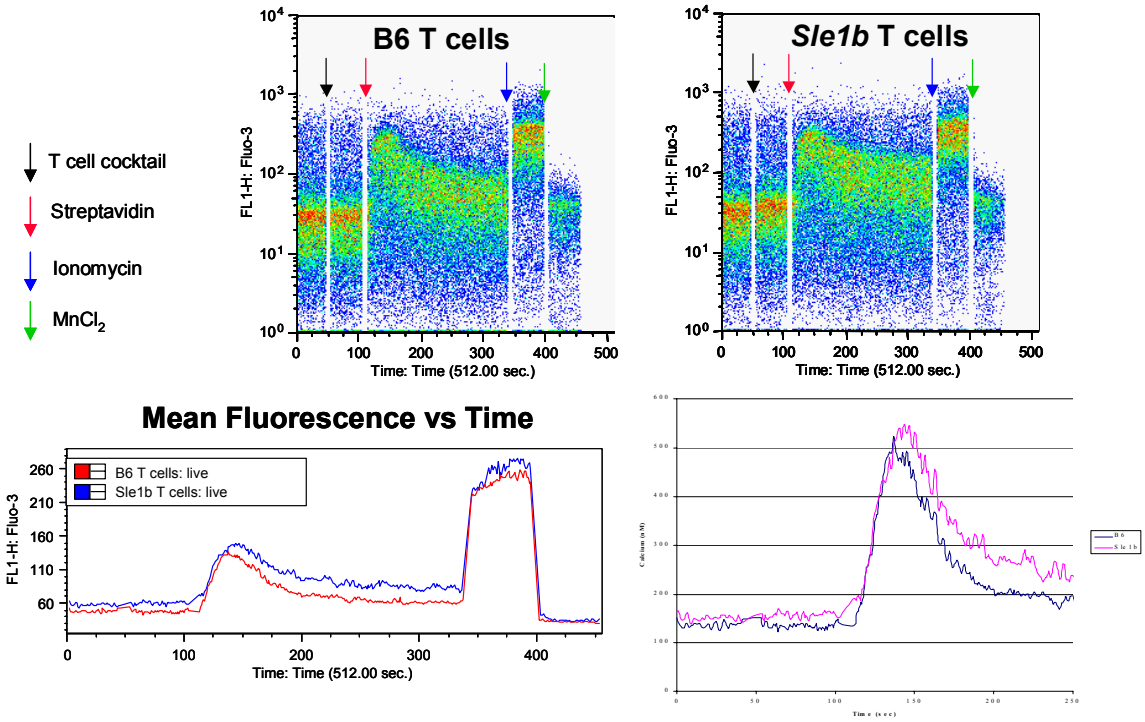


Figure 17. Calcium flux in B6.Sle1b T cells. T cells from B6.*Sle1b* exhibit an altered calcium response to B6. Raw data plotted as a function of time is presented on top. Arrows denote when reagents were added. Mean fluorescence was determined and used to calculate calcium concentrations.

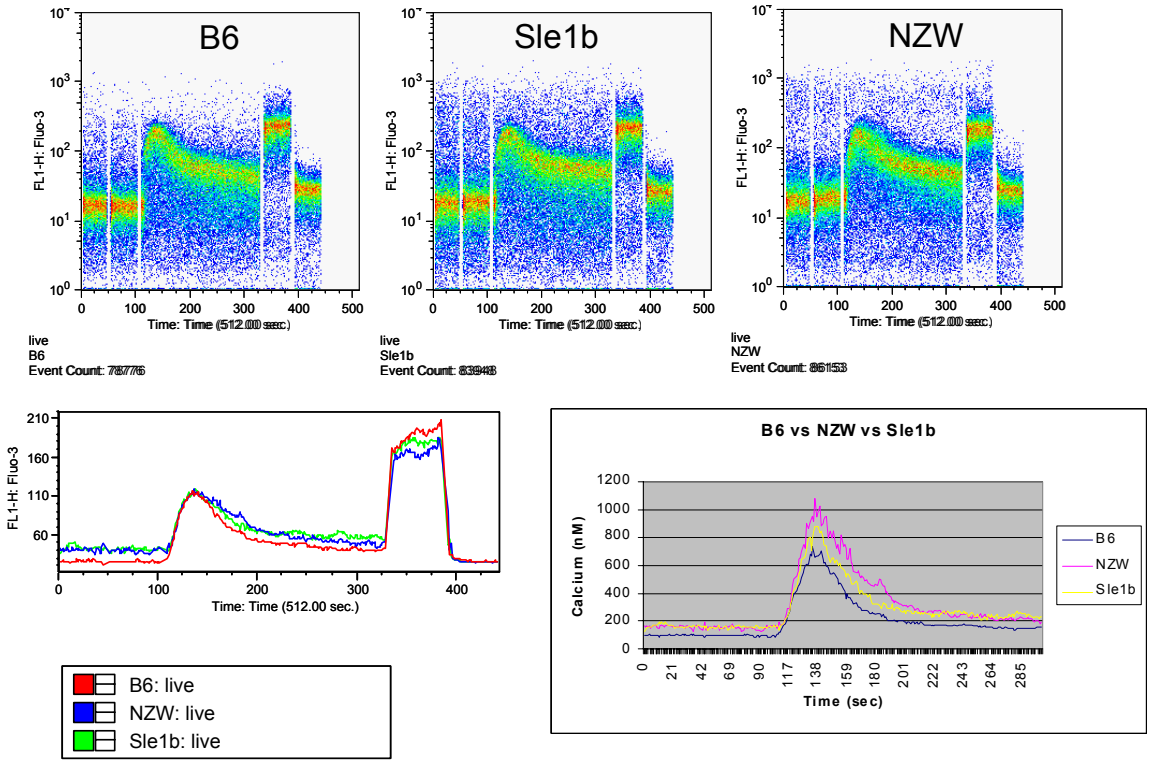


Figure 18. Calcium flux in NZW T cells. NZW T cells also show an abnormal calcium flux when compaied to B6. Raw data plotted as a function of time is presented on top. Mean fluorescence was determined and used to calculate calcium concentrations.

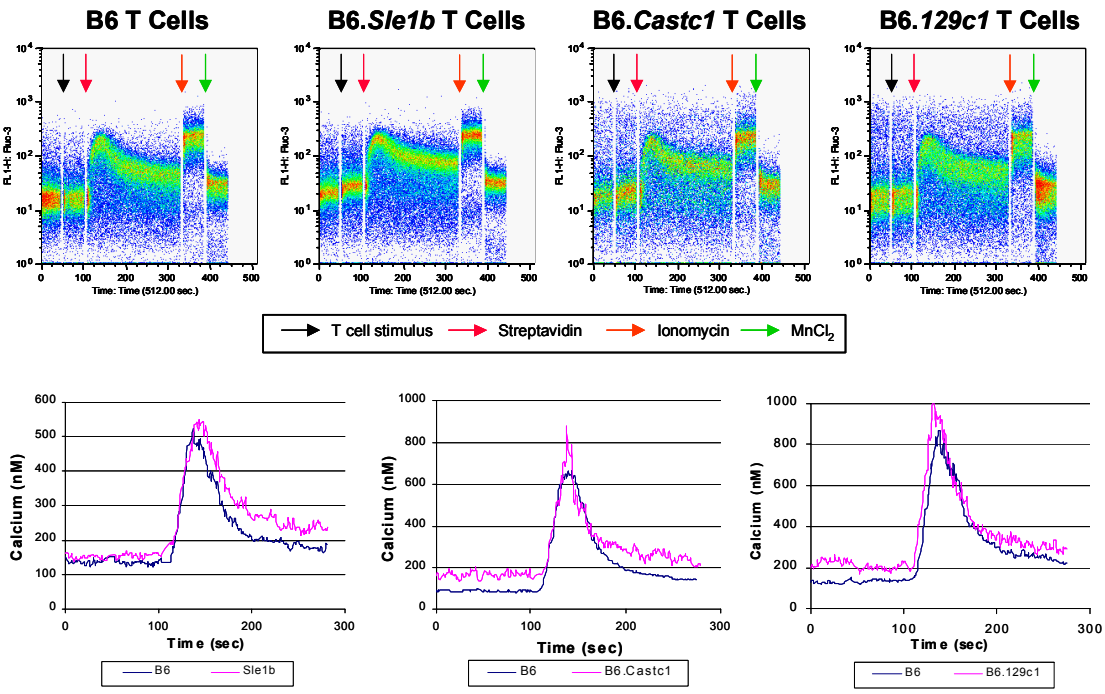


Figure 19. Calcium flux in T cells from B6.129c1 and B6.Castc1. B6.*129c1* and B6.*Castc1* are autoimmune and also exhibit an altered response to T cell stimulation. Raw data plotted as a function of time is presented on top. Arrows denote when reagents were added. Mean fluorescence was determined and used to calculate calcium concentrations.

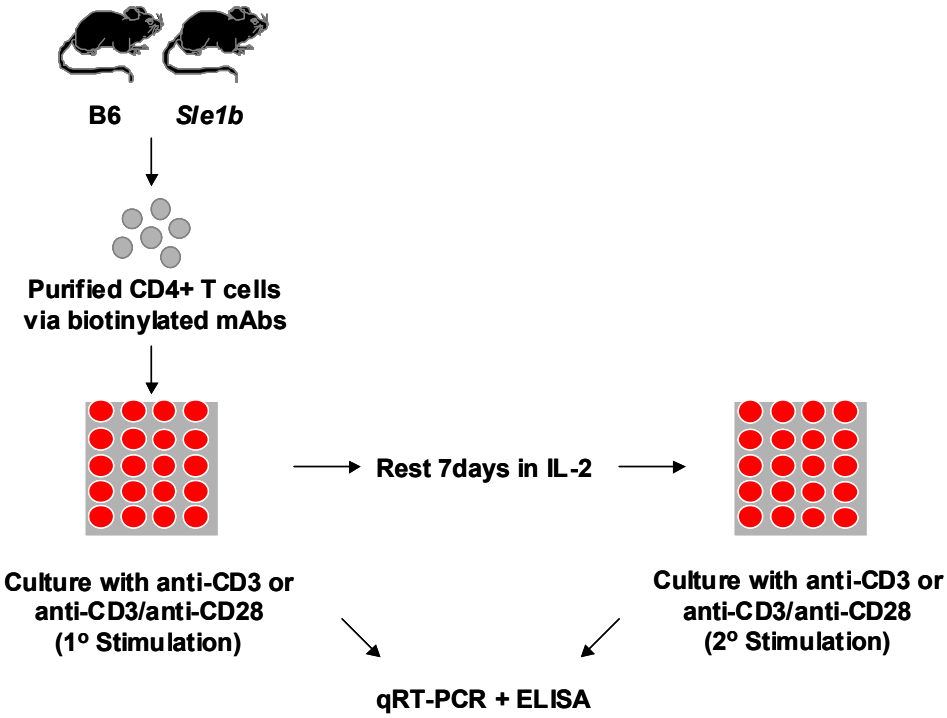


Figure 20. Strategy for cytokine response assays. CD4 T cells are purified by negative selection and plated in the presence of anti-CD3 with or without anti-CD28. Cells are stimulated for up to 72 hrs and then harvested for RNA and cytokine ELISAs. A portion of the cells are allowed to rest for a week with IL-2 and then restimulated again to assess secondary immune response.

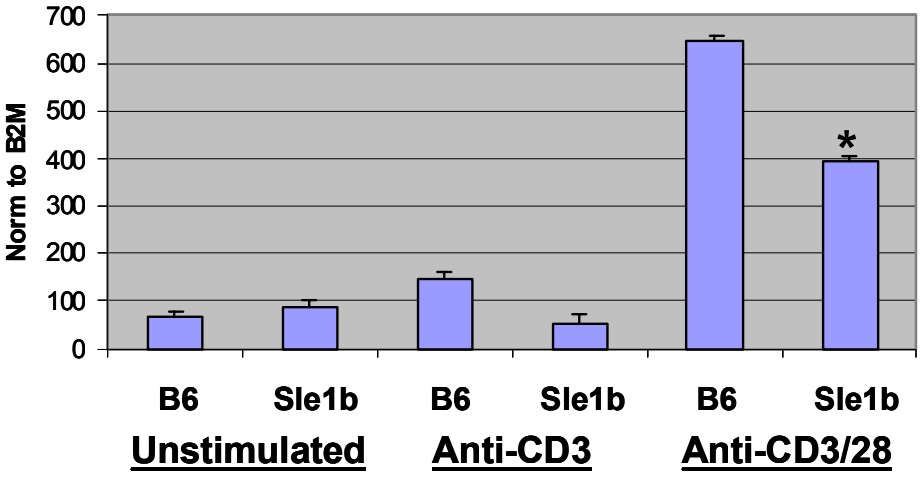


Figure 21A. IL-4 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a downward trend in Sle1b T cells with anti-CD3, and this meets signficance with anti-CD3+CD28 stimulation. * $p<0.05$

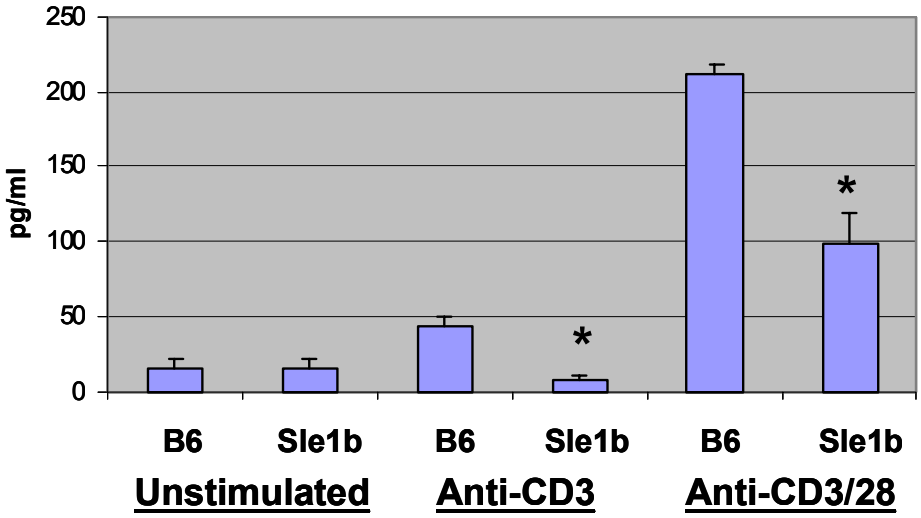


Figure 21B. IL-4 ELISA at 48 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. Supernatants were collected at 48 hrs for cytokine ELISA. There is a decrease in IL-4 produced in B6.*Sle1b* T cells with either anti-CD3 or anti-CD3+CD28 stimulation. * $p<0.05$

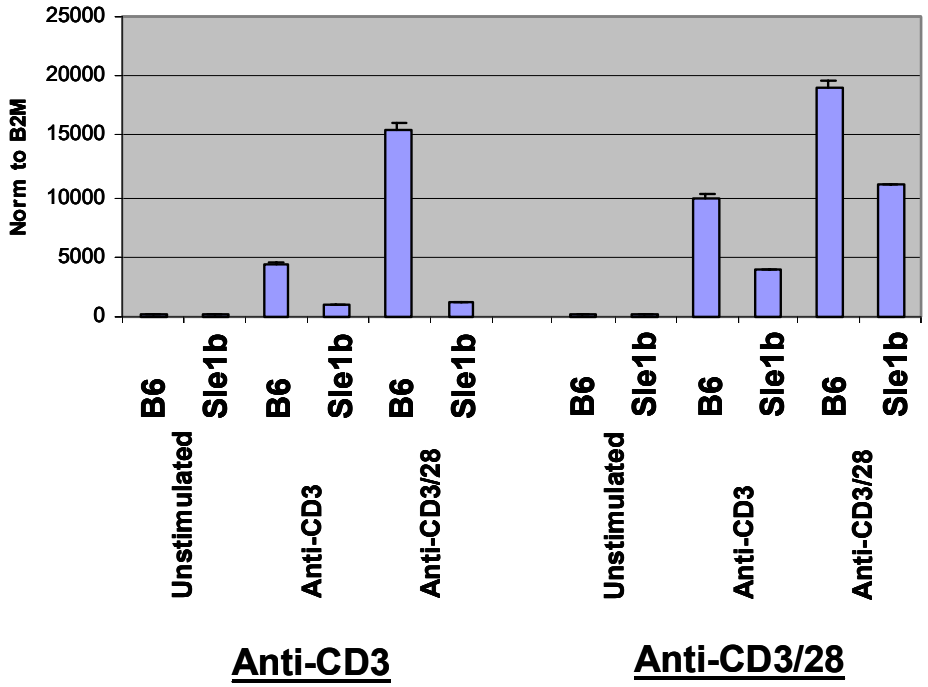


Figure 21C. IL-4 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a downward trend in Sle1b T cells with anti-CD3 and anti-CD3+CD28 stimulation but none attain significance.

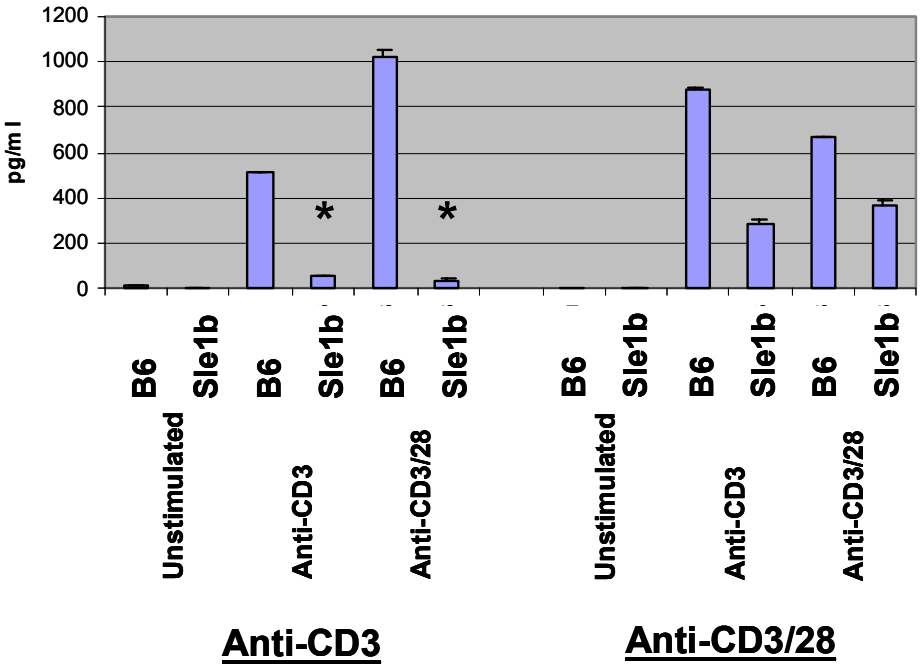


Figure 21D. IL-4 ELISA at 48 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. Supernatants were collected at 48 hrs for cytokine ELISA. There is a significant decrease in IL-4 production in B6.*Sle1b* T cells with either anti-CD3 or anti-CD3+CD28 stimulation under anti-CD3 primary-treated conditions. There is a trend for decreased secretion in B6.*Sle1b* T cells under anti-CD3/28 primary-treated conditions. * $p<0.05$

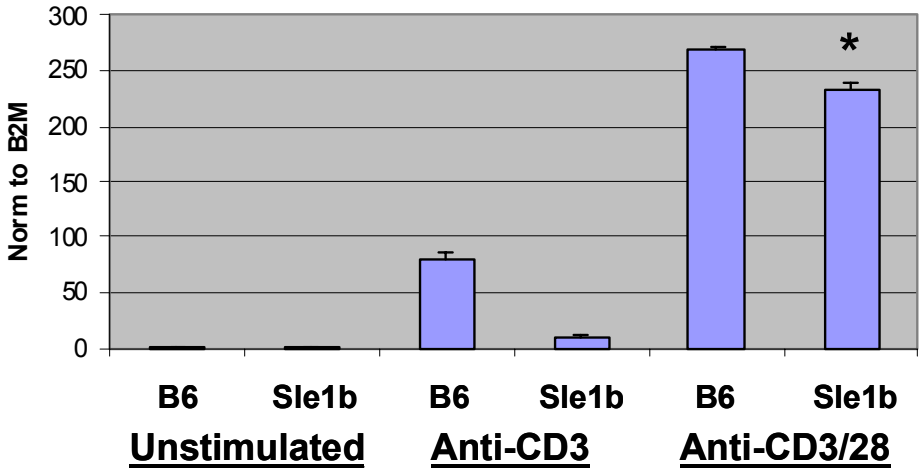


Figure 22A. IFN-gamma expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a downward trend in Sle1b T cells with anti-CD3, and this meets signficance with anti-CD3+CD28 stimulation. * $p<0.05$

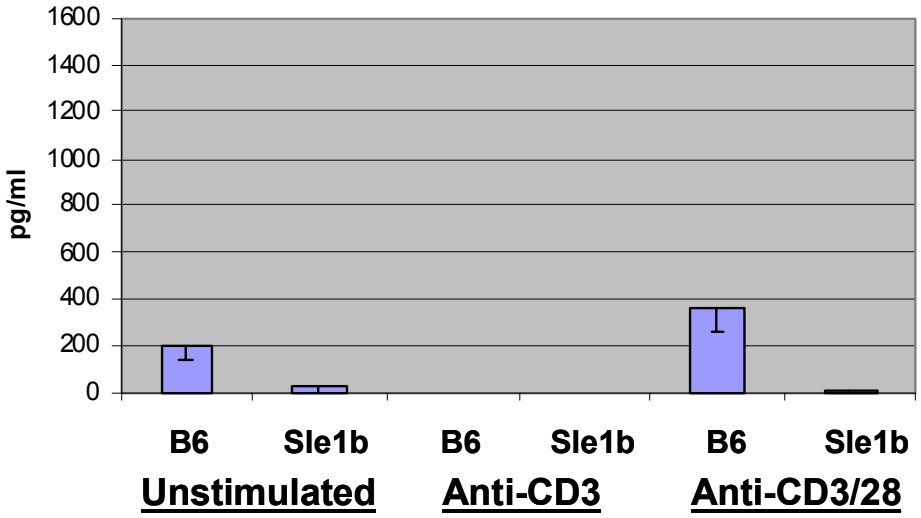


Figure 22B. IFN-gamma ELISA at 48 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. Supernatants were collected at 48 hrs for cytokine ELISA. IFN-gamma levels are barely detectable, even up to 72 hrs (data now shown). While there appears to be a decrease in IFN-gamma levels in B6.*Sle1b*, no consistent trends were observed.

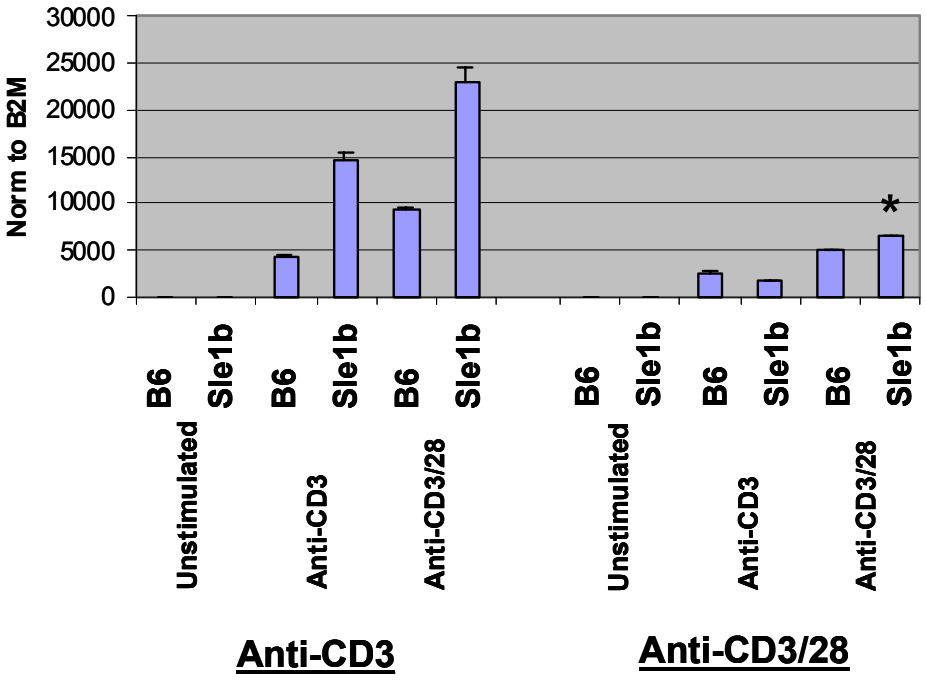


Figure 22C. IFN-gamma expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for increased IFN-gamma expression in B6.*Sle1b* T cells with anti-CD3 and anti-CD3+CD28 stimulation under anti-CD3 primary-treatment. A small but significant increased in IFN-gamma was observed for anti-CD3+CD28 stimulation under anti-CD3/CD28 primary treatment. * $p<0.05$

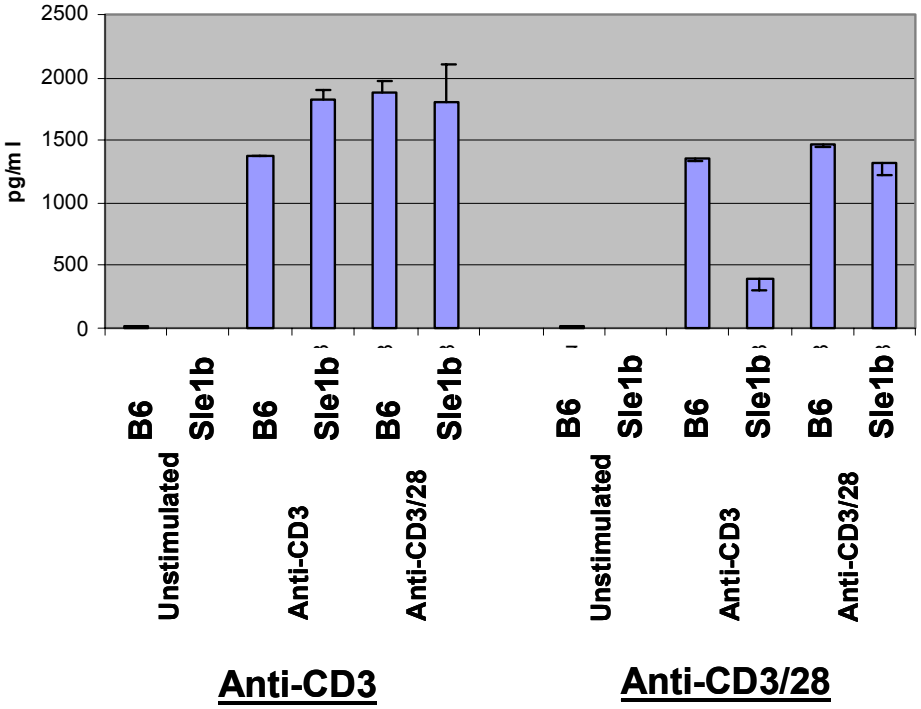


Figure 22D. IFN-gamma ELISA at 48 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. Supernatants were collected at 48 hrs for cytokine ELISA. No trends in the level of IFN-gamma were detected.

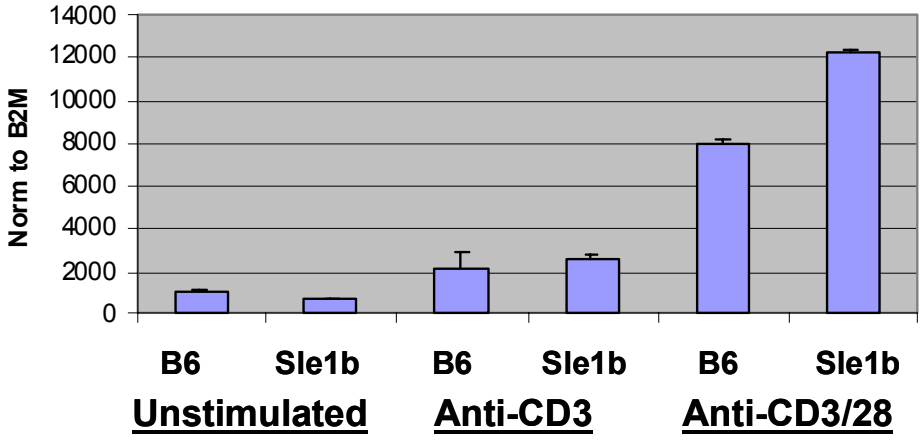


Figure 23A. T-bet expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for increased expression of T-bet in B6.*Sle1b* T cells with anti-CD3+CD28 stimulation.

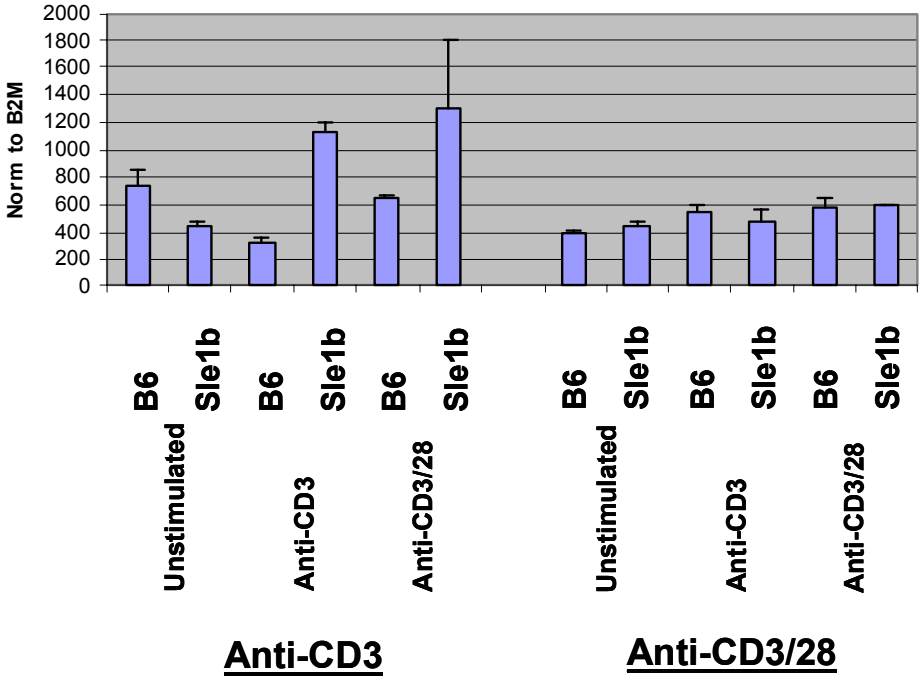


Figure 23B. T-bet expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for increased T-bet expression with anti-CD3 and anti-CD3+CD28 stimulation under anti-CD3 primary treatment.

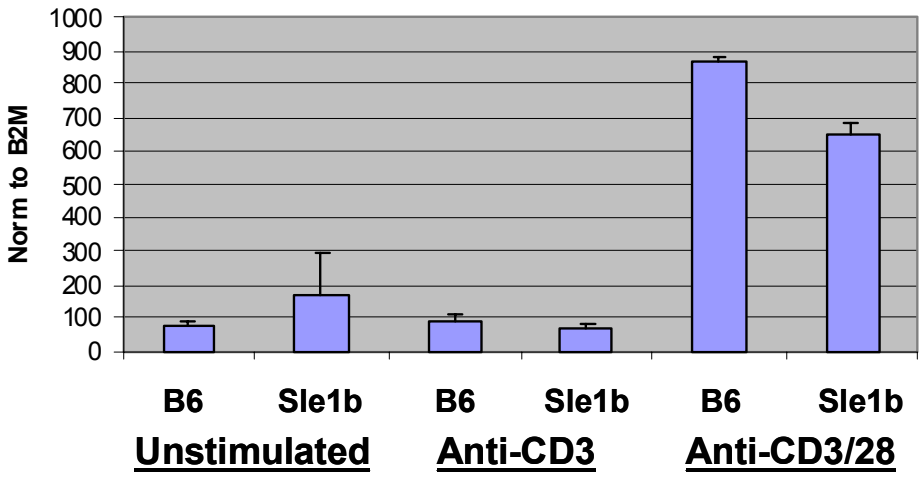


Figure 23C. GATA-3 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. No consistent differences in the level of GATA-3 expression was noted.

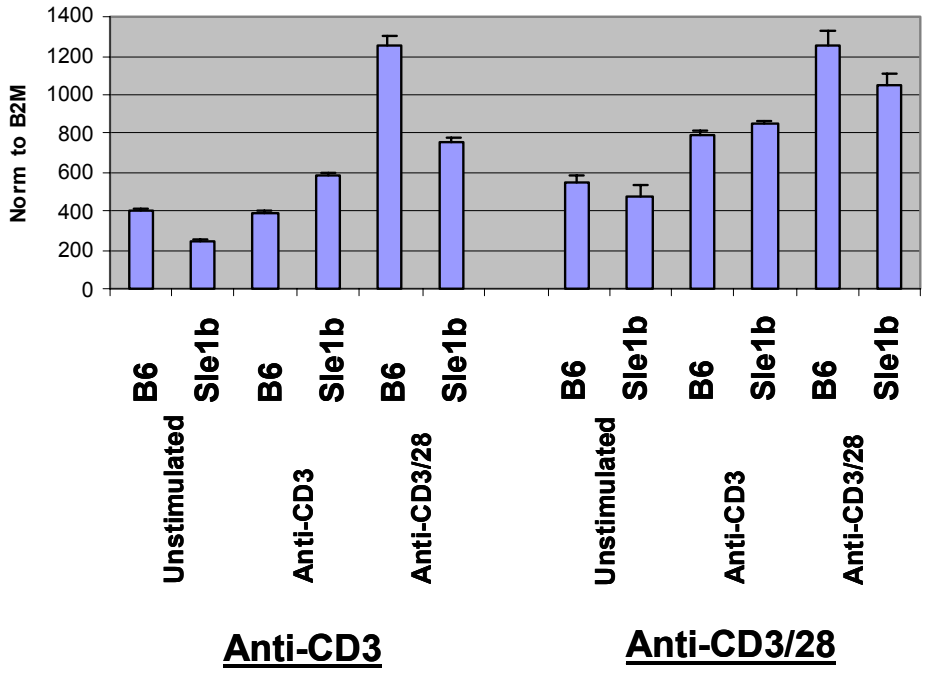


Figure 23D. GATA-3 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. No consistent differences in GATA-3 expression was observed.

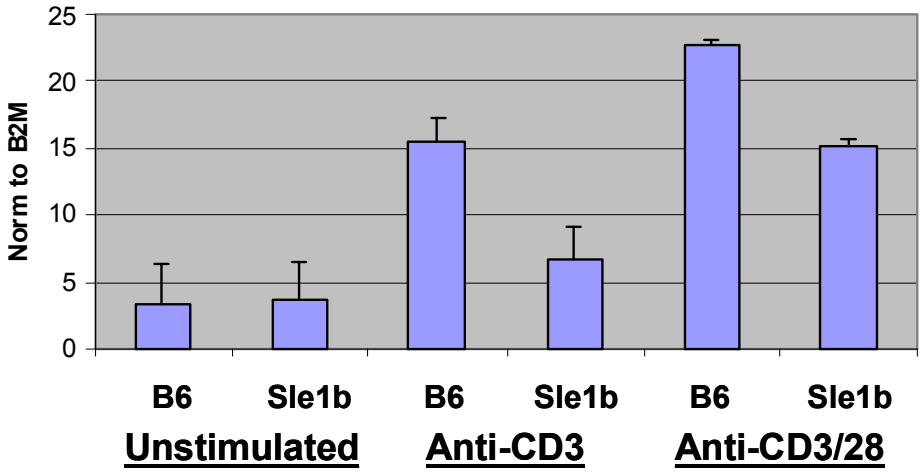


Figure 24A. IL-10 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. B6.*Sle1b* appears to have lower level of IL-10 expression, but this was not consistent across three different experiments.

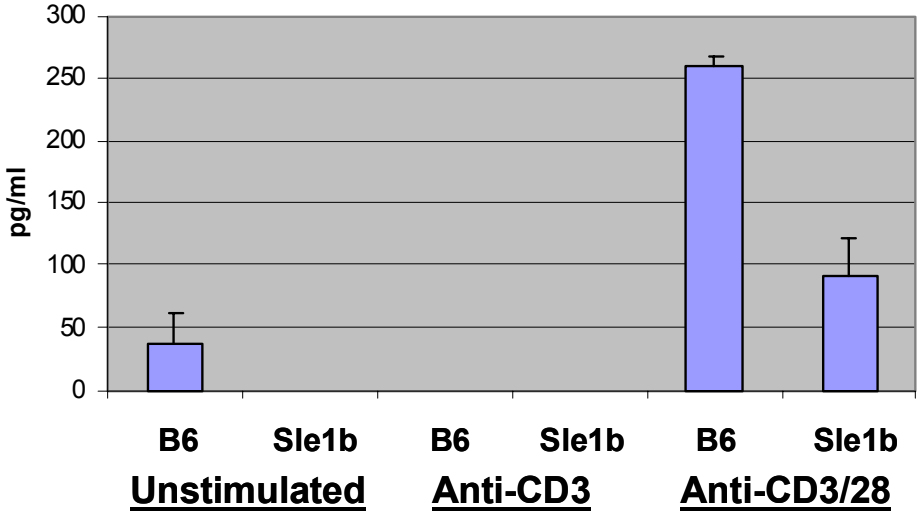


Figure 24B. IL-10 ELISA at 48 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. Supernatants were collected at 48 hrs for cytokine ELISA. B6.*Sle1b* appears to have lower level of IL-10 expression with anti-CD3+CD28 stimulation, but this was not consistent across three different experiments.

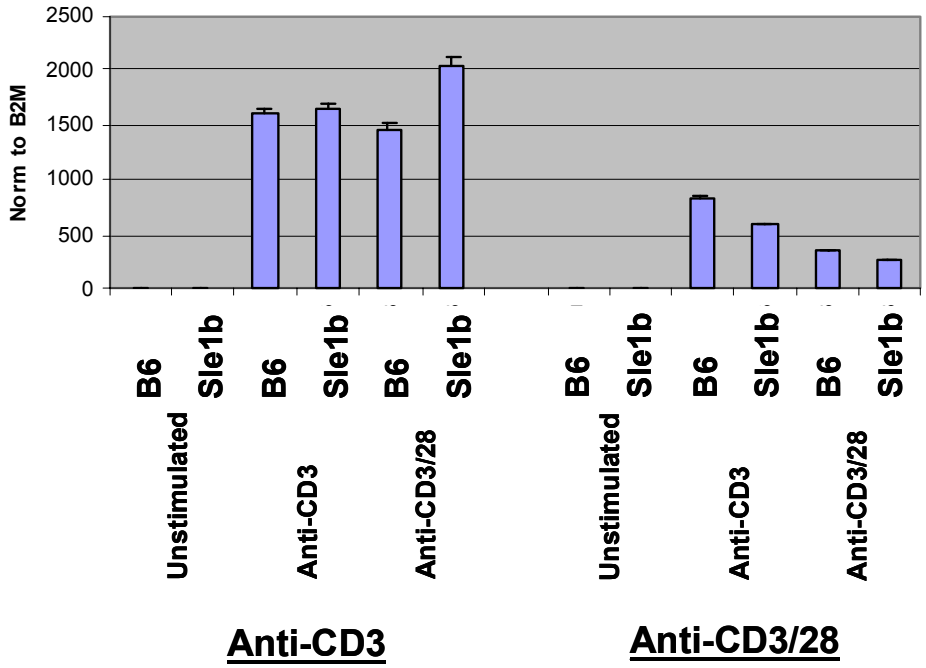


Figure 24C. IL-10 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. No consistent differences in the level of IL-10 expression was observed.

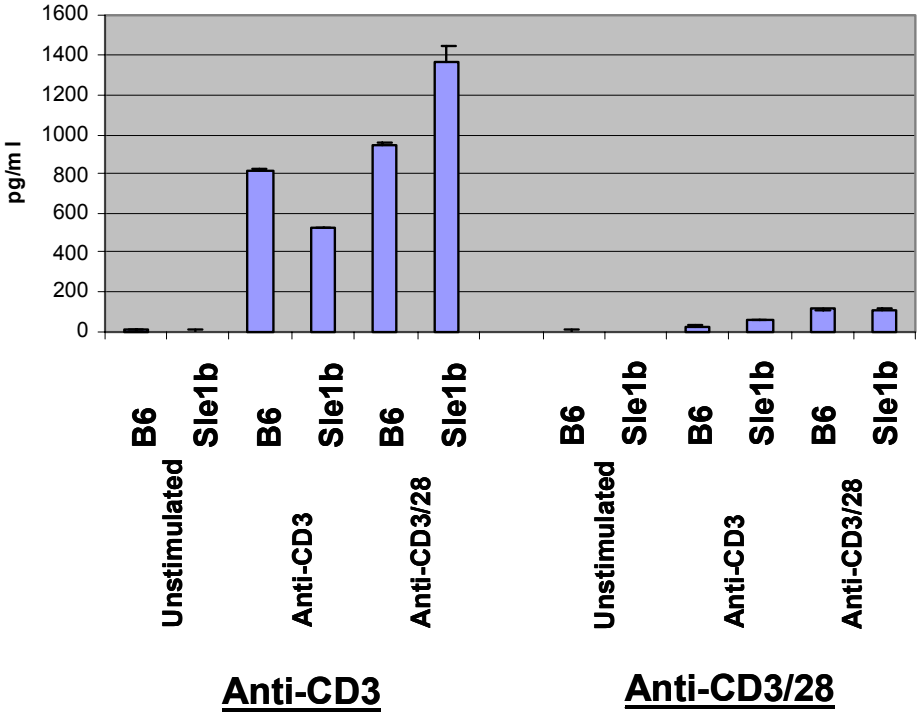


Figure 24D. IL-10 ELISA at 48 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. Supernatants were collected at 48 hrs for cytokine ELISA. No trends in the level of IL-10 were detected.

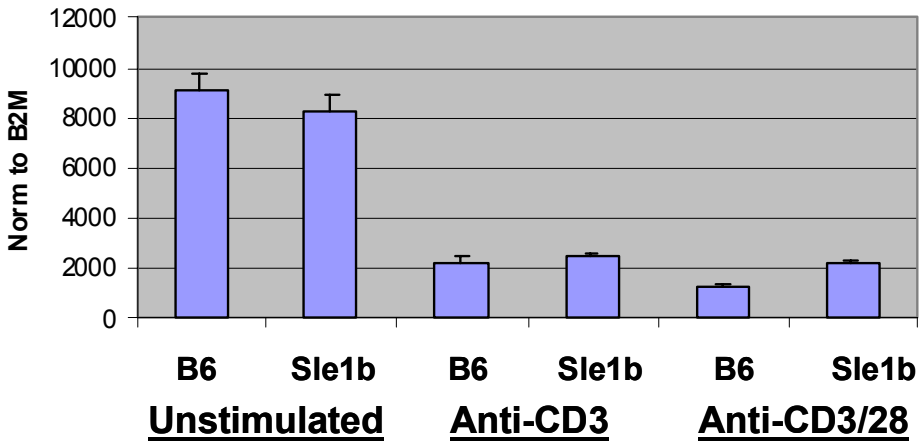


Figure 25A. TNFa expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for increased expression of TNFa in B6.*Sle1b* T cells with anti-CD3+CD28 stimulation.

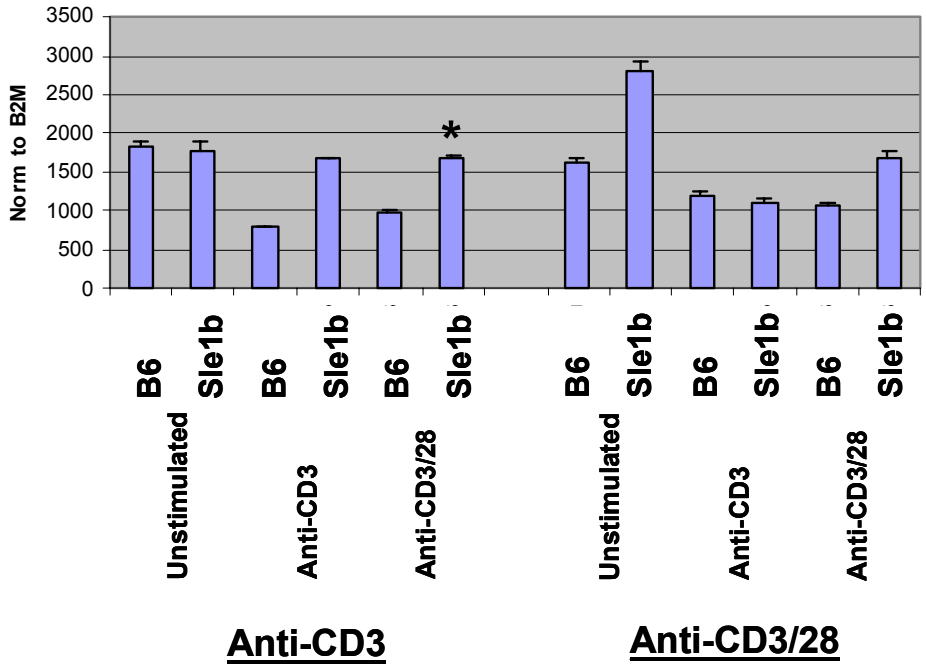


Figure 25B. TNFa expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a significant increase in the level of TNFa expression in B6.*Sle1b* mice with anti-CD3+CD28 stimulation under anti-CD3 primary treatment. There was also a trend for increased expression with anti-CD3+CD28 stimulation under anti-CD3/28 primary treatment. *p<0.05

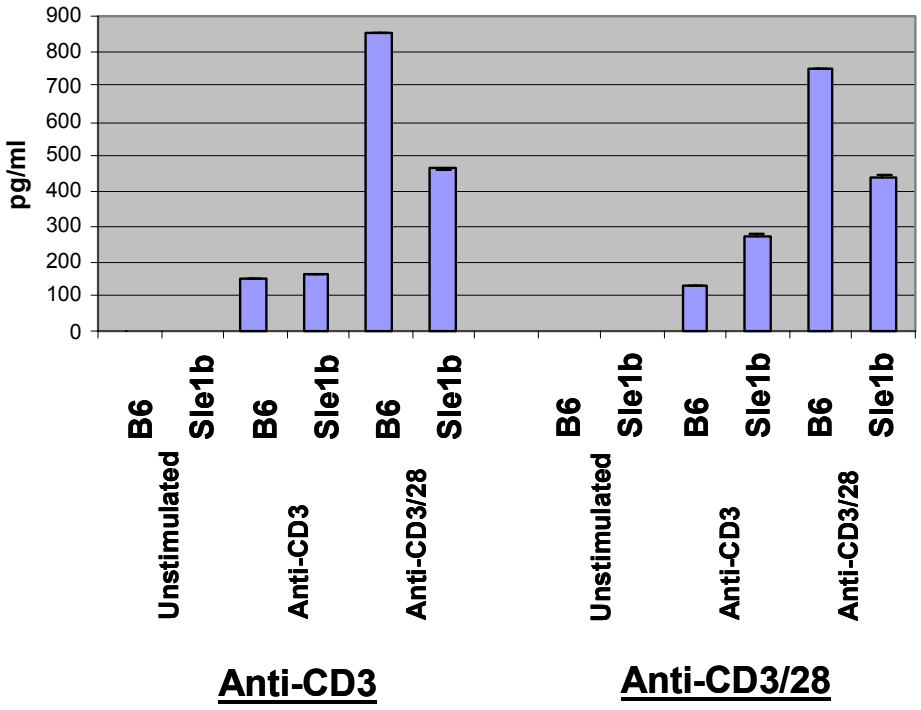


Figure 25C. TNFa ELISA at 48 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. Supernatants were collected at 48 hrs for cytokine ELISA. No consistent differences in the level of TNFa production were detected.

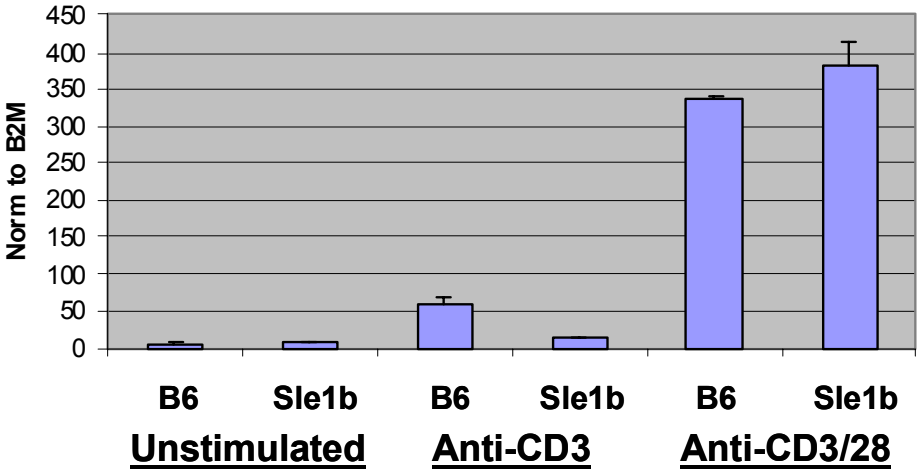


Figure 26A. IL-17 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. No consistent differences in the level of IL-17 expression were observed.

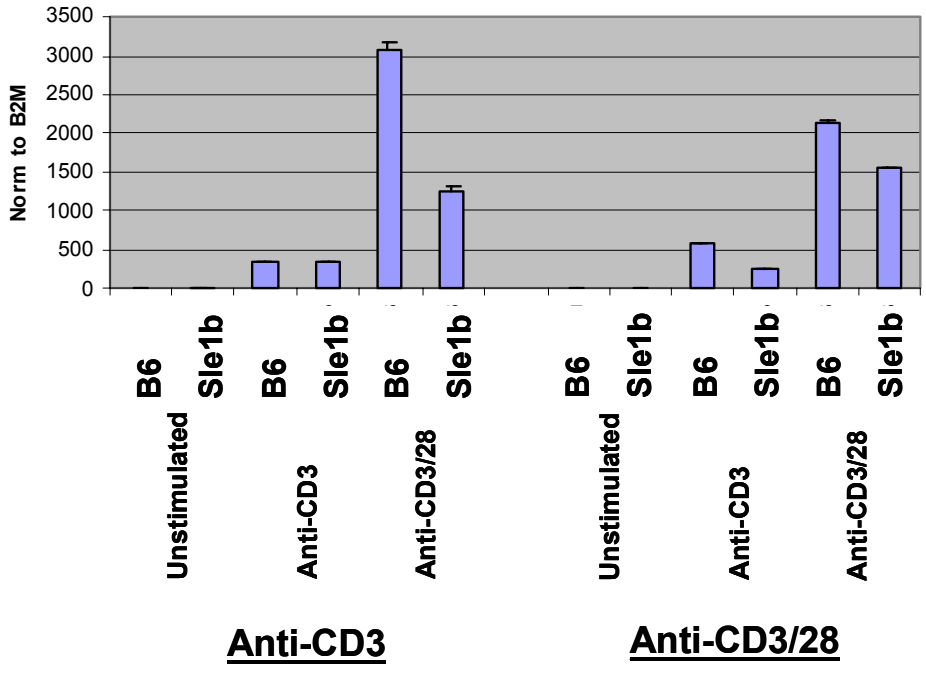


Figure 26B. IL-17 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There are trends for decreased IL-17 expression in B6.*Sle1b* T cells with anti-CD3 and anti-CD3+CD28 under both primary stimulatory treatments.

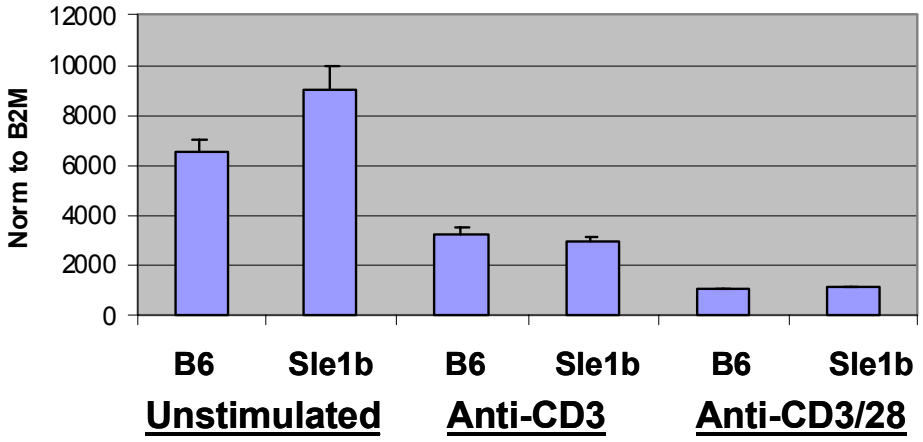


Figure 27A. IL-21 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. No consistent differences in the level of IL-21 expression were observed.

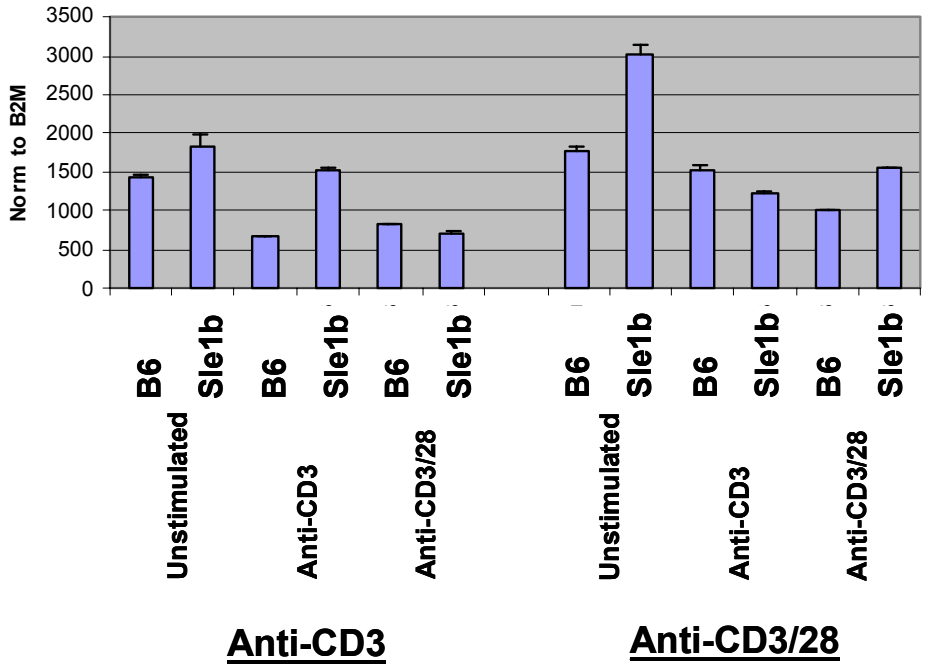


Figure 27B. IL-21 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for increased IL-21 expression in B6.*Sle1b* T cells with anti-CD3 alone under anti-CD3 primary stimulatory treatment. There is also a trend for decreased IL-21 expression with anti-CD3 alone under anti-CD3/28 primary treatment.

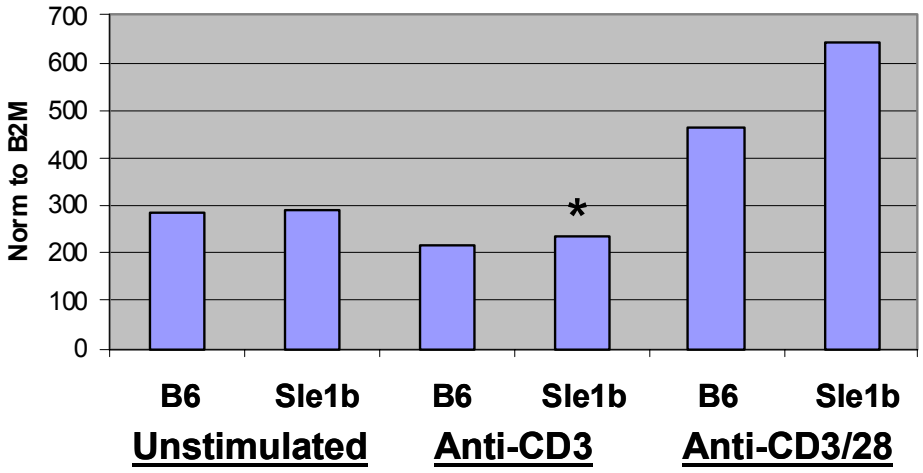


Figure 28A. Cd48 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a trend for decreased expression of Cd48 under resting conditions; however, with anti-CD3 stimulation, there is a subtle but significant increase in the levels of expression in B6.*Sle1b*. *p<0.

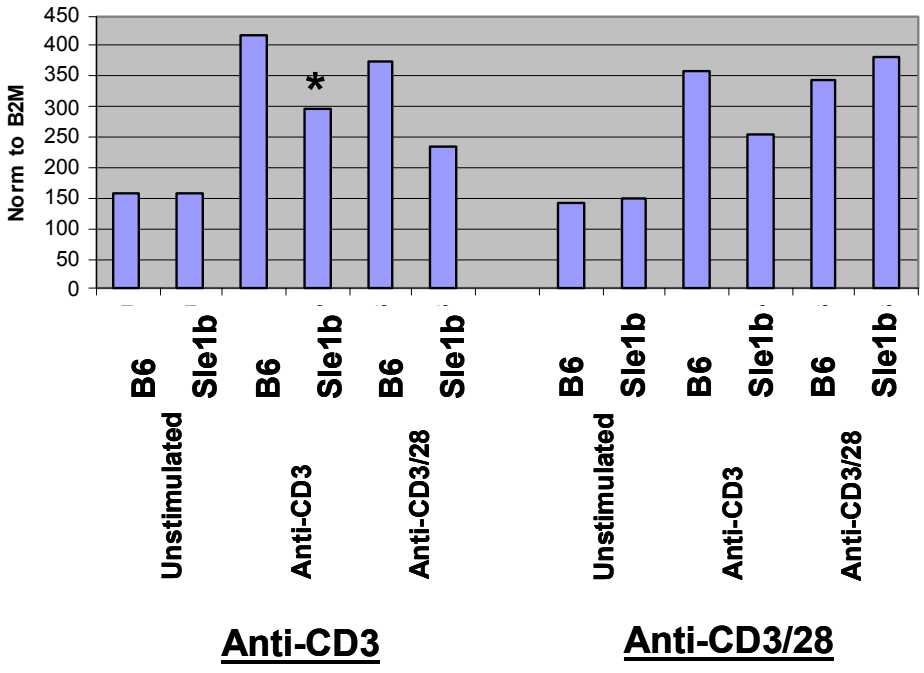


Figure 28B. Cd48 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There is a significant decrease in Cd48 expression with anti-CD3 stimulation under anti-CD3 primary treatment. Also, a trend for decreased expression in B6.*Sle1b* was noted in T cells stimulated with anti-CD3 under anti-CD3/CD28 primary activation conditions. *p<0.0

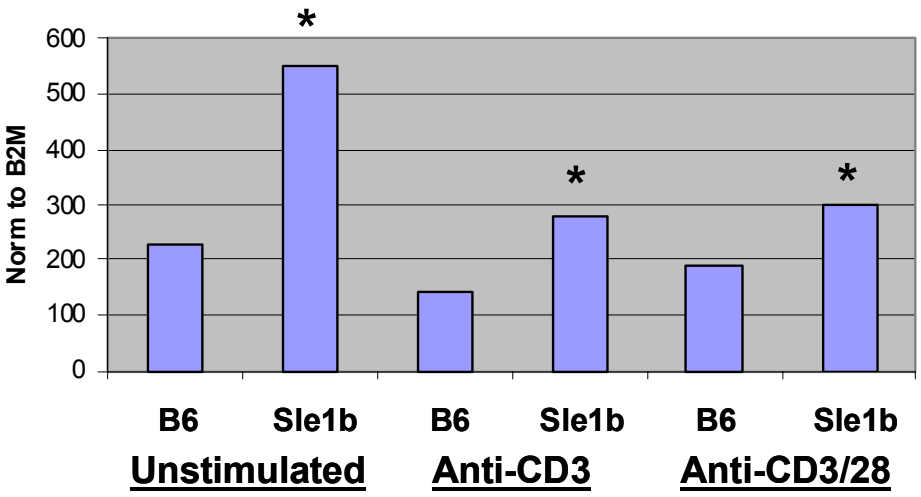


Figure 29A. Cd84 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cd84 is significantly expressed on CD4 T cells from B6.*Sle1b* under resting and activation conditions. *$p<0.05$

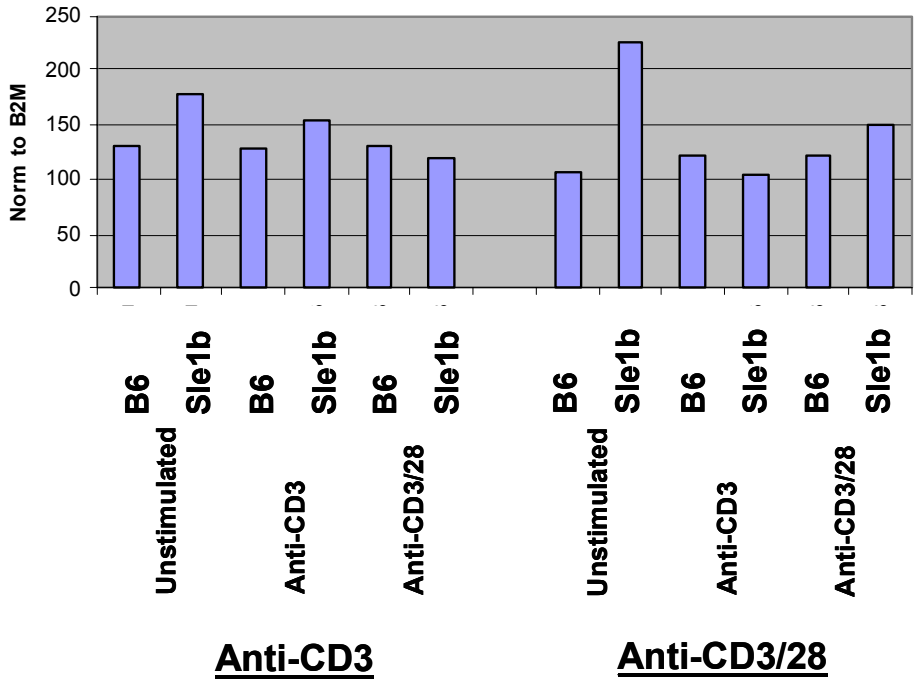


Figure 29B. Cd84 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There are trends for increased Cd84 expression in B6.*Sle1b* mice at resting and anti-CD3+CD28 stimulation conditions.

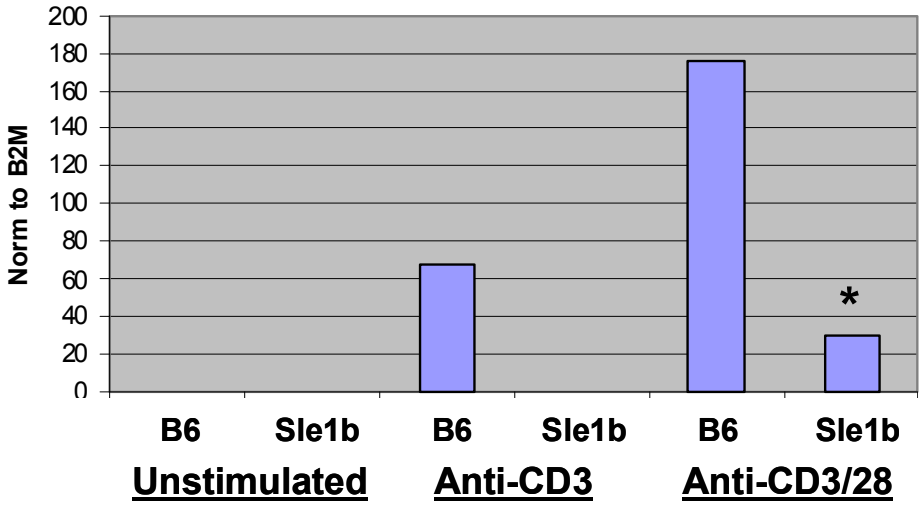


Figure 30A. Cs1 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cs1 is significantly down-regulated on CD4 T cells from B6.*Sle1b* under anti-CD3+CD28 stimulation. *p<0.05

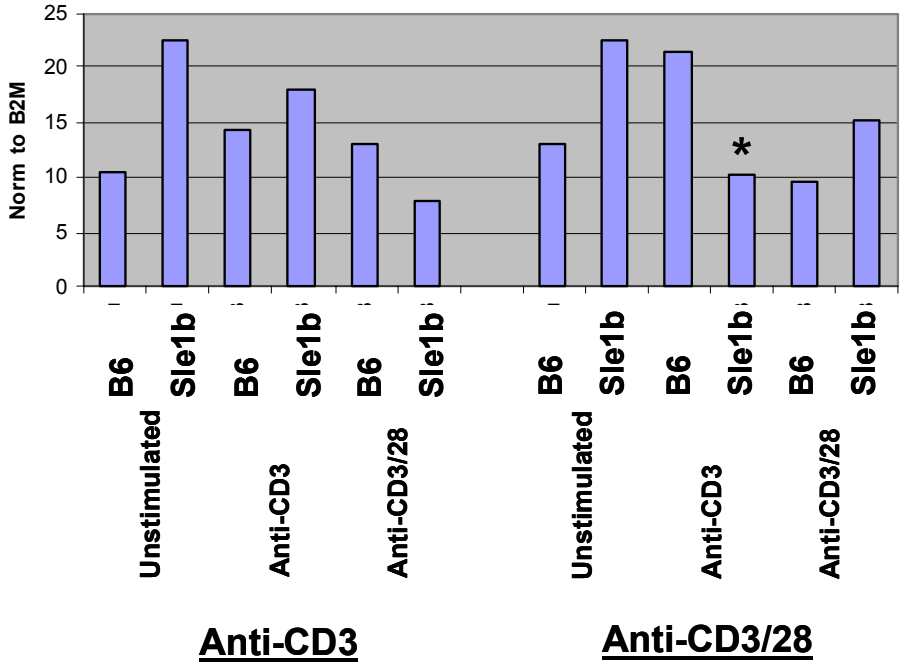


Figure 30B. Cs1 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cs1 is significantly down-regulated in B6.*Sle1b* T cells with anti-CD3 stimulation alone under anti-CD3/CD28 primary treatment. *$p<0.05$

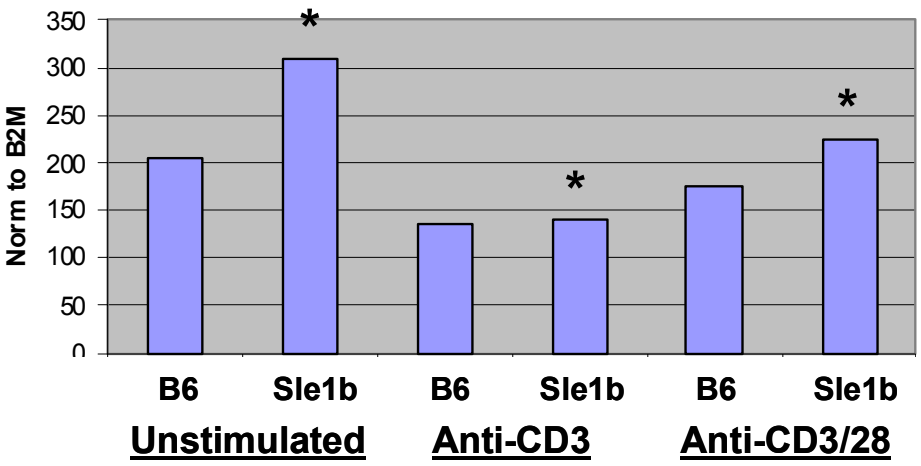


Figure 31A. Cd229 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cs1 is significantly up-regulated on CD4 T cells from B6.*Sle1b* under resting and activating conditions. *$p<0.05$

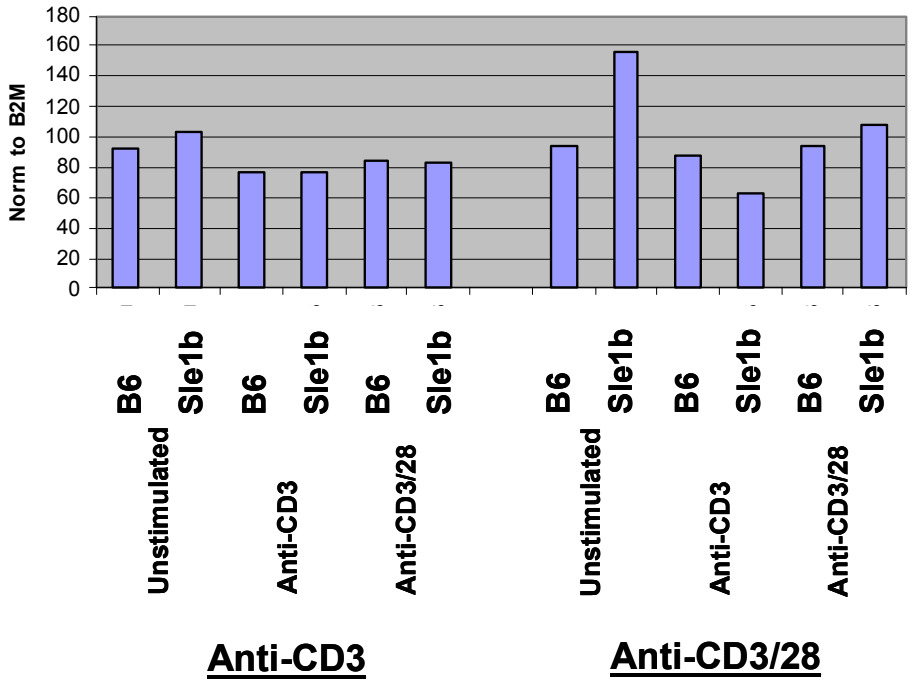


Figure 31B. Cd229 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. There are trends for increased expression of Cd229 in B6.*Sle1b* at resting conditions for both sets of primary stimulatory treatments.

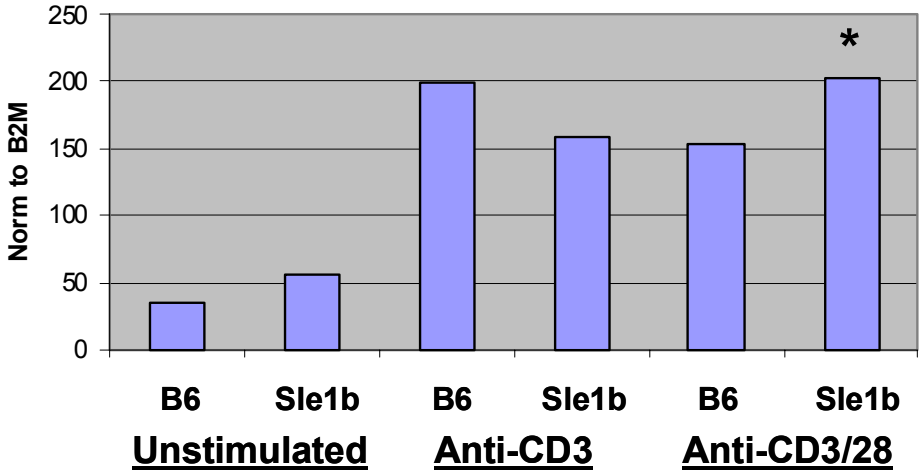


Figure 32A. Cd150 expression at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cd150 is significantly up-regulated on CD4 T cells from B6.*Sle1b* with anti-CD3+CD28 stimulation. *$p<0.05$

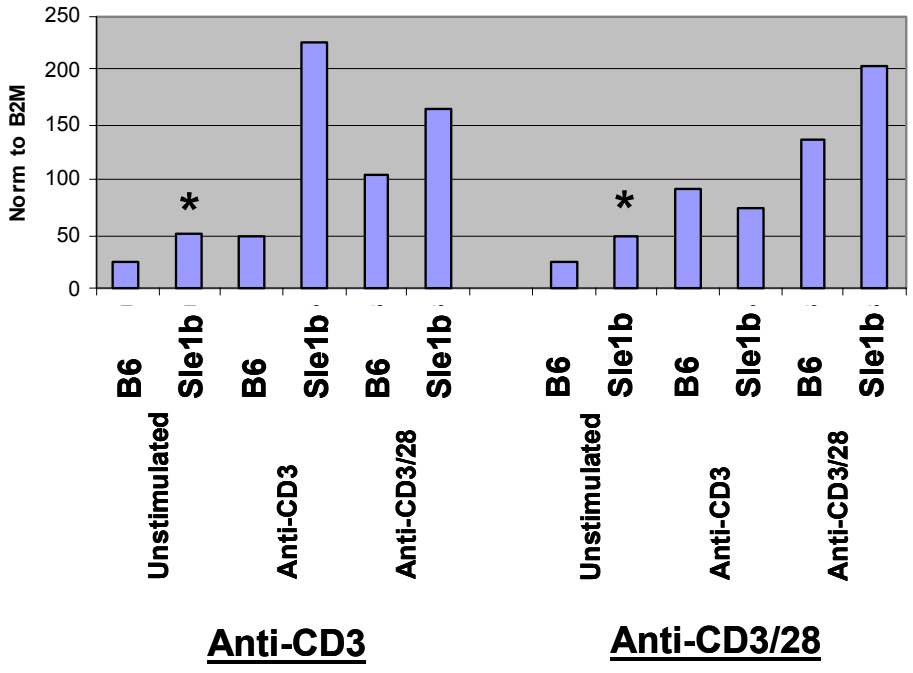


Figure 32B. Cd150 expression at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. Cd150 is significantly up-regulated in *Sle1b* T cells under resting conditions. There are also trends for increased expression with anti-CD3 or anti-CD3+CD28 stimulation under anti-CD3 primary treatment. *$p<0.05$

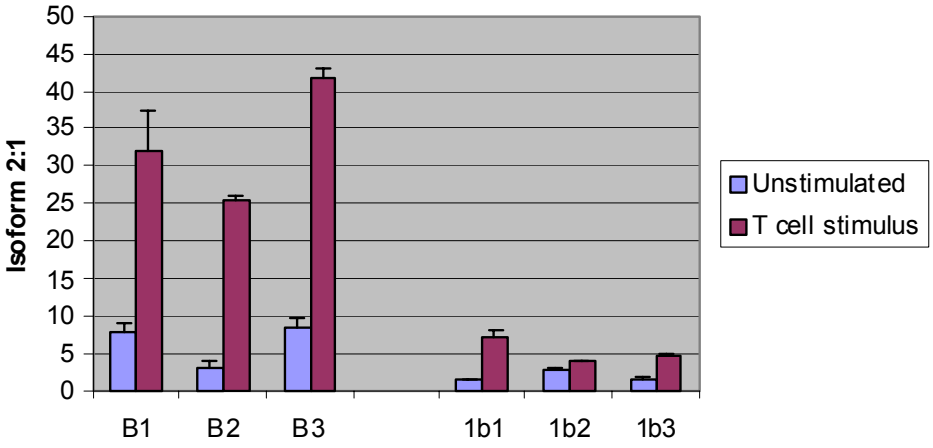


Figure 33A. Real-time PCR of Ly108 isoforms in activated splenocytes. Splenocytes from 2 months-old B6 and B6.*Sle1b* females were cultured with anti-CD3 for 48 hrs. Real-time PCR analysis was performed, and the data is presented as the ratio of isoform 2 to 1.

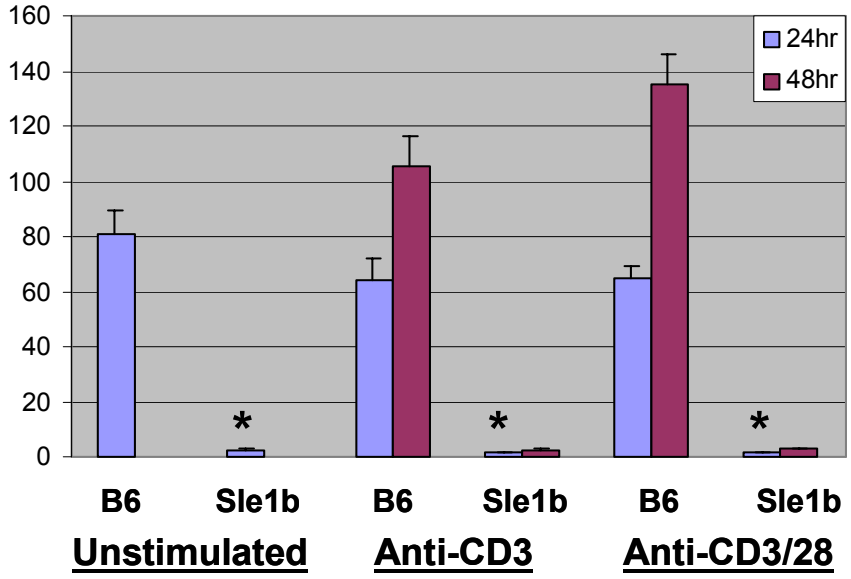


Figure 33B. Ly108 isoform ratios at 24 hrs in CD4 T cells under primary stimulation. Representative data is shown from three separate experiments. RNA was harvested from CD4 T cells collected at 24 and 48 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. The differences in the isoform ratio of 2:1 between B6 and B6.*Sle1b* are significant under resting and activating conditions.

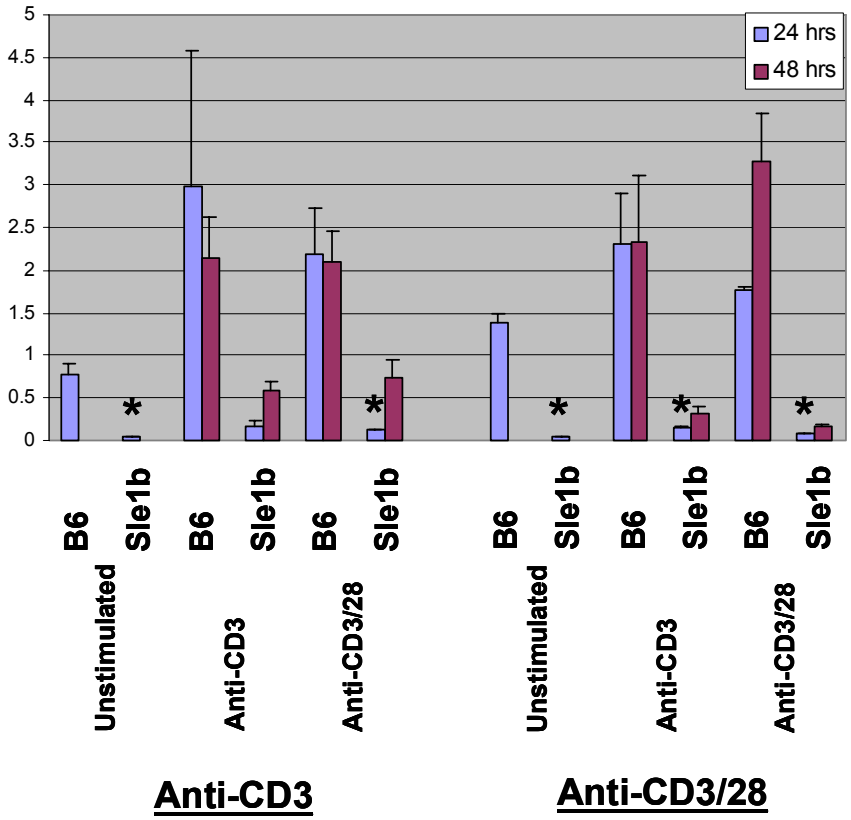


Figure 33C. Ly108 isoform ratios at 24 hrs in CD4 T cells under secondary stimulation. Representative data is shown from three separate experiments. Data on the left of the graph represent cells that were initially stimulated with anti-CD3 and then rested, whereas data on the right represent cells stimulated with both anti-CD3 and costimulatory anti-CD28 and then rested. Cells were restimulated a week later. RNA was harvested from CD4 T cells collected at 24 hrs. Real-time PCR was performed, and β2-microglobulin was used for normalization. The differences in the isoform ratio of 2:1 between B6 and B6.*Sle1b* is significant under resting and activated conditions.

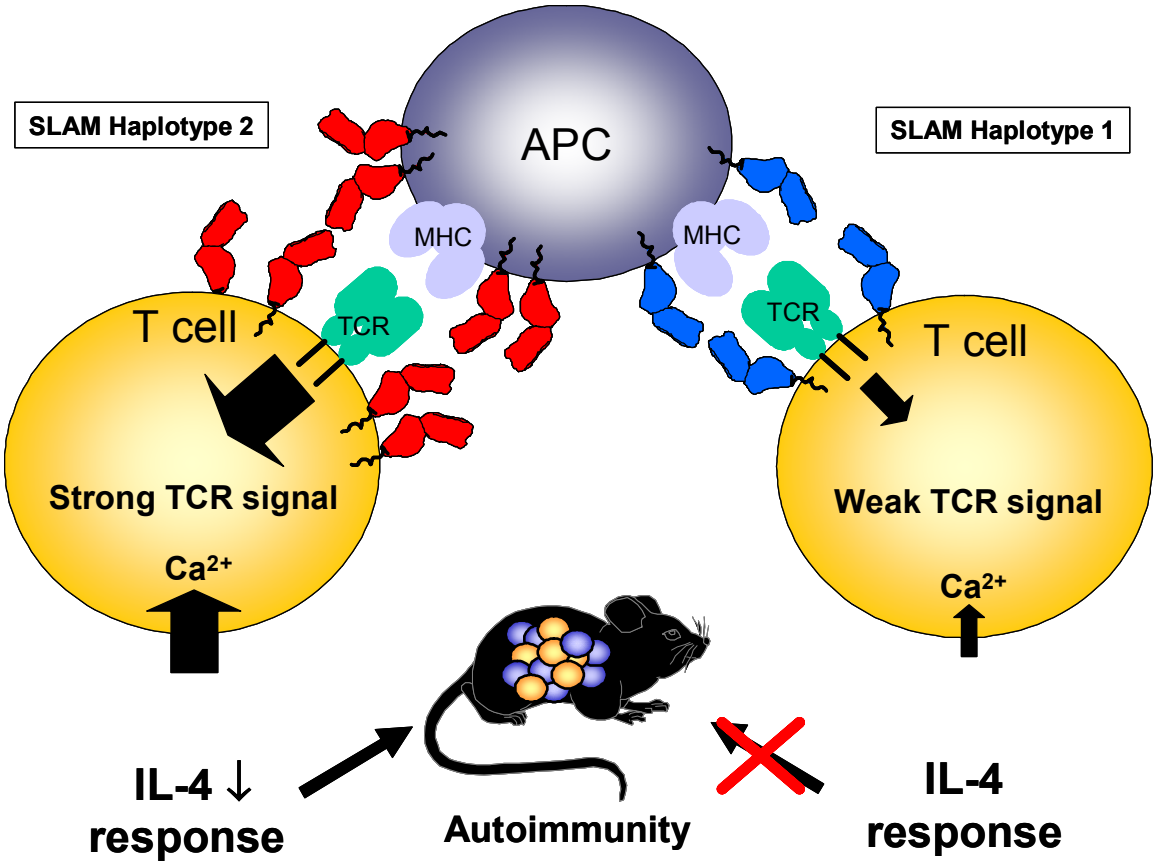


Figure 34. The SLAM/CD2 family haplotype affects T cell response. Haplotype 1 on the B6 background mediates normal TCR signaling events. However, by moving haplotype 2 onto the B6 genome, this causes a potentiation in signaling pathways downstream of TCR activation. This altered cytokine profile is associated with a breach in tolerance by mechanisms yet to be elucidated.

	Unstim	CD3	CD3/28
IL-4	↓*	↓	↓*
Ifn-g	-	↓	↓*
IL-10	-	-	-
IL-17	-	-	-
IL-21	-	-	-
TNFa	-	-	↑
Gata-3	-	-	-
T-bet	-	-	↑
Cd48	↓	↑*	↑
Cd84	↑*	↑*	↑*
Cs1	↓*	-	↓*
Cd229	↑*	↑*	↑*
Cd150	-	-	↑*
Ly108-1	↑*	↑*	↑*
Ly108-2	↓*	↓	↓*
Iso 2:1	↓*	↓*	↓*

- = no trend
↓ = downward trend
↑ = upward trend
* = significant **p<0.05**
n=3 experiments of 3-5 pooled mice in each group

Table 2A. Summary of real-time quantitative RT-PCR data on activated CD4 T cells under primary stimulation.

	Anti-CD3			Anti-CD3+CD28		
	Unstim	**CD3**	**CD3/28**	**Unstim**	**CD3**	**CD3/28**
IL-4	-	↓	↓	-	-	↓
Ifn-g	-	↑	↑	-	-	↑*
IL-10	-	-	-	-	-	-
IL-17	-	↓	↓		↓	↓
IL-21	-	↑	-	-	↓	-
TNFa	-	-	↑*	-	-	↑
Gata-3	-	-	-	-	-	-
T-bet	-	↑	↑	-	-	-
Cd48	-	↓*	-	-	↓	-
Cd84	↑	↑	-	↑	-	↑
Cs1	-	-	-	-	↓*	-
Cd229	↑	-	-	↑	-	-
Cd150	↑*	↑	↑	↑*	-	-
Ly108-1	↑*	↑	↑	↑	↑	↑
Ly108-2	↓*	↓*	↓*	↓*	↓*	↓*
Iso 2:1	↓*	↓	↓*	↓*	↓*	↓*

Table 2B. Summary of real-time quantitative RT-PCR data on activated CD4 T cells under secondary stimulation.

Chapter 5: Discussion

An enormous effort that have been devoted to the identification of *Sle1b* and has led to the identification of a cluster of genes known as the Slam/CD2 family as the strongest candidates for mediating the autoimmunity seen in *Sle1b*. Both the expression data presented in this thesis along with the extensive sequence analysis carried out by Dr. Limaye demonstrate that this gene cluster is highly polymorphic. Furthermore, three independently-derived congenic strains, B6.*Sle1b*, B6.129c1, and B6.Castc1, all of which harbor identical alleles for the telomeric portions of the Slam/CD2 gene family, exhibit ANA production. This strongly supports that the Slam/CD2 haplotype 2-derived alleles for Cd48, Cd150, Cd84, and Ly108 are the susceptibility genes that mediate the loss in tolerance.

Indeed, the B and T cell data on Ly108 expression indicate a dramatic difference in the levels of the two isoforms between B6 and B6.*Sle1b*, which strongly favors Ly108 as the prime candidate gene. This is particularly interesting because it has been demonstrated that splice variants of CD244 play opposing roles in NK cell cytotoxicity (259), and thus, it is tempting to hypothesize that the two isoforms of Ly108 may mediate different functions. Isoform variations of other autoimmune susceptibility genes, such as CTLA-4 (260,261), CD45 (262,263) and CD95 (264), have already been demonstrated to be associated to autoimmunity. Furthermore, the difference in the up-regulation of Ly108 in activated B and T cells between B6 and *Sle1b* is intriguing. The ratio of isoform 2:1 increases in both strains of mice upon cell activation, but this ratio

in *Sle1b* never reaches the same level as B6 (Figure 7, Figure 33). Studies on the long isoform of another Slam/CD2 member, *Cd244*, have indicated its capacity to inhibit NK cell cytotoxcity (265). While there is currently no evidence on the functional properties of the Ly108-2, the long isoform of Ly108, it is tempting to speculate that Ly108-2 may also have some negative regulatory role. If we could assume that Ly108-2 behaves in an inhibitory capacity, we would hypothesize that in non-autoimmune mice such as B6, Ly108-2 becomes up-regulated during cell activation in an attempt to down-regulate the immune response. Such would not be the case in B6.*Sle1b*, where the levels of Ly108-2 are not increased to the same extent as seen in B6, thereby allowing signaling pathways to go unchecked.

Collaborative efforts are underway with the Bennett/Schatzle lab to characterize the role of the individual isoforms. Due to the lack of an available Ly108 antibody at the time, we created epitope-tagged Ly108-1 and Ly108-2 constructs. Initial data on transfected Jurkat cell lines by Jill Mooney from the Schatzle lab suggests a difference in signaling properties between the two Ly108 isoforms (data not shown). Further studies in Jurkats along with retroviral transduction into primary lymphocytes may give us a better picture as to how the individual isoforms function and how their differential expression in the *Sle1b* mice contributes to autoimmunity.

In addition to the transfection studies, *in vivo* models by overexpressing Ly108 isoforms were generated by Dr. Limaye when she established the Ly108 transgenic mouse lines. By overexpressing Ly108-1 on B6 and Ly108-2 on

Sle1b, we had hoped to either recapitulate or suppress, respectively, the autoimmune phenotype. However, only the Ly108-1 transgenic line exhibited high levels of expression on the B6 background (Figure 10b), but in spite of that, these mice showed no developmental abnormalities and failed to produce any autoantibodies (Figure 10e-f). Furthermore, when an antibody that recognized Ly108 was finally made available, we detected no differences in the level of surface expression between the transgenic and non-transgenic littermates. (Figure 10h). Nevertheless, *Ly108* remains a top candidate gene, and while it has already been knocked out (266), no reports on any autoimmune phenotypes have been published.

To understand the process by which the Slam/CD2 family mediates the *Sle1b* phenotype, we assessed cellular proliferation, calcium signaling, and cytokine production in the *Sle1b* mice. While we have demonstrated that *Sle1b* mice have enlarged spleens when compared to B6, even prior to the onset of detectable ANAs, our proliferation studies with CFSE and BrdU labeling have indicated no difference in the capacity to divide in B and T cells from *Sle1b*. It is very likely that the dosage of stimuli used in these experiments are too high to detect any subtle differences, and so, these experiment need to be repeated with an appropriate dose curve. Interestingly, when we looked at mice greater than 9months old, there was a remarkable impairment in the proliferative capacity of *Sle1b* B and T cells. We attribute this to an exhausted phenotype (267,268) that is indicative of a chronically activated immune system.

When no significant difference in the level of proliferation was observed, we turned our attention to signaling pathways and proposed that the Slam/CD2 haplotype might affect the activation threshold of B and T cells. When we examined the mobilization of calcium following antigen receptor stimulation, we observed that B cells did not show any difference between B6 and B6.*Sle1b*. *Sle1b* T cells, on the hand, exhibited a subtle but consistent difference in calcium flux. However, we have not fully teased out which T cell subset is responsible for altering the signaling phenotype since anti-CD4 and anti-CD8 antibodies were used in the cocktail. Further experiments involving one or the other would help narrow down which T cell subset is affected. However, one group has previously reported that T cells from SLE patients exhibit increased calcium responses following the cross-linking of the TCR/CD3 complex, and this phenotype is not unique to any particular T cell subset (269).

The observed increase in calcium flux in the T cells from B6.*Sle1b* mice is intriguing, but how Ly108 isoform expression and/or the presence of a particular Slam/CD2 haplotype mediates this phenotype is uncertain. We have not ruled out the possibility that more than one of these genes may contribute to the overall susceptibility to autoimmunity. It is more than feasible that the overall autoimmune phenotype is more a result of a divergent haplotype of the Slam/CD2 family than *Ly108*. This would not be the first time that the association of autoimmunity with a polymorphic haplotype has been made. There is a tremendous amount of work on the major histocompatibility complex and its association with autoimmunity (212) along with the more recently reported

association of susceptibility to Crohn's disease with a specific common haplotype of the human cytokine gene cluster on chromosome 5q31 (270).

Additional support for *Sle1b* being the Slam/CD2 haplotype has recently emerged from data involving knockouts of particular family members. Both *Cd150* (271) and *Ly108* (272) knockout mice exhibit a reduction in IL-4 production and a modest to no increase in IFN-gamma upon CD4 T cell stimulation with anti-CD3 and anti-CD28 antibodies. Interestingly, this is the phenotype that is observed in our B6.*Sle1b* mice where both receptors are still intact. When purified CD4 T cells from *Sle1b* are subjected to either primary or secondary stimulation, there is a noticeable decrease in the levels of IL-4 produced, both at the RNA and protein level (Figure 21a-d). This suggests a possible redundancy amongst the Slam/CD2 family in the regulation of IL-4. On the other hand, it also possible that the haplotype of the family influences the T cell signaling environment by mechanisms that remain to be determined. In support of this, data on the SAP-deficient mice suggests a similar defect in Th2 cytokine regulation upon CD4 T cell activation (273,274). Given that the SAP adapter molecule interacts with members of the Slam/CD2 family via the ITSM motifs, it is then conceivable that the entire family and its interaction with Sap can overall regulate the cytokine response upon T cell activation.

How the Slam/CD2 family mediates the decreased IL-4 response is not currently known. However, studies on TCR signaling strength and T helper cell differentiation offer a possible model (Figure 34). Brogdon and colleagues have demonstrated that a weak TCR signal mediated by altered peptide ligands lead

to a Th2 differentiation response, whereas a strong TCR signal favors a Th1 response (275). Their report noted that the degree of calcium flux upon TCR stimulation can influence the translocation of nuclear transcription factors that regulate the expression of cytokine genes. Furthermore, they demonstrated that a weak calcium response is associated with a Th2 outcome (276). Given that T cells from B6.*Sle1b* mice exhibit more prolonged calcium flux, we propose that the decreased IL-4 production is a manifestation of a strong TCR signal seen by the *Sle1b* T cells. In such a hypothetical model, the Slam/CD2 haplotype 2 in the B6.*Sle1b* mice mediates a reduction in the activation threshold in T cells, thereby causing a stronger TCR signal to occur. The output of such a stronger signal is made evident as a stronger calcium mobilization response and is translated into a skewed Th response of naïve CD4 T cells as the production of IL-4 is reduced. However, what is problematic with this model is that one might anticipate a consistent increase in the levels of IFN-gamma in the *Sle1b* T cells, but that is not the case, at least under primary stimulatory conditions (Figure 22). This is not too unusual because it was also reported that while SAP deficient mice show an increase in IFN-gamma production, the data has not been always consistent (Dr. Pamela Schwartzberg, personal communication). Still, the question remains as to how this cytokine imbalance contributes to the loss of tolerance seen in B6.*Sle1b*.

Care must be taken when making interpretations of the IL-4 response and its role in mediating the *Sle1b* phenotype. There is no doubt that cytokines are important contributors to the dysregulation of the immune system in lupus-prone

mouse models (277), but categorizing murine lupus, if not the *Sle1b* model, under a Th1 or Th2 paradigm is difficult. The IFN-gamma and IL-4 knockouts on the MRL/lpr background argue that both Th1 and Th2 cells are equally important in SLE (164). Furthermore, the use of anti-IL-4 and anti-IFN-gamma antibodies in B/W mice also suggests that both Th1 and Th2 cells are involved in IgG autoantibody production (278). On the other hand, another study involving IFN-gamma or IL-4-encoding plasmids in B/W mice demonstrated that only the overexpression of IFN-gamma leads to an exacerbation of the phenotype while overexpressing IL-4 had no effect (279). In fact, in a study with BXSB mice that harbored a deletion of the IL-4 receptor, IL-4 was demonstrated not to play a role at all in the development of autoimmunity (280). Therefore, the diminished IL-4 response in the *Sle1b* model may or may not have anything to do with the loss of tolerance to nuclear antigens.

The polymorphisms of gene clusters involved in the regulation of the immune system such as the Slam/CD2 gene family may be the consequence of a pathogen-driven selection. Such selection would favor the maintenance of polymorphisms that diversify the immune response against pathogens (281). The engagement of several of the Slam/CD2 family members, such as Cd224, Cd84, Cd150, and Ly108 leads to IFN-gamma production (282-286), so one would predict that this family plays a significant role in viral immunity. Indeed, there are several studies that have associated polymorphisms of the Slam/CD2 family with resistance to certain viral pathogens, including a prominent role in the regulation of Epstein Barr Virus (EBV) infections (287). Intriguingly, EBV has

been suggested to be a possible environmental trigger for SLE, and that raises the possibility that polymorphisms in the Slam/CD2 family could lead to variations in the response to EBV infections, which could potentiate susceptibility to SLE (288-290).

While there is not an EBV homologue in the mouse, it has been demonstrated that certain infections in the B/W mice improve their autoimmune phenotype. Chen et al. recently reported that infection with Toxoplasma gondii protected B/W mice from developing lupus nephritis (291). B/W mice infected with T. gondii exhibited a reduction in the expression of IFN-gamma in the spleens as well as a concomitant reduction in the levels of IgG2a anti-DNA antibody and IgG2a glomerular deposition (292). Not only that, there have also been studies that support a protective role against malarial infections in B/W mice (293-295). However, in contrast to the T. gondii model, it was reported that infected mice exhibited an increase in the levels of Th2 cytokines, but no changes in IFN-gamma were detected (295). Conclusions as to whether or not the overall levels of IFN-gamma or IL-4 or both are related to the Slam/CD2 haplotype cannot be clearly drawn, but it is tempting to postulate that this particular haplotype, which is found in both non-autoimmune and autoimmune strains of mice, can lead to autoimmunity in the context of permissive genomes. Such allelic variations of receptors that can be potentially harmful in the context of autoimmunity are being maintained because it confers protection against pathogens such as T. gondii and the Plasmodium species.

References

1. Blatt, N. B., and G. D. Glick. 1999. Anti-DNA autoantibodies and systemic lupus erythematosus. *Pharmacol. Ther.* 83:125.

2. Kotzin, B. L. 1996. Systemic lupus erythematosus. . *Cell* 85:303.

3. Sullivan, K. E. 2000. Genetics of systemic lupus erythematosus. Clinical implications. *Rheum Dis Clin North Am* 26:229.

4. Kotzin, B. L., and J. R. O'Dell. 1995. Systemic lupus erythematosus. In *Samter's Immunologic Diseases*. M. M. Frank, K. F. Austen, H. N. Claman, and E. R. Unanue, eds. Little, Brown & Co., Boston, pp. 667.

5. Pickering, M. C., and M. J. Walport. 2000. Links between complement abnormalities and systemic lupus erythematosus. *Rheumatology (Oxford)* 39:133.

6. Yang, Y., K. Lhotta, E. K. Chung, P. Eder, F. Neumair, and C. Y. Yu. 2004. Complete complement components C4A and C4B deficiencies in human kidney diseases and systemic lupus erythematosus. *J. Immunol.* 173:2803.

7. Johnson, G. C., and J. A. Todd. 2000. Strategies in complex disease mapping. *Curr Opin Genet Dev* 10:330.

8. Lindqvist, A. K., and M. E. Alarcon-Riquelme. 1999. The genetics of systemic lupus erythematosus. *Scand J Immunol* 50:562.

9. Johnson, G. C., and J. A. Todd. 2000. Strategies in complex disease mapping. *Curr Opin Genet Dev* 10:330.

10. Lindqvist, A. K., and M. E. Alarcon-Riquelme. 1999. The genetics of systemic lupus erythematosus. *Scand J Immunol* 50:562.

11. Gaffney, P. M., G. M. Kearns, K. B. Shark, W. A. Ortmann, S. A. Selby, M. L. Malmgren, K. E. Rohlf, T. C. Ockenden, R. P. Messner, S. Rich, and T. W. Behrens. 1998. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc Natl Acad Sci USA* 95:14875.

12. Moser, K. L., B. R. Neas, J. E. Salmon, H. Yu, C. Gray-McGuire, N. Asundi, G. R. Bruner, J. Fox, J. Kelly, S. Henshall, D. Bacino, M. Dietz, R. Hogue, G. Koelsch, L. Nightingale, T. Shaver, N. I. Abdou, D. A. Albert, C. Carson, M. Petri, E. L. Treadwell, J. A. James, and J. B. Harley. 1998. Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in african-american pedigrees. *Proc Natl Acad Sci USA* 95:14869.

13. Shai, R., F. Quismorio Jr., L. Li, O.-J. Kwon, J. Morrison, D. Wallace, C. Neuwelt, C. Brautbar, W. Gauderman, and C. O. Jacob. 1999. Genome-

wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Human Molecular Genetics* 8:639.

14. Vyse, T. J., S. J. Rozzo, C. G. Drake, S. Izui, and B. L. Kotzin. 1997. Control of multiple autoantibodies linked with a lupus nephritis susceptibility locus in New Zealand black mice. *J Immunol* 158:5566.

15. Risch, N. J. 2000. Searching for genetic determinants in the new millennium. *Nature* 405:847.

16. Vyse, T. J., and B. L. Kotzin. 1998. Genetic susceptibility to systemic lupus erythematosus. *Annu Rev Immunol* 16:261.

17. Wakeland, E. K., K. Liu, R. R. Graham, and T. W. Behrens. 2001. Delineating the genetic basis of systemic lupus erythematosus. *Immun* 15:397.

18. Xie, S., S. Chang, P. Yang, C. Jacob, A. Kaliyaperumal, S. K. Datta, and C. Mohan. 2001. Genetic contributions of nonautoimmune SWR mice toward lupus nephritis. *J. Immunol.* 167:7141.

19. Morel, L., and E. K. Wakeland. 1998. Susceptibility to lupus nephritis in the NZB/W model system. *Curr. Op. Immun.* 10:718.

20. Xie, S., S. Chang, P. Yang, C. Jacob, A. Kaliyaperumal, S. K. Datta, and C. Mohan. 2001. Genetic contributions of nonautoimmune SWR mice toward lupus nephritis. *J. Immunol.* 167:7141.

21. Xie, S., S. Chang, P. Yang, C. Jacob, A. Kaliyaperumal, S. K. Datta, and C. Mohan. 2001. Genetic contributions of nonautoimmune SWR mice toward lupus nephritis. *J. Immunol.* 167:7141.

22. Wakeland, E. K., K. Liu, R. R. Graham, and T. W. Behrens. 2001. Delineating the genetic basis of systemic lupus erythematosus. *Immun* 15:397.

23. Xie, S., S. Chang, P. Yang, C. Jacob, A. Kaliyaperumal, S. K. Datta, and C. Mohan. 2001. Genetic contributions of nonautoimmune SWR mice toward lupus nephritis. *J. Immunol.* 167:7141.

24. Marrack, P., J. Kappler, and B. L. Kotzin. 2001. Autoimmune disease: why and where it occurs. *Nat. Med.* 7:899.

25. Morel, L., B. P. Croker, K. R. Blenman, C. Mohan, G. Huang, G. Gilkeson, and E. K. Wakeland. 2000. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97:6670.

26. Morel, L., C. Mohan, B. P. Croker, X.-H. Tian, and E. K. Wakeland. 1997. Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J. Immunol.* 158:6019.

27. Mohan, C., E. Alas, L. Morel, P. Yang, and E. K. Wakeland. 1998. Genetic dissection of SLE pathogenesis: *Sle1* on murine chromosome 1 leads to a

selective loss of tolerance to H2A/H2B/DNA subnucelosomes. *J. Clin. Invest.* 101:1362.

28. Mohan, C., L. Morel, P. Yang, and E. K. Wakeland. 1997. Genetic dissection of SLE pathogenesis: *Sle2* on murine chromosome 4 leads to B-cell hyperactivity. *Journal of Immunology* 159:454.

29. Mohan, C., Y. Yu, L. Morel, P. Yang, and E. K. Wakeland. 1999. Genetic dissection of SLE pathogenicity: *Sle3* on murine chromosome 7 impacts T cell activation, differentiation, and cell death. *Journal of Immunology* 162:6492.

30. Morel, L., B. P. Croker, K. R. Blenman, C. Mohan, G. Huang, G. Gilkeson, and E. K. Wakeland. 2000. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97:6670.

31. Morel, L., B. P. Croker, K. R. Blenman, C. Mohan, G. Huang, G. Gilkeson, and E. K. Wakeland. 2000. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97:6670.

32. Morel, L., X.-H. Tian, B. P. Croker, and E. K. Wakeland. 1999. Epistatic modifiers of autoimmunity in a murine model of lupus nephritis. *Immun* 11:131.

33. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

34. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

35. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

36. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

37. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

38. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

39. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr. Opin. Immunol.* 15:277.

40. Tsao, B. P., R. M. Cantor, C. Kalunian, C.-J. Chen, H. Badsha, R. Singh, D. J. Wallace, R. C. Kitridou, S. Chen, N. Shen, Y. W. Song, D. A. Isenberg, C.-L. Yu, B. H. Hahn, and J. I. Rotter. 1997. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* 99:725.

41. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

42. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

43. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

44. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

45. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

46. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

47. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

48. Howie, D., M. Simarro, J. Sayos, M. Guirado, J. Sancho, and C. Terhorst. 2002. Molecular dissection of the signaling and costimulatory functions of CD150 (SLAM): CD150/SAP binding and CD150-mediated costimulation. *Blood* 99:957.

49. Shlapatska, L. M., S. V. Mikhalap, A. G. Berdova, O. M. Zelensky, T. J. Yun, K. E. Nichols, E. A. Clark, and S. P. Sidorenko. 2001. CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *Jour. Imm.* 166:5480.

50. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

51. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

52. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

53. Garni-Wagner, B. A., A. Purohit, P. A. Mathew, M. Bennett, and V. Kumar. 1993. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J Immunol* 151:60.

54. Mathew, P. A., W. Garni, K. Land, A. Takashima, E. Stoneman, M. Bennett, and V. Kumar. 1993. Cloning and characterization of the 2B4 gene encoding a molecule associated with non-MHC-restricted killing mediated by activated natural killer cells and T cells. *J Immunol* 151:5328.

55. Nakajima, H., M. Cella, H. Langen, A. Friedlein, and M. Colonna. 1999. Activating interactions in human NK cell recognition: the role of 2B4-CD48. *Eur. J. Immunol.* 29:1676.

56. Brown, M. H., K. Boles, P. Anton van der Merwe, V. Kumar, P. A. Mathew, and A. N. Barclay. 1998. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med* 188:2083.

57. Latchman, Y., P. F. McKay, and H. Reiser. 1998. Identification of the 2B4 molecule as a counter-receptor for CD48. *J Immunol* 161:5809.

58. Stepp, S. E., J. D. Schatzle, M. Bennett, V. Kumar, and P. A. Mathew. 1999. Gene structure of the murine NK cell receptor 2B4: presence of two

alternatively spliced isoforms with distinct cytoplasmic domains. *Eur J Immunol* 29:2392.

59. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc Natl Acad Sci U S A* 96:3870.

60. Kambayashi, T., E. Assarsson, B. J. Chambers, and H. G. Ljunggren. 2001. Cutting edge: Regulation of CD8(+) T cell proliferation by 2B4/CD48 interactions. *J Immunol* 167:6706.

61. Lee, K. M., M. E. McNerney, S. E. Stepp, P. A. Mathew, J. D. Schatzle, M. Bennett, and V. Kumar. 2004. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J. Exp. Med.* 199:1245.

62. Lee, K. M., M. E. McNerney, S. E. Stepp, P. A. Mathew, J. D. Schatzle, M. Bennett, and V. Kumar. 2004. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J. Exp. Med.* 199:1245.

63. Veillette, A. 2004. SLAM Family Receptors Regulate Immunity with and without SAP-related Adaptors. *J. Exp. Med.* 199:1175.

64. Sandrin, M. S., T. P. Gumley, M. M. Henning, H. A. Vaughan, L. J. Gonez, J. A. Trapani, and I. F. McKenzie. 1992. Isolation and characterization of cDNA clones for mouse Ly-9. *J Immunol* 149:1636.

65. Romero, X., N. Zapater, V. Tovar, C. Ockeloen, and P. Engel. 2004. Cd229 (Ly9) cell-surface molecule functions as a homophilic receptor and relocalizes to the immune synapse.

66. Romero, X., N. Zapater, M. Calvo, S. G. Kalko, M. A. de la Fuente, V. Tovar, C. Ockeloen, P. Pizcueta, and P. Engel. 2005. CD229 (Ly9) Lymphocyte Cell Surface Receptor Interacts Homophilically through Its N-Terminal Domain and Relocalizes to the Immunological Synapse. *J. Immunol.* 174:7033.

67. Sandrin, M. S., T. P. Gumley, M. M. Henning, H. A. Vaughan, L. J. Gonez, J. A. Trapani, and I. F. McKenzie. 1992. Isolation and characterization of cDNA clones for mouse Ly-9. *J Immunol* 149:1636.

68. Wandstrat, A. E., C. Nguyen, N. Limaye, A. Y. Chan, S. Subramanian, X. H. Tian, Y. S. Yim, A. Pertsemlidis, H. R. Garner, Jr., L. Morel, and E. K. Wakeland. 2004. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity.* 21:769.

69. de la Fuente, M. A., V. Tovar, N. Villamor, N. Zapater, P. Pizcueta, E. Campo, J. Bosch, and P. Engel. 2001. Molecular characterization and expression of a novel human leukocyte cell-surface marker homologous to mouse Ly-9. *Blood* 97:3513.

70. Bouchon, A., M. Cella, H. L. Grierson, J. I. Cohen, and M. Colonna. 2001. Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family. *J. Immunol.* 167:5517.

71. Boles, K. S., and P. A. Mathew. 2001. Molecular cloning of CS1, a novel human natural killer cell receptor belonging to the CD2 subset of the immunoglobulin superfamily. *Immunogenetics* 52:302.

72. Tovar, V., V. J. del, N. Zapater, M. Martin, X. Romero, P. Pizcueta, J. Bosch, C. Terhorst, and P. Engel. 2002. Mouse novel Ly9: a new member of the expanding CD150 (SLAM) family of leukocyte cell-surface receptors. *Immunogenetics* 54:394.

73. Bouchon, A., M. Cella, H. L. Grierson, J. I. Cohen, and M. Colonna. 2001. Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family. *J. Immunol.* 167:5517.

74. Bouchon, A., M. Cella, H. L. Grierson, J. I. Cohen, and M. Colonna. 2001. Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family. *J. Immunol.* 167:5517.

75. Bouchon, A., M. Cella, H. L. Grierson, J. I. Cohen, and M. Colonna. 2001. Activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 family. *J. Immunol.* 167:5517.

76. Tovar, V., V. J. del, N. Zapater, M. Martin, X. Romero, P. Pizcueta, J. Bosch, C. Terhorst, and P. Engel. 2002. Mouse novel Ly9: a new member

of the expanding CD150 (SLAM) family of leukocyte cell-surface receptors. *Immunogenetics* 54:394.

77. Lee, K. M., M. E. McNerney, S. E. Stepp, P. A. Mathew, J. D. Schatzle, M. Bennett, and V. Kumar. 2004. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J. Exp. Med.* 199:1245.

78. Lee, J. K., K. S. Boles, and P. A. Mathew. 2004. Molecular and functional characterization of a CS1 (CRACC) splice variant expressed in human NK cells that does not contain immunoreceptor tyrosine-based switch motifs. *Eur. J. Immunol.* 34:2791.

79. Reiser, H. 1990. sgp-60, a signal-transducing glycoprotein concerned with T cell activation through the T cell receptor/CD3 complex. *J. Immunol.* 145:2077.

80. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

81. Gonzalez-Cabrero, J., N. R. de, L. Hernando, and J. Egido. 1991. Expression and synthesis of cross-reactive idiotypic antibodies by peripheral blood lymphocytes and their suppression by anti-idiotypes in patients with IgA nephropathy. *J. Immunol.* 147:4162.

82. Kato, K., M. Koyanagi, H. Okada, T. Takanashi, Y. W. Wong, A. F. Williams, K. Okumura, and H. Yagita. 1992. CD48 is a counter-receptor for mouse CD2 and is involved in T cell activation. *J Exp Med* 176:1241.

83. Brown, M. H., K. Boles, P. Anton van der Merwe, V. Kumar, P. A. Mathew, and A. N. Barclay. 1998. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med* 188:2083.

84. Reiser, H. 1990. sgp-60, a signal-transducing glycoprotein concerned with T cell activation through the T cell receptor/CD3 complex. *J. Immunol.* 145:2077.

85. Kato, K., M. Koyanagi, H. Okada, T. Takanashi, Y. W. Wong, A. F. Williams, K. Okumura, and H. Yagita. 1992. CD48 is a counter-receptor for mouse CD2 and is involved in T cell activation. *J Exp Med* 176:1241.

86. Gonzalez-Cabrero, J., C. J. Wise, Y. Latchman, G. J. Freeman, A. H. Sharpe, and H. Reiser. 1999. CD48-deficient mice have a pronounced defect in CD4(+) T cell activation. *Proc Natl Acad Sci U S A* 96:1019.

87. Gonzalez-Cabrero, J., C. J. Wise, Y. Latchman, G. J. Freeman, A. H. Sharpe, and H. Reiser. 1999. CD48-deficient mice have a pronounced defect in CD4(+) T cell activation. *Proc Natl Acad Sci U S A* 96:1019.

88. Sidorenko, S. P., and E. A. Clark. 1993. Characterization of a cell surface glycoprotein IPO-3, expressed on activated human B and T lymphocytes. *J. Immunol.* 151:4614.

89. Sidorenko, S. P., and E. A. Clark. 1993. Characterization of a cell surface glycoprotein IPO-3, expressed on activated human B and T lymphocytes. *J. Immunol.* 151:4614.

90. Cocks, B. G., C. C. Chang, J. M. Carballido, H. Yssel, V. de, and G. Aversa. 1995. A novel receptor involved in T-cell activation. *Nature* 376:260.

91. Castro, A. G., T. M. Hauser, B. G. Cocks, J. Abrams, S. Zurawski, T. Churakova, F. Zonin, D. Robinson, S. G. Tangye, G. Aversa, K. E. Nichols, J. E. De Vries, L. L. Lanier, and A. O'Garra. 1999. Molecular and functional characterization of mouse signaling lymphocytic activation molecule (SLAM): differential expression and responsiveness in Th1 and Th2 cells. *J Immunol* 163:5860.

92. Mavaddat, N., D. W. Mason, P. D. Atkinson, E. J. Evans, R. J. Gilbert, D. I. Stuart, J. A. Fennelly, A. N. Barclay, S. J. Davis, and M. H. Brown. 2000. Signaling lymphocytic activation molecule (CDw150) is homophilic but self-associates with very low affinity. *J Biol Chem* 275:28100.

93. Cocks, B. G., C. C. Chang, J. M. Carballido, H. Yssel, V. de, and G. Aversa. 1995. A novel receptor involved in T-cell activation. *Nature* 376:260.

94. Castro, A. G., T. M. Hauser, B. G. Cocks, J. Abrams, S. Zurawski, T. Churakova, F. Zonin, D. Robinson, S. G. Tangye, G. Aversa, K. E.

Nichols, J. E. De Vries, L. L. Lanier, and A. O'Garra. 1999. Molecular and functional characterization of mouse signaling lymphocytic activation molecule (SLAM): differential expression and responsiveness in Th1 and Th2 cells. *J Immunol* 163:5860.

95. Aversa, G., C. C. Chang, J. M. Carballido, B. G. Cocks, and J. E. De Vries. 1997. Engagement of the signaling lymphocytic activation molecule (SLAM) on activated T cells results in IL-2-independent, cyclosporin A-sensitive T cell proliferation and IFN-gamma production. *J. Immunol.* 158:4036.

96. Castro, A. G., T. M. Hauser, B. G. Cocks, J. Abrams, S. Zurawski, T. Churakova, F. Zonin, D. Robinson, S. G. Tangye, G. Aversa, K. E. Nichols, J. E. De Vries, L. L. Lanier, and A. O'Garra. 1999. Molecular and functional characterization of mouse signaling lymphocytic activation molecule (SLAM): differential expression and responsiveness in Th1 and Th2 cells. *J Immunol* 163:5860.

97. Howie, D., S. Okamoto, S. Rietdijk, K. Clarke, N. Wang, C. Gullo, J. P. Bruggeman, S. Manning, A. J. Coyle, E. Greenfield, V. Kuchroo, and C. Terhorst. 2002. The role of SAP in murine CD150 (SLAM)-mediated T-cell proliferation and interferon gamma production. *Blood* 100:2899.

98. Quiroga, M. F., G. J. Martinez, V. Pasquinelli, M. A. Costas, M. M. Bracco, A. Malbran, L. M. Olivares, P. A. Sieling, and V. E. Garcia. 2004. Activation of signaling lymphocytic activation molecule triggers a signaling

cascade that enhances Th1 responses in human intracellular infection. *J. Immunol.* 173:4120.

99. Wang, N., A. Satoskar, W. Faubion, D. Howie, S. Okamoto, S. Feske, C. Gullo, K. Clarke, M. R. Sosa, A. H. Sharpe, and C. Terhorst. 2004. The cell surface receptor SLAM controls T cell and macrophage functions. *J. Exp. Med.* 199:1255.

100. de la Fuente, M. A., P. Pizcueta, M. Nadal, J. Bosch, and P. Engel. 1997. CD84 leukocyte antigen is a new member of the Ig superfamily. *Blood* 90:2398.

101. de la Fuente, M. A., V. Tovar, P. Pizcueta, M. Nadal, J. Bosch, and P. Engel. 1999. Molecular cloning, characterization, and chromosomal localization of the mouse homologue of CD84, a member of the CD2 family of cell surface molecules. *Immunogenetics* 49:249.

102. Romero, X., D. Benitez, S. March, R. Vilella, M. Miralpeix, and P. Engel. 2004. Differential expression of SAP and EAT-2-binding leukocyte cell-surface molecules CD84, CD150 (SLAM), CD229 (Ly9) and CD244 (2B4). *Tissue Anti.* 64:132.

103. de la Fuente, M. A., V. Tovar, P. Pizcueta, M. Nadal, J. Bosch, and P. Engel. 1999. Molecular cloning, characterization, and chromosomal localization of the mouse homologue of CD84, a member of the CD2 family of cell surface molecules. *Immunogenetics* 49:249.

104. Martin, M., X. Romero, I. de, V. Tovar, N. Zapater, E. Esplugues, P. Pizcueta, J. Bosch, and P. Engel. 2001. CD84 functions as a homophilic adhesion molecule and enhances IFN-gamma secretion: adhesion is mediated by Ig-like domain 1. *J Immunol* 167:3668.

105. Tangye, S. G., K. E. Nichols, N. J. Hare, and B. C. van de Weerdt. 2003. Functional requirements for interactions between CD84 and Src homology 2 domain-containing proteins and their contribution to human T cell activation. *J. Immunol.* 171:2485.

106. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

107. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

108. Bottino, C., M. Falco, S. Parolini, E. Marcenaro, R. Augugliaro, S. Sivori, E. Landi, R. Biassoni, L. D. Notarangelo, L. Moretta, and A. Moretta. 2001. NTB-A [correction of GNTB-A], a novel SH2D1A-associated surface molecule contributing to the inability of natural killer cells to kill Epstein-Barr virus-infected B cells in X-linked lymphoproliferative disease. *J Exp Med* 194:235.

109. Wandstrat, A. E., C. Nguyen, N. Limaye, A. Y. Chan, S. Subramanian, X. H. Tian, Y. S. Yim, A. Pertsemlidis, H. R. Garner, Jr., L. Morel, and E. K.

Wakeland. 2004. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity.* 21:769.

110. Valdez, P. A., H. Wang, D. Seshasayee, C. M. van Lookeren, A. Gurney, W. P. Lee, and I. S. Grewal. 2004. NTB-A, a new activating receptor in T cells that regulates autoimmune disease. *J. Biol. Chem.* 279:18662.

111. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

112. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

113. Wandstrat, A. E., C. Nguyen, N. Limaye, A. Y. Chan, S. Subramanian, X. H. Tian, Y. S. Yim, A. Pertsemlidis, H. R. Garner, Jr., L. Morel, and E. K. Wakeland. 2004. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity.* 21:769.

114. Valdez, P. A., H. Wang, D. Seshasayee, C. M. van Lookeren, A. Gurney, W. P. Lee, and I. S. Grewal. 2004. NTB-A, a new activating receptor in T cells that regulates autoimmune disease. *J. Biol. Chem.* 279:18662.

115. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

116. Nagy, N., C. Cerboni, K. Mattsson, A. Maeda, P. Gogolak, J. Sumegi, A. Lanyi, L. Szekely, E. Carbone, G. Klein, and E. Klein. 2000. SH2D1A and SLAM protein expression in human lymphocytes and derived cell lines. *Int. J. Cancer* 88:439.

117. Hwang, P. M., C. Li, M. Morra, J. Lillywhite, D. R. Muhandiram, F. Gertler, C. Terhorst, L. E. Kay, T. Pawson, J. D. Forman-Kay, and S. C. Li. 2002. A "three-pronged" binding mechanism for the SAP/SH2D1A SH2 domain: structural basis and relevance to the XLP syndrome. *EMBO J.* 21:314.

118. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

119. Coffey, A. J., R. A. Brooksbank, O. Brandau, T. Oohashi, G. R. Howell, J. M. Bye, A. P. Cahn, J. Durham, P. Heath, P. Wray, R. Pavitt, J. Wilkinson, M. Leversha, E. Huckle, S. Shaw, A. Dunham, S. Rhodes, V. Schuster, G. Porta, L. Yin, P. Serafini, B. Sylla, M. Zollo, B. Franco, and D. R. Bentley. 1998. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* 20:129.

120. Nichols, K. E., D. P. Harkin, S. Levitz, M. Krainer, K. A. Kolquist, C. Genovese, A. Bernard, M. Ferguson, L. Zuo, E. Snyder, A. J. Buckler, C. Wise, J. Ashley, M. Lovett, M. B. Valentine, A. T. Look, W. Gerald, D. E. Housman, and D. A. Haber. 1998. Inactivating mutations in an SH2

domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc. Natl. Acad. Sci. U. S. A* 95:13765.

121. Czar, M. J., E. N. Kersh, L. A. Mijares, G. Lanier, J. Lewis, G. Yap, A. Chen, A. Sher, C. S. Duckett, R. Ahmed, and P. L. Schwartzberg. 2001. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. *Proc Natl Acad Sci U S A* 98:7449.

122. Wu, C., K. B. Nguyen, G. C. Pien, N. Wang, C. Gullo, D. Howie, M. R. Sosa, M. J. Edwards, P. Borrow, A. R. Satoskar, A. H. Sharpe, C. A. Biron, and C. Terhorst. 2001. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat Immunol* 2:410.

123. Cannons, J. L., L. J. Yu, B. Hill, L. A. Mijares, D. Dombroski, K. E. Nichols, A. Antonellis, G. A. Koretzky, K. Gardner, and P. L. Schwartzberg. 2004. SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity.* 21:693.

124. Cannons, J. L., L. J. Yu, B. Hill, L. A. Mijares, D. Dombroski, K. E. Nichols, A. Antonellis, G. A. Koretzky, K. Gardner, and P. L. Schwartzberg. 2004. SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity.* 21:693.

125. La, C. A., and N. Sarvetnick. 1999. The role of cytokines in autoimmunity. *Curr. Dir. Autoimmun.* 1:56.

126. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

127. Malek, T. R., and A. L. Bayer. 2004. Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* 4:665.

128. Dauphinee, M. J., S. B. Kipper, D. Wofsy, and N. Talal. 1981. Interleukin 2 deficiency is a common feature of autoimmune mice. *J. Immunol.* 127:2483.

129. Dauphinee, M. J., S. B. Kipper, D. Wofsy, and N. Talal. 1981. Interleukin 2 deficiency is a common feature of autoimmune mice. *J. Immunol.* 127:2483.

130. Kelley, V. E., G. N. Gaulton, M. Hattori, H. Ikegami, G. Eisenbarth, and T. B. Strom. 1988. Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. *J. Immunol.* 140:59.

131. Owen, K. L., T. Shibata, S. Izui, and S. E. Walker. 1989. Recombinant interleukin-2 therapy of systemic lupus erythematosus in the New Zealand black/New Zealand white mouse. *J. Biol. Response Mod.* 8:366.

132. Abe, C., A. Koyama, and T. Nishimura. 1987. Interleukin-2 induction, response and therapy on murine lupus lesions in the MRL/l strain. *Prog. Clin. Biol. Res.* 229:147.

133. Podolin, P. L., P. Denny, C. J. Lord, N. J. Hill, J. A. Todd, L. B. Peterson, L. S. Wicker, and P. A. Lyons. 1997. Congenic mapping of the Insulin-Dependent Diabetes (*Idd*) gene, *Idd10*, localizes two genes mediating the *Idd10* effect and eliminates the candidate *Fcrg1*. *J. Immunol.* 159:1835.

134. Ikegami, H., T. Fujisawa, T. Sakamoto, S. Makino, and T. Ogihara. 2004. Idd1 and Idd3 are necessary but not sufficient for development of type 1 diabetes in NOD mouse. *Diabetes Res. Clin. Pract.* 66 Suppl 1:S85-S90.

135. Sadlack, B., J. Lohler, H. Schorle, G. Klebb, H. Haber, E. Sickel, R. J. Noelle, and I. Horak. 1995. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur. J. Immunol.* 25:3053.

136. Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume. 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J. Leukoc. Biol.* 75:163.

137. Prud'homme, G. J., D. H. Kono, and A. N. Theofilopoulos. 1995. Quantitative polymerase chain reaction analysis reveals marked overexpression of interleukin-1 beta, interleukin-1 and interferon-gamma mRNA in the lymph nodes of lupus-prone mice. *Mol. Immunol.* 32:495.

138. Lin, L. C., Y. C. Chen, C. C. Chou, K. H. Hsieh, and B. L. Chiang. 1995. Dysregulation of T helper cell cytokines in autoimmune prone NZB x NZW F1 mice. *Scand. J. Immunol.* 42:466.

139. Jacob, C. O., P. H. van der Meide, and H. O. McDevitt. 1987. In vivo treatment of (NZB X NZW)F1 lupus-like nephritis with monoclonal antibody to gamma interferon. *J. Exp. Med.* 166:798.

140. Ozmen, L., D. Roman, M. Fountoulakis, G. Schmid, B. Ryffel, and G. Garotta. 1995. Experimental therapy of systemic lupus erythematosus: the treatment of NZB/W mice with mouse soluble interferon-gamma receptor inhibits the onset of glomerulonephritis. *Eur. J. Immunol.* 25:6.

141. Haas, C., B. Ryffel, and H. M. Le. 1998. IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB x NZW)F1 mice. *J. Immunol.* 160:3713.

142. Balomenos, D., R. Rumold, and A. N. Theofilopoulos. 1998. Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J Clin Invest* 101:364.

143. Krakowski, M., and T. Owens. 1996. Interferon-gamma confers resistance to experimental allergic encephalomyelitis. *Eur. J. Immunol.* 26:1641.

144. Liu, Y., and C. A. Janeway, Jr. 1990. Interferon gamma plays a critical role in induced cell death of effector T cell: a possible third mechanism of self-tolerance. *J. Exp. Med.* 172:1735.

145. Mihara, M., N. Takagi, Y. Takeda, and Y. Ohsugi. 1998. IL-6 receptor blockage inhibits the onset of autoimmune kidney disease in NZB/W F1 mice. *Clin. Exp. Immunol.* 112:397.

146. Hirano, T., K. Yasukawa, H. Harada, T. Taga, Y. Watanabe, T. Matsuda, S. Kashiwamura, K. Nakajima, K. Koyama, A. Iwamatsu, and . 1986. Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. *Nature* 324:73.

147. Suematsu, S., T. Matsuda, K. Aozasa, S. Akira, N. Nakano, S. Ohno, J. Miyazaki, K. Yamamura, T. Hirano, and T. Kishimoto. 1989. IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc. Natl. Acad. Sci. U. S. A* 86:7547.

148. Mihara, M., H. Fukui, Y. Koishihara, M. Saito, and Y. Ohsugi. 1990. Immunologic abnormality in NZB/W F1 mice. Thymus-independent expansion of B cells responding to interleukin-6. *Clin. Exp. Immunol.* 82:533.

149. Ryffel, B., B. D. Car, H. Gunn, D. Roman, P. Hiestand, and M. J. Mihatsch. 1994. Interleukin-6 exacerbates glomerulonephritis in (NZB x NZW)F1 mice. *Am. J. Pathol.* 144:927.

150. Tang, B., T. Matsuda, S. Akira, N. Nagata, S. Ikehara, T. Hirano, and T. Kishimoto. 1991. Age-associated increase in interleukin 6 in MRL/lpr mice. *Int. Immunol.* 3:273.

151. McMurray, R. W., R. W. Hoffman, W. Nelson, and S. E. Walker. 1997. Cytokine mRNA expression in the B/W mouse model of systemic lupus

erythematosus--analyses of strain, gender, and age effects. *Clin. Immunol. Immunopathol.* 84:260.

152. Liu, K., C. Liang, Z. Liang, K. Tus, and E. K. Wakeland. 2005. Sle1ab Mediates the Aberrant Activation of STAT3 and Ras-ERK Signaling Pathways in B Lymphocytes. *J. Immunol.* 174:1630.

153. Mihara, M., N. Takagi, Y. Takeda, and Y. Ohsugi. 1998. IL-6 receptor blockage inhibits the onset of autoimmune kidney disease in NZB/W F1 mice. *Clin. Exp. Immunol.* 112:397.

154. Finck, B. K., P. S. Linsley, and D. Wofsy. 1994. Treatment of murine lupus with CTLA4Ig. *Science* 265:1225.

155. Ryffel, B., B. D. Car, H. Gunn, D. Roman, P. Hiestand, and M. J. Mihatsch. 1994. Interleukin-6 exacerbates glomerulonephritis in (NZB x NZW)F1 mice. *Am. J. Pathol.* 144:927.

156. Mosmann, T. R., and S. Sad. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* 17:138.

157. McMurray, R. W., R. W. Hoffman, W. Nelson, and S. E. Walker. 1997. Cytokine mRNA expression in the B/W mouse model of systemic lupus erythematosus--analyses of strain, gender, and age effects. *Clin. Immunol. Immunopathol.* 84:260.

158. Lin, L. C., Y. C. Chen, C. C. Chou, K. H. Hsieh, and B. L. Chiang. 1995. Dysregulation of T helper cell cytokines in autoimmune prone NZB x NZW F1 mice. *Scand. J. Immunol.* 42:466.

159. Umland, S., R. Lee, M. Howard, and C. Martens. 1989. Expression of lymphokine genes in splenic lymphocytes of autoimmune mice. *Mol. Immunol.* 26:649.

160. Shirai, A., J. Conover, and D. M. Klinman. 1995. Increased activation and altered ratio of interferon-gamma: interleukin-4 secreting cells in MRL-lpr/lpr mice. *Autoimm.* 21:107.

161. Takahashi, S., L. Fossati, M. Iwamoto, R. Merino, R. Motta, T. Kobayakawa, and S. Izui. 1996. Imbalance towards Th1 predominance is associated with acceleration of lupus-like autoimmune syndrome in MRL mice. *J. Clin. Invest* 97:1597.

162. Santiago, M. L., L. Fossati, C. Jacquet, W. Muller, S. Izui, and L. Reininger. 1997. Interleukin-4 protects against a genetically linked lupus-like autoimmune syndrome. *J. Exp. Med.* 185:65.

163. Nakajima, A., S. Hirose, H. Yagita, and K. Okumura. 1997. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol.* 158:1466.

164. Peng, S. L., J. Moslehi, and J. Craft. 1997. Roles of interferon-gamma and interleukin-4 in murine lupus. *J. Clin. Invest.* 99:1936.

165. Beebe, A. M., D. J. Cua, and M. R. de Waal. 2002. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). *Cytokine Growth Factor Rev.* 13:403.

166. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

167. Chong, W. P., W. K. Ip, W. H. Wong, C. S. Lau, T. M. Chan, and Y. L. Lau. 2004. Association of interleukin-10 promoter polymorphisms with systemic lupus erythematosus. *Genes Immun.* 5:484.

168. Prud'homme, G. J., D. H. Kono, and A. N. Theofilopoulos. 1995. Quantitative polymerase chain reaction analysis reveals marked overexpression of interleukin-1 beta, interleukin-1 and interferon-gamma mRNA in the lymph nodes of lupus-prone mice. *Mol. Immunol.* 32:495.

169. Ozaki, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F. Qi, P. Hwu, D. J. Shaffer, S. Akilesh, D. C. Roopenian, H. C. Morse, III, P. E. Lipsky, and W. J. Leonard. 2004. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J. Immunol.* 173:5361.

170. Ishida, T., M. Inaba, H. Hisha, K. Sugiura, Y. Adachi, N. Nagata, R. Ogawa, R. A. Good, and S. Ikehara. 1994. Requirement of Donor-Derived Stomal Cells in the Bone Marrow for Successful Allogeneic Bone Marrow

Transplantation - Complete Prevention of Recurrence of Autoimmune Diseases in MRL/MP-Lpr/Lpr Mice by Transplantation of Bone Marrow Plus Bones (Stromal Cells) from the Same Donor. *J. Immunol.* 152:3119.

171. Beebe, A. M., D. J. Cua, and M. R. de Waal. 2002. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). *Cytokine Growth Factor Rev.* 13:403.

172. Aringer, M., and J. S. Smolen. 2004. Tumour necrosis factor and other proinflammatory cytokines in systemic lupus erythematosus: a rationale for therapeutic intervention. *Lupus* 13:344.

173. Jacob, C. O., and H. O. McDevitt. 1988. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 331:356.

174. Jacob, C. O., and H. O. McDevitt. 1988. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 331:356.

175. Boswell, J. M., M. A. Yui, D. W. Burt, and V. E. Kelley. 1988. Increased tumor necrosis factor and IL-1 beta gene expression in the kidneys of mice with lupus nephritis. *J. Immunol.* 141:3050.

176. Moore, K. J., K. Yeh, T. Naito, and V. R. Kelley. 1996. TNF-alpha enhances colony-stimulating factor-1-induced macrophage accumulation in autoimmune renal disease. *J. Immunol.* 157:427.

177. Brennan, D. C., M. A. Yui, R. P. Wuthrich, and V. E. Kelley. 1989. Tumor necrosis factor and IL-1 in New Zealand Black/White mice. Enhanced gene expression and acceleration of renal injury. *J. Immunol.* 143:3470.

178. Moore, K. J., K. Yeh, T. Naito, and V. R. Kelley. 1996. TNF-alpha enhances colony-stimulating factor-1-induced macrophage accumulation in autoimmune renal disease. *J. Immunol.* 157:427.

179. Brennan, D. C., M. A. Yui, R. P. Wuthrich, and V. E. Kelley. 1989. Tumor necrosis factor and IL-1 in New Zealand Black/White mice. Enhanced gene expression and acceleration of renal injury. *J. Immunol.* 143:3470.

180. Brennan, D. C., M. A. Yui, R. P. Wuthrich, and V. E. Kelley. 1989. Tumor necrosis factor and IL-1 in New Zealand Black/White mice. Enhanced gene expression and acceleration of renal injury. *J. Immunol.* 143:3470.

181. Jacob, C. O., and H. O. McDevitt. 1988. Tumour necrosis factor-alpha in murine autoimmune 'lupus' nephritis. *Nature* 331:356.

182. Grewal, I. S., K. D. Grewal, F. S. Wong, D. E. Picarella, C. A. Janeway, Jr., and R. A. Flavell. 1996. Local expression of transgene encoded TNFalpha in islets prevents autoimmune diabetes in nonobese diabetic (NOD) mice by preventing the development of auto-reactive islet-specific T cells. *J. Exp. Med.* 184:1963.

183. Hunger, R. E., C. Carnaud, I. Garcia, P. Vassalli, and C. Mueller. 1997. Prevention of autoimmune diabetes mellitus in NOD mice by transgenic

expression of soluble tumor necrosis factor receptor p55. *Eur. J. Immunol.* 27:255.

184. Aggarwal, S., and A. L. Gurney. 2002. IL-17: prototype member of an emerging cytokine family. *J. Leukoc. Biol.* 71:1.

185. Wong, C. K., C. Y. Ho, E. K. Li, and C. W. Lam. 2000. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. *Lupus* 9:589.

186. Aggarwal, S., and A. L. Gurney. 2002. IL-17: prototype member of an emerging cytokine family. *J. Leukoc. Biol.* 71:1.

187. Witowski, J., K. Ksiazek, and A. Jorres. 2004. Interleukin-17: a mediator of inflammatory responses. *Cell Mol. Life Sci.* 61:567.

188. Parrish-Novak, J., S. R. Dillon, A. Nelson, A. Hammond, C. Sprecher, J. A. Gross, J. Johnston, K. Madden, W. Xu, J. West, S. Schrader, S. Burkhead, M. Heipel, C. Brandt, J. L. Kuijper, J. Kramer, D. Conklin, S. R. Presnell, J. Berry, F. Shiota, S. Bort, K. Hambly, S. Mudri, C. Clegg, M. Moore, F. J. Grant, C. Lofton-Day, T. Gilbert, F. Rayond, A. Ching, L. Yao, D. Smith, P. Webster, T. Whitmore, M. Maurer, K. Kaushansky, R. D. Holly, and D. Foster. 2000. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* 408:57.

189. Ozaki, K., R. Spolski, C. G. Feng, C. F. Qi, J. Cheng, A. Sher, H. C. Morse, III, C. Liu, P. L. Schwartzberg, and W. J. Leonard. 2002. A critical role for IL-21 in regulating immunoglobulin production. *Science* 298:1630.

190. Ozaki, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F. Qi, P. Hwu, D. J. Shaffer, S. Akilesh, D. C. Roopenian, H. C. Morse, III, P. E. Lipsky, and W. J. Leonard. 2004. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J. Immunol.* 173:5361.

191. Mosmann, T. R., and R. L. Coffman. 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7:145.

192. Romagnani, S. 1997. The Th1/Th2 paradigm. *Immunol. Today* 18:263.

193. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

194. Liblau, R. S., S. M. Singer, and H. O. McDevitt. 1995. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today* 16:34.

195. Takahashi, S., L. Fossati, M. Iwamoto, R. Merino, R. Motta, T. Kobayakawa, and S. Izui. 1996. Imbalance towards Th1 predominance is

associated with acceleration of lupus-like autoimmune syndrome in MRL mice. *J. Clin. Invest* 97:1597.

196. Nakajima, A., S. Hirose, H. Yagita, and K. Okumura. 1997. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol.* 158:1466.

197. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

198. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

199. Theofilopoulos, A. N., S. Koundouris, D. H. Kono, and B. R. Lawson. 2001. The role of IFN-gamma in systemic lupus erythematosus: a challenge to the Th1/Th2 paradigm in autoimmunity. *Arthritis Res* 3:136.

200. Prud'homme, G. J., D. H. Kono, and A. N. Theofilopoulos. 1995. Quantitative polymerase chain reaction analysis reveals marked overexpression of interleukin-1 beta, interleukin-1 and interferon-gamma mRNA in the lymph nodes of lupus-prone mice. *Mol. Immunol.* 32:495.

201. Balomenos, D., R. Rumold, and A. N. Theofilopoulos. 1998. Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J Clin Invest* 101:364.

202. Nakajima, A., S. Hirose, H. Yagita, and K. Okumura. 1997. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol.* 158:1466.

203. Mosmann, T. 2000. Complexity or coherence? Cytokine secretion by B cells. *Nat. Immunol.* 1:465.

204. Wakeland, E. K., L. Morel, K. Achey, M. Yui, and J. Longmate. 1997. Speed congenics: A classic technique moves into the fast lane (relatively speaking). *Immunol. Today.* 18:473.

205. Osoegawa, K., M. Tateno, P. Y. Woon, E. Frengen, A. G. Mammoser, J. J. Catanese, Y. Hayashizaki, and J. de. 2000. Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. *Genome Res* 10:116.

206. Shizuya, H., B. Birren, U. J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. *Proc. Natl. Acad. Sci. U. S. A* 89:8794.

207. Stanier, P., H. Abu, J. N. Murdoch, J. Eddleston, and A. J. Copp. 1998. Paralogous sm22alpha (Tagln) genes map to mouse chromosomes 1 and 9: further evidence for a paralogous relationship. *Genomics* 51:144.

208. Vandenberghe, P. A., and J. L. Ceuppens. 1990. Flow cytometric measurement of cytoplasmic free calcium in human peripheral blood T

lymphocytes with fluo-3, a new fluorescent calcium indicator. *J. Immunol. Methods* 127:197.

209. Rock, K. L., and B. Benacerraf. 1983. Inhibition of Antigen-Specific T Lymphocyte Activation by Structurally Related Ir Gene-Controlled Polymers. *J. Exp. Med.* 157:1618.

210. Wandstrat, A. E., and E. K. Wakeland. 2001. The Genetics of Complex Autoimmune Diseases: non-MHC Susceptibility Genes. *Nature Immunology* 2:802.

211. Wakeland, E. K., K. Liu, R. R. Graham, and T. W. Behrens. 2001. Delineating the genetic basis of systemic lupus erythematosus. *Immun* 15:397.

212. Vyse, T. J., and J. A. Todd. 1996. Genetic analysis of autoimmune disease. . *Cell* 85:311.

213. Morel, L., U. H. Rudofsky, J. A. Longmate, J. Schiffenbauer, and E. K. Wakeland. 1994. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immun* 1(3):219.

214. Morel, L., B. P. Croker, K. R. Blenman, C. Mohan, G. Huang, G. Gilkeson, and E. K. Wakeland. 2000. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97:6670.

215. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

216. Croker, B. P., G. Gilkeson, and L. Morel. 2003. Genetic interactions between susceptibility loci reveal epistatic pathogenic networks in murine lupus. *Genes Immun.* 4:575.

217. Sobel, E. S., C. Mohan, L. Morel, J. Schiffenbauer, and E. K. Wakeland. 1999. Genetic dissection of SLE pathogenesis: Adoptive transfer of *Sle1* mediates the loss of tolerance by bone marrow-derived B cells. *Journal of Immunology* 162:2415.

218. Sobel, E. S., M. Satoh, Y. Chen, E. K. Wakeland, and L. Morel. 2002. The major murine systemic lupus erythematosus susceptibility locus Sle1 results in abnormal functions of both B and T cells. *J. Immunol.* 169:2694.

219. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr. Opin. Immunol.* 15:277.

220. Veillette, A. 2004. SLAM Family Receptors Regulate Immunity with and without SAP-related Adaptors. *J. Exp. Med.* 199:1175.

221. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

222. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr. Opin. Immunol.* 15:277.

223. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin Immunol* 12:149.

224. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

225. Osoegawa, K., M. Tateno, P. Y. Woon, E. Frengen, A. G. Mammoser, J. J. Catanese, Y. Hayashizaki, and J. de. 2000. Bacterial artificial chromosome libraries for mouse sequencing and functional analysis. *Genome Res* 10:116.

226. Shizuya, H., B. Birren, U. J. Kim, V. Mancino, T. Slepak, Y. Tachiiri, and M. Simon. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. *Proc. Natl. Acad. Sci. U. S. A* 89:8794.

227. Stanier, P., H. Abu, J. N. Murdoch, J. Eddleston, and A. J. Copp. 1998. Paralogous sm22alpha (Tagln) genes map to mouse chromosomes 1 and 9: further evidence for a paralogous relationship. *Genomics* 51:144.

228. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

229. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

230. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

231. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

232. Mock, B. A., J. Hartley, P. Le Tissier, J. S. Wax, and M. Potter. 1997. The plasmacytoma resistance gene, *Pctr2*, delays the onset of tumorigenesis and resides in the telomeric region of chromosome 4. *Blood* 10:4092.

233. Peck, S. R., and H. E. Ruley. 2000. Ly108: a new member of the mouse CD2 family of cell surface proteins. *Immunogenetics* 52:63.

234. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

235. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr. Opin. Immunol.* 15:277.

236. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

237. Reininger, L., T. H. Winkler, C. P. Kalberer, M. Jourdan, F. Melchers, and A. G. Rolink. 1996. Intrinsic B cell defects in NZB and NZW mice contribute to systemic lupus erythematosus in (NZB X NZW)F1 mice. *J. Exp. Med.* 184:853.

238. Vratsanos, G. S., S. Jung, Y. M. Park, and J. Craft. 2001. CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: a model to explain spontaneous T cell activation in lupus. *J. Exp. Med.* 193:329.

239. Huang, Y. P., L. H. Perrin, P. A. Miescher, and R. H. Zubler. 1988. Correlation of T and B cell activities in vitro and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol.* 141:827.

240. Yi, Y., M. McNerney, and S. K. Datta. 2000. Regulatory defects in Cbl and mitogen-activated protein kinase (extracellular signal-related kinase) pathways cause persistent hyperexpression of CD40 ligand in human lupus T cells. *J. Immunol.* 165:6627.

241. Morel, L., K. R. Blenman, B. P. Croker, and E. K. Wakeland. 2001. The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98:1787.

242. Vassilopoulos, D., B. Kovacs, and G. C. Tsokos. 1995. TCR/CD3 complex-mediated signal transduction pathway in T cells and T cell lines from patients with systemic lupus erythematosus. *J. Immunol.* 155:2269.

243. Liossis, S. N., X. Z. Ding, G. J. Dennis, and G. C. Tsokos. 1998. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. *J. Clin. Invest* 101:1448.

244. De Albuquerque, D. A., F. M. Ebling, D. W. Lienesch, R. R. Singh, and B. H. Hahn. 2004. Analysis of serum levels of type 1, type 2, and type 3 cytokines reveal multiple abnormalities in lupus-prone (NZB X NZW) F1 mice., pp. 3.

245. Cannons, J. L., L. J. Yu, B. Hill, L. A. Mijares, D. Dombroski, K. E. Nichols, A. Antonellis, G. A. Koretzky, K. Gardner, and P. L. Schwartzberg. 2004. SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity.* 21:693.

246. Davidson, D., X. Shi, S. Zhang, H. Wang, M. Nemer, N. Ono, S. Ohno, Y. Yanagi, and A. Veillette. 2004. Genetic evidence linking SAP, the X-linked lymphoproliferative gene product, to Src-related kinase FynT in T(H)2 cytokine regulation. *Immunity.* 21:707.

247. Jenkins, M. K., C. A. Chen, G. Jung, D. L. Mueller, and R. H. Schwartz. 1990. Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J. Immunol.* 144:16.

248. Jenkins, M. K., C. A. Chen, G. Jung, D. L. Mueller, and R. H. Schwartz. 1990. Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J. Immunol.* 144:16.

249. Murphy, K. M., and S. L. Reiner. 2002. The lineage decisions of helper T cells. *Nat. Rev. Immunol.* 2:933.

250. Chong, W. P., W. K. Ip, W. H. Wong, C. S. Lau, T. M. Chan, and Y. L. Lau. 2004. Association of interleukin-10 promoter polymorphisms with systemic lupus erythematosus. *Genes Immun.* 5:484.

251. D', A. S., M. Giordano, M. Mellai, M. Lanceni, N. Barizzone, M. Marchini, R. Scorza, M. G. Danieli, M. Cappelli, P. Rovere, M. G. Sabbadini, and P. Momigliano-Richiardi. 2002. Association tests with systemic lupus erythematosus (SLE) of IL10 markers indicate a direct involvement of a CA repeat in the 5' regulatory region. *Genes Immun.* 3:454.

252. Beebe, A. M., D. J. Cua, and M. R. de Waal. 2002. The role of interleukin-10 in autoimmune disease: systemic lupus erythematosus (SLE) and multiple sclerosis (MS). *Cytokine Growth Factor Rev.* 13:403.

253. Alecu, M., G. Coman, and S. Alecu. 2000. Serological levels of apoptotic bodies, sFAS and TNF in lupus erythematosus. *Rom. J. Intern. Med.* 38-39:83.

254. Ozaki, K., R. Spolski, R. Ettinger, H. P. Kim, G. Wang, C. F. Qi, P. Hwu, D. J. Shaffer, S. Akilesh, D. C. Roopenian, H. C. Morse, III, P. E. Lipsky, and W. J. Leonard. 2004. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J. Immunol.* 173:5361.

255. Engel, P., M. J. Eck, and C. Terhorst. 2003. The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat. Rev. Immunol.* 3:813.

256. Veillette, A., and S. Latour. 2003. The SLAM family of immune-cell receptors. *Curr. Opin. Immunol.* 15:277.

257. Valdez, P. A., H. Wang, D. Seshasayee, C. M. van Lookeren, A. Gurney, W. P. Lee, and I. S. Grewal. 2004. NTB-A, a new activating receptor in T cells that regulates autoimmune disease. *J. Biol. Chem.* 279:18662.

258. Harber, M., A. Sundstedt, and D. Wraith. 2000. The role of cytokines in immunological tolerance: potential for therapy. *Expert. Rev. Mol. Med.* 2000:1.

259. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc Natl Acad Sci U S A* 96:3870.

260. Blomhoff, A., B. A. Lie, A. G. Myhre, E. H. Kemp, A. P. Weetman, H. E. Akselsen, E. S. Huseby, and D. E. Undlien. 2004. Polymorphisms in the

Cytotoxic T Lymphocyte Antigen-4 Gene Region Confer Susceptibility to Addison's Disease. *J. Clin. Endocrinol. Metab* 89:3474.

261. Ueda, H., J. M. Howson, L. Esposito, J. Heward, H. Snook, G. Chamberlain, D. B. Rainbow, K. M. Hunter, A. N. Smith, G. G. Di, M. H. Herr, I. Dahlman, F. Payne, D. Smyth, C. Lowe, R. C. Twells, S. Howlett, B. Healy, S. Nutland, H. E. Rance, V. Everett, L. J. Smink, A. C. Lam, H. J. Cordell, N. M. Walker, C. Bordin, J. Hulme, C. Motzo, F. Cucca, J. F. Hess, M. L. Metzker, J. Rogers, S. Gregory, A. Allahabadia, R. Nithiyananthan, E. Tuomilehto-Wolf, J. Tuomilehto, P. Bingley, K. M. Gillespie, D. E. Undlien, K. S. Ronningen, C. Guja, C. Ionescu-Tirgoviste, D. A. Savage, A. P. Maxwell, D. J. Carson, C. C. Patterson, J. A. Franklyn, D. G. Clayton, L. B. Peterson, L. S. Wicker, J. A. Todd, and S. C. Gough. 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423:506.

262. Jacobsen, M., D. Schweer, A. Ziegler, R. Gaber, S. Schock, R. Schwinzer, K. Wonigeit, R. B. Lindert, O. Kantarci, J. Schaefer-Klein, H. I. Schipper, W. H. Oertel, F. Heidenreich, B. G. Weinshenker, N. Sommer, and B. Hemmer. 2000. A point mutation in PTPRC is associated with the development of multiple sclerosis. *Nat. Genet.* 26:495.

263. Jacobsen, M., D. Schweer, A. Ziegler, R. Gaber, S. Schock, R. Schwinzer, K. Wonigeit, R. B. Lindert, O. Kantarci, J. Schaefer-Klein, H. I. Schipper, W. H. Oertel, F. Heidenreich, B. G. Weinshenker, N. Sommer, and B.

Hemmer. 2000. A point mutation in PTPRC is associated with the development of multiple sclerosis. *Nat. Genet.* 26:495.

264. Muschen, M., U. Warskulat, A. Perniok, J. Even, C. Moers, B. Kismet, N. Temizkan, D. Simon, M. Schneider, and D. Haussinger. 1999. Involvement of soluble CD95 in Churg-Strauss syndrome. *Am. J. Pathol.* 155:915.

265. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc. Natl. Acad. Sci. U. S. A* 96:3870.

266. Howie, D., F. S. Laroux, M. Morra, A. R. Satoskar, L. E. Rosas, W. A. Faubion, A. Julien, S. Rietdijk, A. J. Coyle, C. Fraser, and C. Terhorst. 2005. Cutting edge: the SLAM family receptor Ly108 controls T cell and neutrophil functions. *J. Immunol.* 174:5931.

267. Huang, Y. P., L. H. Perrin, P. A. Miescher, and R. H. Zubler. 1988. Correlation of T and B cell activities in vitro and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol.* 141:827.

268. Yi, Y., M. McNerney, and S. K. Datta. 2000. Regulatory defects in Cbl and mitogen-activated protein kinase (extracellular signal-related kinase) pathways cause persistent hyperexpression of CD40 ligand in human lupus T cells. *J. Immunol.* 165:6627.

269. Liossis, S. N., X. Z. Ding, G. J. Dennis, and G. C. Tsokos. 1998. Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus. Deficient expression of the T cell receptor zeta chain. *J. Clin. Invest* 101:1448.

270. Rioux, J. D., M. J. Daly, M. S. Silverberg, K. Lindblad, H. Steinhart, Z. Cohen, T. Delmonte, K. Kocher, K. Miller, S. Guschwan, E. J. Kulbokas, S. O'Leary, E. Winchester, K. Dewar, T. Green, V. Stone, C. Chow, A. Cohen, D. Langelier, G. Lapointe, D. Gaudet, J. Faith, N. Branco, S. B. Bull, R. S. McLeod, A. M. Griffiths, A. Bitton, G. R. Greenberg, E. S. Lander, K. A. Siminovitch, and T. J. Hudson. 2001. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat. Genet.* 29:223.

271. Wang, N., A. Satoskar, W. Faubion, D. Howie, S. Okamoto, S. Feske, C. Gullo, K. Clarke, M. R. Sosa, A. H. Sharpe, and C. Terhorst. 2004. The cell surface receptor SLAM controls T cell and macrophage functions. *J. Exp. Med.* 199:1255.

272. Howie, D., F. S. Laroux, M. Morra, A. R. Satoskar, L. E. Rosas, W. A. Faubion, A. Julien, S. Rietdijk, A. J. Coyle, C. Fraser, and C. Terhorst. 2005. Cutting edge: the SLAM family receptor Ly108 controls T cell and neutrophil functions. *J. Immunol.* 174:5931.

273. Cannons, J. L., L. J. Yu, B. Hill, L. A. Mijares, D. Dombroski, K. E. Nichols, A. Antonellis, G. A. Koretzky, K. Gardner, and P. L. Schwartzberg. 2004.

SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. *Immunity.* 21:693.

274. Davidson, D., X. Shi, S. Zhang, H. Wang, M. Nemer, N. Ono, S. Ohno, Y. Yanagi, and A. Veillette. 2004. Genetic evidence linking SAP, the X-linked lymphoproliferative gene product, to Src-related kinase FynT in T(H)2 cytokine regulation. *Immunity.* 21:707.

275. Brogdon, J. L., D. Leitenberg, and K. Bottomly. 2002. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. *J. Immunol.* 168:3825.

276. Brogdon, J. L., D. Leitenberg, and K. Bottomly. 2002. The potency of TCR signaling differentially regulates NFATc/p activity and early IL-4 transcription in naive CD4+ T cells. *J. Immunol.* 168:3825.

277. Theofilopoulos, A. N., and B. R. Lawson. 1999. Tumour necrosis factor and other cytokines in murine lupus. *Ann Rheum Dis* 58 Suppl 1:I49-I55.

278. Nakajima, A., S. Hirose, H. Yagita, and K. Okumura. 1997. Roles of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol.* 158:1466.

279. Hasegawa, K., T. Hayashi, and K. Maeda. 2002. Promotion of lupus in NZB x NZWF1 mice by plasmids encoding interferon (IFN)-gamma but not by those encoding interleukin (IL)-4. *J. Comp Pathol.* 127:1.

280. Kono, D. H., D. Balomenos, M. S. Park, and A. N. Theofilopoulos. 2000. Development of lupus in BXSB mice is independent of IL-4. *J Immunol* 164:38.

281. Slev, P. R., and W. K. Potts. 2002. Disease consequences of pathogen adaptation. *Curr. Opin. Immunol.* 14:609.

282. Chuang, S. S., M. H. Kim, L. A. Johnson, P. Albertsson, R. P. Kitson, U. Nannmark, R. H. Goldfarb, and P. A. Mathew. 2000. 2B4 stimulation of YT cells induces natural killer cell cytolytic function and invasiveness. *Immunology* 100:378.

283. Martin, M., X. Romero, I. de, V. Tovar, N. Zapater, E. Esplugues, P. Pizcueta, J. Bosch, and P. Engel. 2001. CD84 functions as a homophilic adhesion molecule and enhances IFN-gamma secretion: adhesion is mediated by Ig-like domain 1. *J Immunol* 167:3668.

284. Howie, D., S. Okamoto, S. Rietdijk, K. Clarke, N. Wang, C. Gullo, J. P. Bruggeman, S. Manning, A. J. Coyle, E. Greenfield, V. Kuchroo, and C. Terhorst. 2002. The role of SAP in murine CD150 (SLAM)-mediated T-cell proliferation and interferon gamma production. *Blood* 100:2899.

285. Falco, M., E. Marcenaro, E. Romeo, F. Bellora, D. Marras, F. Vely, G. Ferracci, L. Moretta, A. Moretta, and C. Bottino. 2004. Homophilic interaction of NTBA, a member of the CD2 molecular family: induction of

cytotoxicity and cytokine release in human NK cells. *Eur. J. Immunol.* 34:1663.

286. Valdez, P. A., H. Wang, D. Seshasayee, C. M. van Lookeren, A. Gurney, W. P. Lee, and I. S. Grewal. 2004. NTB-A, a new activating receptor in T cells that regulates autoimmune disease. *J. Biol. Chem.* 279:18662.

287. Sidorenko, S. P., and E. A. Clark. 2003. The dual-function CD150 receptor subfamily: the viral attraction. *Nat. Immunol.* 4:19.

288. James, J. A., B. R. Neas, K. L. Moser, T. Hall, G. R. Bruner, A. L. Sestak, and J. B. Harley. 2001. Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum* 44:1122.

289. Moon, U. Y., S. J. Park, S. T. Oh, W. U. Kim, S. H. Park, S. H. Lee, C. S. Cho, H. Y. Kim, W. K. Lee, and S. K. Lee. 2004. Patients with systemic lupus erythematosus have abnormally elevated Epstein-Barr virus load in blood. *Arthritis Res. Ther.* 6:R295-R302.

290. Kang, I., T. Quan, H. Nolasco, S. H. Park, M. S. Hong, J. Crouch, E. G. Pamer, J. G. Howe, and J. Craft. 2004. Defective control of latent Epstein-Barr virus infection in systemic lupus erythematosus. *J. Immunol.* 172:1287.

291. Chen, M., F. Aosai, K. Norose, H. S. Mun, H. Ishikura, S. Hirose, L. X. Piao, H. Fang, and A. Yano. 2004. Toxoplasma gondii infection inhibits the

development of lupus-like syndrome in autoimmune (New Zealand Black x New Zealand White) F1 mice. *Int. Immunol.* 16:937.

292. Chen, M., F. Aosai, K. Norose, H. S. Mun, H. Ishikura, S. Hirose, L. X. Piao, H. Fang, and A. Yano. 2004. Toxoplasma gondii infection inhibits the development of lupus-like syndrome in autoimmune (New Zealand Black x New Zealand White) F1 mice. *Int. Immunol.* 16:937.

293. Greenwood, B. M., and A. Voller. 1970. Suppression of autoimmune disease in New Zealand mice associated with infection with malaria. I. (NZBxNZW) F1 hybrid mice. *Clin. Exp. Immunol.* 7:793.

294. Hentati, B., M. N. Sato, B. Payelle-Brogard, S. Avrameas, and T. Ternynck. 1994. Beneficial effect of polyclonal immunoglobulins from malaria-infected BALB/c mice on the lupus-like syndrome of (NZB x NZW)F1 mice. *Eur. J. Immunol.* 24:8.

295. Yu, G., M. Nishimura, S. Arawaka, D. Levitan, L. Zhang, A. Tandon, Y. Q. Song, E. Rogaeva, F. Chen, T. Kawarai, A. Supala, L. Levesque, H. Yu, D. S. Yang, E. Holmes, P. Milman, Y. Liang, D. M. Zhang, D. H. Xu, C. Sato, E. Rogaev, M. Smith, C. Janus, Y. Zhang, R. Aebersold, L. S. Farrer, S. Sorbi, A. Bruni, P. Fraser, and P. St George-Hyslop. 2000. Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaATP processing. *Nature* 407:48.

VITAE

Charles Minh Nguyen was born in Wichita Falls, Texas, on September 28, 1975, the son of Can Nguyen and Lien Nguyen. After graduating from Nimitz High School, Irving, Texas in 1993, he enrolled at The Johns Hopkins University and graduated with a Bachelor of Arts degree with a major in chemistry in 1997. That year, he entered the Medical Scientist Training Program at the University of Texas Southwestern Medical Center of Dallas and earned his degree of Doctor of Philosophy with an emphasis on immunology as well as Doctor of Medicine in the spring of 2007.

Permanent Address: 1806 Glenwick Lane
Irving, Texas 75060