[Frontiers in Bioscience 12, 1707-1721, January 1, 2007]

Genetics of Autoreactive B Cells

Zhiwei Xu, Biyan Duan, Laurence Morel

Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, 32610, USA

TABLE OF CONTENTS

1. ABSTRACT

It is now well-accepted that autoimmune diseases develop as a result of interactions between a complex genetic basis and environmental triggers. Autoreactive B cells play a major role in many autoimmune diseases, by secreting autoantibodies or cytokines and/or presenting auto-antigens to T cells. Studies performed with human patients and murine models have accumulated evidence that B-cell autoreactivity, or its manifestation as the presence of autoantibodies, are also supported by multiple genetic determinants. These studies will be summarized in this review and presented in a critical perspective of the approaches used to obtain these results, and their significance for our understanding of B-cell tolerance.

2. INTRODUCTION

It is now well accepted that many autoimmune diseases such as type 1 diabetes (T1D), systemic lupus erythematosus (SLE), or rheumatoid arthritis (RA) have a genetic basis. The disease phenotypes result from the synergistic interactions of many susceptibility alleles that individually contribute only modestly to the overall phenotype (1). This conclusion has been reached from the convergent findings of many studies conducted with both human patients and well-established animal models. The identification of the specific genes contributing to the autoimmune process is a complex task, with forward and reverse genetic approaches having made significant contributions. In humans, genome-wide linkage analyses

of autoimmune families have identified large genomic regions that were significantly linked to the transmission of the disease. In animal models, similar studies have been conducted on specific crosses between susceptible and resistant strains. The next step, which is to pinpoint which genes within these regions are responsible for the linkage, has been a much more difficult task. Reverse genetics approaches have been conducted with association studies on candidate genes, based on the known or suspected coincidence between the function of a gene and the etiology of an autoimmune disease. The conceptual equivalent in animal models, which consists of genetic targeting, either with transgenes to induce over-expression, or knock-outs resulting in a genetic deficiency, has been also extensively used. Although these studies are inherently biased to a small subset of genes, they have been extremely useful to delineate the functional pathways that are genetically altered in human and animal diseases. Using either approach, only a small number of susceptibility genes, such as *NOD2* in Crohn's disease (2), *CTLA-4* in Graves' disease and T1D (3), *PTPN22* in T1D, RA and SLE (4-6), PDCD1 in SLE and RA (7,8), and SUMO4 in T1D (9), have been identified in human autoimmune diseases.

Autoimmune diseases are complex not only from a genetic point of view, but also from a functional point of view in that many cellular processes involving the immune system and the target organs participate in the pathogenesis. If the disease as a whole has a genetic basis, it implies logically that the various mechanisms that lead to its development must also have a genetic basis. Furthermore, these mechanisms should be more proximal to the individual genetic defects than the clinical presentation of the disease, which is often heterogeneous and evolves with time. Autoreactive B cells are a common feature to most autoimmune diseases, which are characterized by the presence of autoantibodies (AAbs). Recently, autoreactive B cells have enjoyed considerable interest, largely due to the promising therapeutic results obtained with B-cell ablation treatments in RA, SLE and other autoimmune diseases (see the reviews by Silvermann and Sanz in this issue). The relatively small clinical trials that have been conducted so far with rituxan have highlighted the need for a better understanding of the role of B cells, autoreactive or not, in autoimmune pathogenesis. Deciphering the genetic networks that are mobilized by the lost of tolerance in B cells is most likely to contribute greatly to this endeavor.

Although the generation of autoreactive B cells is probably the best characterized autoimmune process from a genetic point of view, as will be detailed in this review, its genetic basis remains relatively unexplored. The genetics of other major effector mechanisms, such as autoreactive T cells, have been given far less attention, although results are starting to emerge in diseases such as lupus (10). The reasons why there is less information available on the genetics of individual effector mechanisms than on the diseases themselves are not clear. One may speculate that the complexity of the genetics of multifactorial diseases has pushed investigators to make choices and focus on the "big picture", the presence or absence of the disease, at least initially. One other reason may be that it is sometimes difficult to distinguish primary from secondary phenotypes, i. e. whether a specific phenotype is the direct primary consequence from a genetic defect, or rather a secondary effect of the disease process. It has been shown that the control of the production of particular autoantibody specificities, such as anti-Sm (for Smith antigen), is genetically determined (11). This could be considered as the most primary checkpoint for the production of autoreactive B cells. But many other checkpoints, both B-cell intrinsic and non-intrinsic, are involved in controlling whether the autoreactive B cells will be selected, expanded, and will express AAbs. The autoimmune process results in tissue damage that may release large quantities of normally sequestered autoantigens, or in the presentation of autoantigens in an inflammatory milieu that may break self-tolerance. In this case, B-cell autoreactivity is most likely a secondary process that is not directly linked to B-cell autoreactivity is a secondary process that is most likely not directly linked to any genetic defect affecting B cells. This review will focus on the identification and characterization of primary genetic defects associated with the generation of autoreactive B cells in humans and murine models.

3. THE GENETICS OF HUMAN AUTOREACTIVE B CELLS

A familial aggregation has been reported for the presence of anti-nuclear antibodies (ANA) among the relative of children with SLE (12) and inflammatory bowel disease (IBD) (13), suggesting that B-cell autoreactivity against nuclear antigens has a genetic basis. Since then, familial aggregation of a large number of antibodies has been reported, including against neutrophils in ulcerative colitis (14), heart-specific antigens in dilated cardiomyopathy patients and their relatives (15), goblet cells in IBD (16), thyroid antigens in autoimmune thyroid disease (AITD) (17,18), phospholipids in a family with anti-phospholipid syndrome (19), Ro60/SS-A in SLE-affected families (20), and β-cell antigens in T1D-affected families (21). These studies conducted across a broad spectrum of diseases argue in favor of a genetic basis for B-cell autoreactivity, but also point to the strong possibility of non-B cell factors that expand autoreactive B cell clones directed against specific antigens. Genomic mapping via linkage analysis has been initiated for the production of lupus-related AAbs (22). Interestingly in this study, AAbs with different specificities mapped to different regions and anti-Ro/SSA or anti-La/SSB mapped to 4q34-35, a locus that has been previously identified in multiple other autoimmune diseases, including SLE patients with dermatological manifestations. To the best of our knowledge, no other genome-wide mapping of autoantibody production or any other B-cell associated phenotypes has been performed in humans.

Two linkage analyses performed in AITD families of Japanese (23) or Caucasian (24) descent have identified a locus on 8q23-24. Although mutations in the thyroglobulin gene are likely to be the major contributor to this locus (25), an additional association study has

Table 1. Quantitative trait loci (QTLs) linked to autoreactive B-cell phenotypes.

QTL	Position (chr, cM)	Strain	Phenotype	Disease model	References
Sle1	1 (76-110)	NZM2410/NZW	ANA, B cell hyperactivity	SLE	51,57,56
Nba2	1 (75-100)	NZB	B cell survival, AAbs	SLE	87
NZBc1	1 (35-106)	NZB	B cell activation, AAbs	SLE	90
Ltk[1]	2 (65.5-69)	NZB	High B-1 cell numbers	SLE	33
Sle2	4 (21-57)	NZM2410/NZW/NZB	Increased B-1a cell numbers, lower threshold for B cell activation	SLE	50,83
Imh-1, Mott-1	4 (62-69)	NZB	Hypergammaglobulinimia, Mott cell formation	SLE	34,35
Lbw2	4 (59-62)	NZB	B cell hyperactivity, IgM AAbs	SLE	144
Idd11	4 (59-60)	NOD	Increased MZ B-cell numbers	T1D	36,4
Sle3	7 (23-53)	NZM2410/NZW	Ig repertoire	SLE	145
Bpal-2	13 (8-35)	NZW	High B-1 cell numbers	SLE, B-CLL	42
NZBc13	13 (24-73)	NZB	B cell hyperactivity, autoAbs	SLE	146
Bpal-1	17 (H-2^z linked)	NZW	High B-1 cell numbers	SLE, B-CLL	42

Susceptibility gene identified within the QTL

identified a single nucleotide polymorphism (SNP) in the promoter of a B-cell specific anti-sense transcript, SAS-ZFAT (26). ZFAT is a novel zinc finger protein of unknown function. The expression of the anti-sense transcript is B-cell specific and its disease-associated allele results in a reduced expression of a truncated ZFAT isoform (TR-ZFAT). The elucidation of ZFAT and TR-ZFAT functions promise to uncover new functional pathways that are implicated in B cell tolerance.

Two other association studies have been performed between B-cell expressed genes and autoimmune manifestations. These two genes belong to the Fc receptor family and both map to the 1q23 region, which has been implicated with susceptibility to multiple autoimmune diseases, including SLE (27) and psoriasis (28). *FCGR2B* encodes for a low-affinity Fc receptor that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) that mediates negative regulation of B cells (29). A reduced *FCGR2B* expression due to a -343 G/C SNP in the promoter was significantly associated with SLE, and consequently, with the presence of autoreactive B cells (30). Furthermore, the murine ortholog gene has been associated with systemic autoimmunity and autoreactive B cells (see below). *FCRL3* is a yet poorly characterized member of the Fc receptor family that is expressed on lymphocytes, primarily on B cells. A -169 C/T polymorphism controls the high expression of *FCRL3*, which is associated with RA, SLE, and AITD and the production of high titer autoantibodies in patients afflicted with these diseases (31). This association across multiple diseases suggests that the elucidation of *FCRL3* functions will provide important clues on the regulation of B-cell tolerance.

4. MAPPING THE GENETICS OF MURINE B-CELL AUTOREACTIVITY BY LINKAGE ANALYSES

Only a few linkage analyses were purposely designed to map genomic regions linked to autoreactive B-cell phenotypes (Table 1). High numbers of B-1a cells were mapped in an (NZB x NZW) cross to a region on NZB chromosome 2. A gain-of-function mutation was found in the NZB allele of the *Ltk* gene, which maps within the quantitative trait locus (QTL) and encodes for leukocyte tyrosine kinase. This gain of function polymorphism affects a binding motif for the p85 subunit of phosphatidylinositol 3-kinase (PI3K). PI3K plays a major role in B cell development and activation, and p85 gene targeting results in B-1 cell depletion (32). Interestingly, the same *Ltk* polymorphism was also found in humans and its frequency, albeit low, is significantly higher in SLE patients than in controls (33).

Hypergammaglobulinia was mapped in the autoimmune strain NZB to a major locus on chromosome 4, *Imh-1* (34). The same group later mapped to the same region a locus, *Mott-1*, linked to the presence of B-1 cell derived Mott cells (35). Mott cells represent pathologic state of plasma cells containing intracellular inclusions of immunoglobulin (Russell bodies), and they are frequent in lymphoid tissues in murine and human autoimmune diseases. It is likely that *Imh-1* and *Mott-1* correspond to the same genetic determinant, since an increase in plasma cells is likely to result in an increased immunoglobulin secretion. Interestingly, an overlapping region on chromosome 4 has been also linked to an increased number of marginal zone (MZ) B cells in the NOD mouse, the primary model of T1D (36). B cells are required for disease development in the NOD mouse, and several reports have suggested that the essential contribution of B cells to T1D is through their antigen presentation capacity (see review by Silveira in this issue). Due to their specific phenotypes, including the ability to prime naïve T cells (37), the expansion of MZB cells in the NOD mouse have been proposed to be critical for disease development (38). This genomic interval on chromosome 4 around 59-60 cM away from the centromere was already identified as the *Idd11* diabetes susceptibility locus (39,40), suggesting that the MZB phenotype could directly contribute to disease susceptibility. The analysis of MZB-cell numbers in congenic strains in which the NOD *Idd11* region was replaced by the corresponding segment from the non-autoimmune C57BL/6 (B6) strain revealed that the *Idd11* region was not sufficient to affect the MZB-cell numbers (41). As the significance of the original mapping puts the odds of a false positive finding at less than 1:25,000 (36), these latter findings clearly indicate a complex genetic determination of the MZB-cell numbers in the NOD mouse, requiring the interactions between *Idd11* and other yet unknown loci. Finally, a linkage analysis was performed to map the high number of B-1 cells found in the

lupus prone strain NZW. This study linked this phenotype to the *H-2*^{z} haplotype (which was called *Bpal-1* for this linkage), and another locus on chromosome 13, *Bpal-2* (42). B-1 cells constitute a B cell compartment with distinctive functional and developmental properties (43). Their increased numbers in lupus-prone strains and some patients with SLE, Sjogren's syndrome and RA, and their polyreactive/autoreactive repertoire have suggested that they may participate to the autoimmune process, although the subject is still controversial (44). The *H-2*^{z} haplotype is a major contributor to SLE pathogenesis, either as a susceptibility (45) or resistance (46) locus, and an increased B-1 compartment may be a way by which it is achieved. However, it should be noted that, similarly to *Idd11* and the size of the MZB compartment, congenic analysis has shown that the *H-2*^{z} locus was not sufficient by itself to increase the size of the B-1 pool (47).

A large number of linkage analyses have been performed in animal models of autoimmune diseases to map susceptibility loci. Congenic strains have been produced to validate some of these loci. When susceptibility locus were bred on a resistant strain, detailed phenotypic analyses were carried out to assess their functional contribution to the disease (48,1). Several SLE susceptibility loci characterized in that fashion affect B cells (Table 1). We have mapped the position of four lupus nephritis QTLs, *Sle1-4* in NZM2410, an inbred strain combining about 70% NZW and 30% NZB genomes (49), and produced four congenic strains, B6.NZM*Sle1*, -*Sle2*, -*Sle3*, and -*Sle4,* each carrying the corresponding NZM2410-derived genomic interval on the C57BL/6 (B6) genome (47). The phenotypes contributed by each locus have been determined via detailed analysis of the immunological properties of each congenic strain in comparison to B6 (50-57).

The locus with the strongest linkage to lupus nephritis was *Sle1* on telomeric chromosome 1, a region that has been independently linked to SLE in several mouse models and human patients (58). Functional analyses of the B6.*Sle1* congenic mice carrying *Sle1* on a non-autoimmune B6 background have shown that this locus mediates a loss of tolerance to nuclear antigens (51,53), primarily directed to the H2A/H2B/DNA sub-nucleosomal particles, which are the primary target in the early production of anti-nuclear antibodies (59). Genetic experiments have demonstrated that *Sle1* was necessary for the development of nephritis in the NZM2410 model (46,60). Moreover, co-expression of *Sle1* with a number of single mutations such as *Yaa* (60), *Lpr* (61), or *FcrγRIIb*$^{-/-}$ (62), or other SLE-susceptibility locus such as *Sle3* (63) produced a highly penetrant clinical pathology. By using both mixed bone-marrow chimeras and genetic ablation of the T cell compartment, we have shown that the anti-nuclear AAbs production and B-cell activation mediated by *Sle1* were due to B-cell intrinsic defects (57,56). *Sle1* congenic recombinants have shown that production of anti-nuclear AAbs corresponds to at least three independent loci, *Sle1a*, *Sle1b* and *Sle1c* (64). The *Sle1a* locus represents a 2.5-Mb segment that is relatively gene-poor, with 14 reported known genes or predicted transcripts, but no strong candidate gene identified yet for *Sle1a*. We have shown that *Sle1a* contributes to the production of autoreactive T cells (65), and we have not been able to show a direct contribution of *Sle1a* to B cell phenotypes (Morel et al, unpublished). The *Sle1b* locus is allelic with polymorphisms in a cluster of four genes (*Cd48*, *Cd150*, *Cd84* and *Ly108*) from the CD150/SLAM family (66). The contribution of the CD150 family to immune regulation and tolerance is not well understood. These genes are expressed on T cells and antigen presenting cells, including B cells (67). Our knowledge of the effects of the SLAM family members on B cell functions is still sketchy. It has been recently shown that B cells can activate NK cell activation via CD48/CD244 interactions (68), and that CD150 ligation induces the activation of the ERK pathway (69). Interestingly, *Sle1b* has been associated with an aberrant activation of the Ras-ERK pathway in B lymphocytes (70). CD84 is up-regulated on human memory B cells, and its ligation induces the recruitment of the SH2-domain containing adaptor proteins SAP and EAT-2 (71). It has been recently shown that the differential expression of the two Ly108 isoforms, Ly108^{1} and Ly108^{2}, accounted for the apoptosis resistance in immature B cells expressing *Sle1b*, and that this phenotype was associated with a loss of peripheral B cells tolerance (72). Although these results do not exclude the involvement of the other SLAM family genes, it definitively places *Ly108* as a strong contributor to *Sle1b* phenotypes.

Sle1c is located in a 7 Mb interval on the chromosome 1 telomere. A candidate gene approach has proposed that polymorphisms in the complement receptor 2 (*Cr2*) gene were responsible for *Sle1c* phenotypes (73). The CR1 and CR2 proteins, which are isoforms from the same *Cr2* gene, function as B-cell co-receptor and increase germinal center efficiency through antigen trapping by the follicular dendritic cells (74). *Cr2* deficiency has been associated with loss of B cell tolerance and autoantibody production, especially in the absence of FAS expression (75,76). Finally, CR1/CR2 levels are significantly decreased in lupus patients (77,78), and in certain cases, inversely related to disease activity (79). Similarly, decreased CR2 expression has been reported on B cells before the onset of disease in the MRL/lpr (80) and chronic graft versus host disease (cGVHD) models of SLE (81). *Sle1c* recombinants have shown that several susceptibility genes are located within *Sle1c*, some of them inducing the production of autoreactive T and B cells (82). The impaired humoral response to T-dependent antigens and the abnormal formation of germinal centers are still consistent with the involvement of *Cr2* in the *Sle1c* phenotypes, but other genes are also clearly involved in the overall *Sle1c* contribution to NZM2410 disease.

NZM2410 SLE-susceptibility locus *Sle2* leads to B-cell hyperactivity and elevated B-1 cell numbers in the peritoneal cavity and later in the spleen (50). These phenotypes are intrinsic to *Sle2*-expressing B cells, and we have shown that *Sle2* mediates an accumulation of B-1 cells through four mechanisms: Increased output from fetal liver, continuous output from adult lymphoid organs, increased spontaneous *in vivo* proliferation, and decreased

apoptosis (83). *Sle2* congenic recombinants have shown that at least three loci mediate these phenotypes, NZW-derived *Sle2a* and *Sle2b*, and NZB-derived *Sle2c* (84). *Sle2c* is the most penetrant locus in terms of B-1-cell numbers; surprisingly, however, *Sle2a* and *Sle2b*, but not *Sle2c*, aggravate disease when co-expressed with both *Sle1* and *Sle3*, indicating that the mere expansion of the B-1 pool does not contribute to autoimmune pathology (84). Additional work is being conducted to identified the genes responsible for these phenotypes and to better characterize the mechanisms by which they contribute, or not, to pathogenesis.

*Sle*3 leads to increased T cell activation and decreased activation-induced cell death in $CD4^+$ T cells (52), with evidence that this phenotype is induced by defects in the myeloid compartment (85). Most attention has been given to the phenotypes that *Sle3* confers to T cells and myeloid cells. A detailed analysis of the B-cell repertoire after hapten-immunization has shown, however, that *Sle3* affected V_HDJ_H junctional diversity and V_H mutational diversity, and that it led to the recombinational activation of allelically excluded IgH genes in the periphery (86). Such characteristics of somatic IgH diversification have been shown to contribute to the repertoire of autoreactive B cells in other systems, and we propose that they also do in the NZM2410 model. It is not currently known whether 1) the effects of *Sle3* on the B-cell repertoire are intrinsic to *Sle3*-expressing B cells and 2) the same gene(s) is responsible for the myeloid and B-cell phenotypes. The production and characterization of bone marrow chimeras and *Sle3* congenic recombinants will be necessary to address these questions.

Several QTLs mapped in the NZB strain affect B-cell functions. Among them, *Nba-2*, which was mapped by two independent groups to the same region of telomeric chromosome 1 (87,88), results in the production of anti-nuclear AAbs, spontaneous IgM production and B-cell activation (88,89). It has been proposed that the interferon inducible gene *Ifi202* gene is responsible for the *Nba-2* phenotype by mediating apoptosis resistance in autoreactive B cells (87). Sub-congenic strains showed that, as in NZM2410, there are probably multiple susceptibility genes on NZB chromosome 1 and that both T and B cells are affected (90,91). It is not known whether the NZB-derived and NZW-derived SLE susceptibility loci are allelic or correspond to different genes. One hand, we have shown that NZB and NZW share the same *Sle1b* haplotype (66), and that both *Sle1* and *Nba2* result in severe disease when bred to either NZW or *Yaa*, and are suppressed by the $H\text{-}2^{z/z}$ locus (46,92,93). On the other hand, detailed side by side characterization of the *Sle1* and *Nba-2* congenics revealed some phenotypic differences (94,95).

Lbw2 was initially mapped in a NZB x NZW cross (which implies that it is different between the two strains) as being linked to lupus nephritis and splenomegaly but not to IgG anti-chromatin AAbs (96). This locus is telomeric to *Sle2c*, but overlaps with *Imh-1* and *Idd11*. The absence of *Lbw2* in BWF1 congenic mice resulted in a significantly reduced nephritis, total IgM and IgM AAbs levels and B-cell response to LPS, but not of total and anti-dsDNA IgG, suggesting that *Lbw2* corresponds to a novel B cell activation gene (97). Another QTL, *Adnz1*, was mapped in virtually the same chromosome 4 region of the NZM2328 mouse, a region which is also derived from NZB (98). Surprisingly, this locus was linked to anti-dsDNA IgG, but not nephritis (99), which is the opposite of *Lbw2*. It should be noted that congenic strains used in these two studies were produced by replacing the susceptibility locus by the resistance locus on an autoimmune background. These genomic combinations allow for the expression and interactions of the other susceptibility loci, which complicate phenotypic interpretation, and may explain some of the divergent results. As with telomeric chromosome 1, the complexity of this chromosome 4 region which probably contains several genes affecting B cells, as well as its sharing among several autoimmune strains, underscore the need for further characterization, and the identification of the susceptibility gene(s) will be ultimately necessary for a full understanding of its contribution to the development of autoreactive B cells.

Finally, an NZB chromosome 13 region was linked to an increased expression of co-stimulatory molecules on B cells (88). This locus has been previously linked to disease susceptibility in NZB (100) and NZM2410 (101,49), which has an NZB-derived chromosome 13. Congenic analysis of this locus expressed on a B6 background showed that it was associated not only to increased B cell activation, but also to an expanded marginal zone and B-1a compartments, and increased total IgM and IgM AAbs production (102).

5. AUTOREACTIVE B CELLS RESULTING FROM SINGLE GENE TARGETING OR NATURAL VARIANTS

A large number of single gene targeting experiments in mice have resulted in lupus-like systemic autoimmunity (103,104). Since lupus is a disease in which autoreactive B cells play a major role, it is not surprising that a significant number of these genes affect B cells (Table 2). A few naturally occurring polymorphisms affecting B-autoreactivity have been described, and they will be discussed in this section along with the results obtained with genetic engineering.

Only a minority of B-cell specific genes affect directly autoreactive B cells. Among them, polymorphisms in the immunoglobulin *Kappa* locus may contribute to the autoreactive repertoire, specifically anti-insulin AAbs, in the NOD mouse, and that some of these alleles are shared with the NZB strains (105), which produces a wide variety of AAbs. Other genes have a more widespread expression, such as *Ptprc* or *Cr2*, and we have restricted this review to those with a strong B-cell component. Most of the genetic ablation experiments, a.k.a. knockouts (KO), were not performed in a B-cell specific manner, and the observed autoreactive B cells may result from a number of cellular pathways, including B cells.

Table 2. B-cell expressed genes whose targeting is associated with autoreactivity in rodent models

Gene	Protein	Position (chr, cM)	Method	Allele type	Function	Associated disease	References
bcl2	Bcl-2	1 (59.8)	Eu-hBcl2 tg	over-expression in B cells	apoptosis	Lupus	147
Ptprc	CD45	1 (74)	KO	null allele	signaling	Lupus	148
Fcgr2b	FcγRIIB	1 (93)	KO on B6	null allele	signaling	Lupus	149,62
Fcgr2b	FcγRIIB	1 (93)	association	NZB, BXSB, MRL, and NOD allele	signaling	Lupus	150,151,117
Fcgr2b	FcγRIIB	1 (93)	Association, retroviral transduction	NZW/NZM2410 allele	signaling	Lupus	152,153
Cr2	CD21/CD35	1 (106.6)	KO	Null on lpr background	BCR co-receptor	Accelerated lupus	75,76
E2f1	E2F transcription factor 1	2 (84)	KO on Eu-hBcl2 tg	null allele	apoptosis	Lupus	154
Plcg2	phospholipase C, gamma 2	8 (62)	ENU	gain of function	signaling	arthritis, dermatitis	155
Lyn	Lyn	4 (0)	KO	null allele	signaling	Lupus	156,157
Lyn	Lyn	4 (0)	Tg	gain of function	signaling	Lupus	158
Lyn	Lyn	4 (0)	polygenic hypomorph	hemizygote	signaling	Lupus	112
Igk	Ig kappa	6 (30)	candidate gene	NOD allele	antibody gene	T1D	159
Hcph	SHP-1	6 (60)	motheaten	me and me^{v}	signaling	Lupus	160,161
Hcph	SHP-1	6 (60)	polygenic hypomorph	hemizygote	signaling	Lupus	112
CD22	CD22	7 (9)	polygenic hypomorph	hemizygote	signaling	Lupus	112
Cd22	CD22	7 (9)	KO	null allele	signaling	Lupus	162,163
CD19	CD19	7 (59)	Tg	hypermorph	signaling	Lupus	107
Tnfsf13b	BAFF	8 (3)	Tg	over-expression	growth factor	Lupus	164
Tnfsf13b	BAFF	8 (3)	Tg on B6.Sle1 or B6.Nba2	over-expression	growth factor	Accelerated lupus	95
Fli-1	Fli-1	9 (16)	Tg (class I promoter)	over-expression	apoptosis	Lupus	165

With a few exceptions involving apoptosis, growth, and co-stimulation, all of which have been abundantly covered in past reviews (106,103), the vast majority of genes whose over-expression or deficiency produce autoreactive B cells are involved in B-cell signaling. Generally, either over-expression of positive regulators of the B-cell receptor (BCR) such as CD19 (107), or decreased expression of negative regulators such CD22 (108,109) result in autoreactive B cells, presumably by lowering the BCR activation threshold and allowing autoreactive B cells to respond to what would be sub-threshold signals in wild type littermates. Gene targeting experiments result in drastic changes in expression levels which are not likely to correspond to naturally occurring disease alleles. In fact, variations in proteins corresponding to only a few of these targeted genes, such as CR2 (77,78), BAFF (110), CD154 (111), or FcγRIIB (see below), have been validated in autoimmune patients. It has been demonstrated, however, that the combination of hemizygous null alleles of 3 genes encoding for BCR negative regulators, Lyn, CD22, and SHP-1, results in a dose-dependent production of autoreactive B cells (112). These results validated the use of single gene targeting approaches to explore functional pathways involved in spontaneous autoimmunity, and showed the power of interactions between susceptibility alleles, which are singly devoid of phenotypes, but produce strong synergistic effects in combination.

One of these genes affecting B cells, *Fcgr2b*, has crystallized more attention than others in the recent years. *Fcgr2b* encodes for FcγRIIB, a low affinity IgG Fc receptor expressed on B cells that contains an inhibitory ITIM motif (113) that recruits SHIP and initiates a signaling cascade that precludes the intracellular calcium mobilization necessary for B-cell activation (114). Promoter polymorphisms identified in the *Fcgr2b* promoter in a number of autoimmune strains, including NZB, have been associated with a reduced expression and function of FcγRIIB (115,116,117). This result fits with the expected function of this receptor and with its promoter polymorphisms associating the human ortholog FCGR2B with lupus (30). Another polymorphism found in the third intronic region in NZB, NZW, and 129/SVJ is associated with the failed up-regulation of FcγRIIB on GC B cells (118). The importance of this latter polymorphism was dramatically illustrated by the complete prevention of autoimmune pathogenesis achieved with the retroviral transduction of a normal *Fcgr2b* allele in the bone marrow cells of lupus-prone NZM2410 mice, which carry an NZW *Fcgr2b* allele (119). This result suggested that restoration of FCGRIIB expression on B cells from lupus patients to a level equivalent to that of normal controls may be a promising therapeutic venue.

A growing body of data is accumulating on the functions of FcγRIIB through the analysis of *Fcgr2b* null mice. FcγRIIB deficiency results in the spontaneous production of antinuclear AAbs and severe autoimmune disease on a B6 but not on a BALB/c background (62). The differences between the B6 and BALB/c genome in controlling FcγRIIB$^{-/-}$ mediated autoimmunity have been mapped to three loci on chromosomes 9, 12, and 17 (62). This strain difference is in fact due to a more robust receptor editing by BALB/c mice, which are therefore able to censure most autoreactive clones (120). This result is very interesting as it demonstrates a genetic regulation of receptor editing, and the characterization of the BALB/c loci that silence FcγRIIB$^{-/-}$ generated autoreactive B cell clones, as well as *Sle3* in NZM2410 (86), has the potential to reveal the genes involved in this process. The analysis of the fate of 56R B cells bearing a transgenic BCR with

Table 3. Genetics of BCR trangenic B cells

Transgene	Specificity	Permissive background	Resistant background	References
AM14	Ig2a[1]	B6/lpr/IgH[1] MRL/lpr/IgH[1]	BALB/c BALB/c	172 173
glD42	dsDNA	NZB/WF1	B6	174
3-32	dsDNA	NZB/WF1	B6	175
V3H9	dsDNA	MRL.lpr BALB/c/lpr, BALB/c/gld	MRL$^{+/+}$, BALB/c BALB/c	176 177
V3H9/56R	dsDNA	MRL.lpr	BALB/c	178
V3H9/56R	dsDNA	B6.FcgRIIb	BALB/c.FcgRIIb	120
2-12H	Sm	MRL.lpr	B6	179
MD4	HEL	B6.CD5$^{-/-}$	B6	132
MD4	HEL	NZB	B6.H-2^{d}	131

anti-DNA specificity has shown that FcγRIIB controls a novel tolerance checkpoint between the germinal center and the plasma cell stage (120). This result fits nicely with data obtained with the NZW allele in the B6.*Sle1* congenic mice, which is associated with a failed FcγRIIB up-regulation on GC B cells (118).

The *Fcgr2b* gene is located in a region that include the *Sle1* locus, and for which either the NZB, NZW, or 129/SV haplotype mediates autoreactivity on a B6 background (121). This can be explained by the fact that NZW, NZB and 129/SV share the same alleles for some genes such as the SLAM family members in the *Sle1b* region (66). As for most KO strains, 129/SV embryonic stem cells (ESC) were used to target *Fcgr2b,* and B6.FcγRIIB$^{-/-}$ therefore carries a 129/SV-derived chromosome 1 region flanking this gene (122). Although the FcγRIIB deficiency in the NZB and NZW-derived strains and its functional consequence for autoimmunity have been demonstrated without a doubt (118,119), 129SV alleles of flanking genes may also contribute to the strong autoimmune phenotype displayed by B6.FcγRIIB$^{-/-}$ mice.

Finally, the *Yaa* (for Y chromosome accelerated autoimmunity) locus has been known for a long time to affect B cells (123). The identity of the gene responsible for the *Yaa*-phenotypes has been elusive until two recent papers have recently determined that Yaa results from the translocation of X-chromosome encoded TLR7, a single-stranded RNA-binding innate immune receptor (124,125). This is an important finding as it bridges the innate immune response to pathogen to the amplification of autoreactivity, a concept that has been previously postulated, but never as beautifully demonstrated.

6. TRANSGENIC MODELS OF AUTOREACTIVE B CELLS

Immunoglobulin transgenic models have been used very successfully to determine the fate of autoreactive B cells. Many different antigenic specificities have been used, covering both neo-autoantigens, such as hen egg lysozyme (HEL) expressed as a transgene, and self-antigens, such as dsDNA or Sm. These models have revealed different mechanisms by which tolerance is maintained and autoreactive clones eliminated or silenced on a non-autoimmune background (126,127). When these transgenic autoimmune BCRs are expressed in lupus-prone strains, the overall result is that the transgenic autoreactive clones are expressed (Table 3). Defects in FAS or FASL (as *lpr* or *gld* mutations, respectively) have consistently been permissive for the expression of autoreactive clones of several specificities. It is unclear however if this is due to a defective FAS pathway in the B cell themselves. FAS plays a critical role in the ability of B cells to regulate T cells and other B cells (128). It has been recently shown however that FAS-regulated apoptosis of dendritic cells, and not in lymphocytes, is critical for maintenance of self tolerance (129). Furthermore, although the MRL background controls the production of anti-SM AAbs (11), it is not sufficient in FAS-sufficient mice to allow the follicular entry and secretion of anti-dsDNA AAbs by V3H9 B cells (130). In summary, FAS plays a critical role in controlling autoreactive B cells, but it is surprisingly not clear how it is achieved.

Anti-HEL B cells are normally anergized in the presence of soluble HEL (126). This tolerance is however breached by the NZB genetic background, which allows the proliferation and differentiation of the transgenic B cells into Ab-producing cells in spite of their follicular exclusion (131). Deficiency in a single gene, CD5, which is a negative regulator of lymphocyte signaling, also breaches tolerance of the anti-HEL transgenic B cells (132). The mechanisms involved in these two models are probably different since CD5 seems to regulate anergy induction.

With the exception of FAS and CD5, the identity of genes that control the fate of these transgenic autoreactive B cells is unknown. Efforts in that direction are ongoing, with a recent report that the *Sle2* locus is permissive for the expression of V3H9/56R cells (133). In that specific case, this "break of tolerance for anti-dsDNA specific B cells" should provide an addition tool in the ongoing efforts to identify the *Sle2* genes.

7. CONCLUSIONS AND PERSPECTIVES

Strong evidence has emerged that the generation of autoreactive B cells is controlled at the genetic level from data collected mostly from autoimmune patients and murine models. Phenotypic analysis of mutations, either spontaneous or engineered, in genes affecting B cells has also been very useful in establishing a genetic basis of B cell autoreactivity. As reported in this review, most of this data has been associated with lupus research. This may be the result of a heightened attention to B cells in that field, since B cells play such an important role in this disease.

On the other hand, it is possible that in other autoimmune diseases, the production of AAbs is a secondary process, and therefore not under direct genetic control. One recent result in favor of this latter hypothesis is that B cells expressing an anti-insulin antibody as a transgenic receptor are similarly tolerized both in the NOD strain, which normally produces anti-insulin AAbs, and B6, which does not (134).

Progress in the identification and characterization of the genes involved in the generation and survival of pontaneous autoreactive B cells is uneven, from large genomic segments with several dozens of genes (Table 1), to a single gene such as *Ly108* or *Fcgr2b,* for which the mechanisms by which it is involved in B-cell tolerance is rapidly unraveling. The pursuit of the existing leads will certainly achieve a better understanding of B-cell tolerance. The emergence of new tools is also re-energizing the field. The large amount of the genome that is still waiting for annotation, and the limitations of the current approaches, both in forward and reverse genetics, have pushed for combining alternative approaches with high through-put engineering. This high-tech revamping of vetted concepts is showing promises. The high-through put screen of ethylnitrosourea (ENU)-generated point mutations for autoimmune phenotypes (135,136) has recently started to make significant contributions. ENU has first assigned new functions to known genes, such as *NF-κB2* regulating the number of circulating follicular B cells (137), or a gain of function in *Plcg2* resulting in B-cell autoreactivity (138). Another known gene, *Carma-1*, has been identified through this approach as a key regulator of the plasticity in antigen receptor signaling that underpins opposing mechanisms of immunity and tolerance (139). ENU mutagenesis has also identified a novel gene, *roquin* (*Rc3h1*), that encodes a highly conserved member of the RING-type ubiquitin ligase protein family and controls follicular T helper cells in providing help to autoreactive B cells (140). It is likely that novel genes that directly affect B-cell tolerance will be identified in the near future through the continuing ENU screens.

Microarray analyses are now widely used to define molecular signatures of B cell lymphomas and leukemias. Transcriptional signatures have also been used to define the normal peripheral B-cell compartments in humans (141) and memory B cells in the mouse (142). It is likely that transcriptional profiling of autoreactive B cells, providing the adequate model is used, would provide a wealth of new data that would push this field forward.

Finally, whole genome association studies (143), in which large numbers (100,000) of SNPs are being simultaneously tested on the same patient or control, are being conducted on various cohorts of autoimmune patients. Although no result has been published yet, it is widely expected that the strong statistical power of association studies combined with high-throughput technology will deliver the identity of new susceptibility genes in these diseases. It is very likely that some of these genes will directly affect B-cell tolerance. It is also possible that whole genome association studies will be conducted directly with autoreactive B cells, or AAb production as a phenotype.

8. ACKNOWLEDGEMENTS

This review was supported in part by grants from the NIH (AI045050 and AI05850) to LM.

9. REFERENCES

1. G. Morahan and L. Morel: Genetics of autoimmune diseases in humans and in animal models. *Curr Opin Immunol* 14, 803-811 (2002)
2. Y. Ogura, D. K. Bonen, N. Inohara, D. L. Nicolae, F. F. Chen, R. Ramos, H. Britton, T. Moran, R. Karaliuskas, R. H. Duerr, J. P. Achkar, S. R. Brant, T. M. Bayless, B. S. Kirschner, S. B. Hanauer, G. Nunez and J. H. Cho: A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411, 603-606 (2001)
3. H. Ueda, J. M. M. Howson, L. Esposito, J. Heward, H. Snook, G. Chamberlain, D. B. Rainbow, K. M. D. Hunter, A. N. Smith, G. Di Genova, M. H. Herr, I. Dahlman, F. Payne, D. Smyth, C. Lowe, R. C. J. 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. L. Gough: Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 423, 506-511 (2003)
4. S. Onengut-Gumuscu, K. G. Ewens, R. S. Spielman and P. Concannon: A functional polymorphism (1858C/T) in the PTPN22 gene is linked and associated with type 1 diabetes in multiplex families. *Genes and Immunity* 5, 678-680 (2004)
5. C. Kyogoku, C. D. Langefeld, W. A. Ortmann, A. Lee, S. Selby, V. E. Carlton, M. Chang, P. Ramos, E. C. Baechler, F. M. Batliwalla, J. Novitzke, A. H. Williams, C. Gillett, P. Rodine, R. R. Graham, K. G. Ardlie, P. M. Gafney, K. L. Moser, M. Petri, A. B. Begovitch, P. K. Gregersen and T. W. Behrens: Genetic association of the R620W polymorphism of protein tyrosine phosphatase PTPN22 with human SLE. *Am J Hum Genet* 75, 504-507 (2004)
6. D. Jawaheer, R. Graham, W. Chen, A. Damle, N. Kohn, T. W. Behrens, J. Monteiro, A. B. Begovich, M. F. Seldin, L. A. Criswell, J. T. Elder, K. Pillaipakkamnatt, C. I. Amos and P. K. Gregersen: Dense mapping within the MHC suggests a complex pattern of haplotype associations with rheumatoid arthritis. *Arthritis and Rheumatism* 44, S101-S101 (2001)
7. L. Prokunina, C. Castillejo-Lopez, F. Oberg, I. Gunnarsson, L. Berg, V. Magnusson, A. J. Brookes, D. Tentler, H. Kristjansdottir, G. Grondal, A. I. Bolstad, E. Svenungsson, I. Lundberg, G. Sturfelt, A. Jonssen, L. Truedsson, G. Lima, J. Alcocer-Varela, R. Jonsson, U. B. Gyllensten, J. B. Harley, D. Alarcon-Segovia, K. Steinsson

and M. E. Alarcon-Riquelme: A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nature Genetics* 32, 666-669 (2002)
8. L. Prokunina, L. Padyukov, A. Bennet, U. de Faire, B. Wiman, J. Prince, L. Alfredsson, L. Klareskog and M. Alarcon-Riquelme: Association of the PD-1.3A allele of the PDCD1 gene in patients with rheumatoid arthritis negative for rheumatoid factor and the shared epitope. *Arthritis and Rheumatism* 50, 1770-1773 (2004)
9. D. H. Guo, M. Y. Li, Y. Zhang, P. Yang, S. Eckenrode, D. Hopkins, W. P. Zheng, S. Purohit, R. H. Podolsky, A. Muir, J. Z. Wang, Z. Dong, T. Brusko, M. Atkinson, P. Pozzilli, A. Zeidler, L. J. Raffel, C. O. Jacob, Y. Park, M. Serrano-Rios, M. T. M. Larrad, Z. X. Zhang, H. J. Garchon, J. F. Bach, J. I. Rotter, J. X. She and C. Y. Wang: A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabetes. *Nature Genetics* 36, 837-841 (2004)
10. Y. F. Chen and L. Morel: Genetics of T cell defects in lupus. *Cell Mol Immunol* 2, 403-409 (2005)
11. V. N. Kakkanaiah, E. S. Sobel, G. C. MacDonald, R. L. Cheek, P. L. Cohen and R. A. Eisenberg: B cell genotype determines the fine specificity of autoantibody in lpr mice. *J Immunol* 159, 1027-1035 (1997)
12. T. J. Lehman, J. G. Curd, N. J. Zvaifler and V. Hanson: The association of antinuclear antibodies, antilymphocyte antibodies, and C4 activation among the relatives of children with systemic lupus erythematosus. *Arthritis and Rheumatism* 25, 556-561 (1982)
13. C. Folwaczny, N. Noehl, S. P. Endres, W. Heldwein, K. Loeschke and H. Fricke: Antinuclear autoantibodies in patients with inflammatory bowel disease (high prevalence in first-degree relatives). *Digestive Diseases and Sciences* 42, 1593-1597 (1997)
14. F. Shanahan, R. H. Duerr, J. I. Rotter, H. Y. Yang, L. R. Sutherland, C. Mcelree, C. J. Landers and S. R. Targan: Neutrophil autoantibodies in ulcerative-colitis - Familial aggregation and genetic-heterogeneity. *Gastroenterol*103, 456-461 (1992)
15. A. L. Caforio, J. H. Goldman, A. J. Haven, K. M. Baig and W. J. McKenna: Evidence for autoimmunity to myosin and other heart-specific autoantigens in patients with dilated cardiomyopathy and their relatives. *Int J Cardiol* 54, 157-163 (1996)
16. C. Folwaczny, N. Noehl, K. Tschop, S. P. Endres, W. Heldwein, K. Loeschke and H. Fricke: Goblet cell autoantibodies in patients with inflammatory bowel disease and their first-degree relatives. *Gastroenterol* 113, 101-106 (1997)
17. M. P. Desai and S. Karandikar: Autoimmune thyroid disease in childhood: a study of children and their families. *Indian Pediatr* 36, 659-658 (1999)
18. T. Heiberg Brix, P. Skov Hansen, K. Ohm Kyvik and L. Hegedus: Aggregation of thyroid autoantibodies in first-degree relatives of patients with autoimmune thyroid disease is mainly due to genes: a twin study. *Clin Endocrinol* 60, 329-334 (2004)
19. I. ElHajj, I. Uthman, A. Chahel, F. Khoury, T. Arayssi and A. Taher: Familial antiphospholipid antibodies and acquired circulating anticoagulants. *Lupus* 13, 812-814 (2004)
20. R. Ferreira, M. Barreto, E. Santos, C. Pereira, B. Martins, R. Andreia, F. Crespo, J. F. Viana, C. Vasconcelos and C. Ferreira: Heritable factors shape natural human IgM reactivity to Ro60/SS-A and may predispose for SLE-associated IgG anti-Ro and anti-La autoantibody production. *J Autoimmun* 25, 155-163 (2005)
21. M. Kukko, A. Toivonen, A. Kupila, S. Korhonen, P. Keskinen, R. Veijola, S. M. Virtanen, J. Ilonen, O. Simell and M. Knip: Familial clustering of beta-cell autoimmunity in initially non-diabetic children. *Diabetes-Metabolism Res Rev* 22, 53-58 (2006)
22. P. S. Ramos, J. A. Kelly, C. Gray-McGuire, R. R. Graham, C. D. Gillett, K. J. Espe, W. A. Ortmann, G. R. Bruner, T. W. Behrens, J. B. Harley and K. L. Moser: Familial aggregation and linkage analysis of lupus-related autoantibody traits. *Arthritis and Rheumatism* 52, S202-S202 (2005)
23. K. Sakai, S. Shirasawa, N. Ishikawa, K. Ito, H. Tamai, K. Kuma, T. Akamizu, M. Tanimura, K. Furugaki, K. Yamamoto and T. Sasazuki: Identification of susceptibility loci for autoimmune thyroid disease to 5q31-q133 and Hashimoto's thyroiditis to 8q23-q24 by multipoint affected sib-pair linkage analysis in Japanese. *Hum Mol Genet* 10, 1379-1386 (2001)
24. Y. Tomer, Y. Ban, E. Concepcion, G. Barbesino, R. Villanueva, D. A. Greenberg and T. F. Davies: Common and unique susceptibility loci in Graves and Hashimoto diseases: Results of whole-genome screening in a data set of 102 multiplex families. *Amer J Hum Genet* 73, 736-747 (2003)
25. Y. Tomer, D. A. Greenberg, E. Concepcion, Y. Ban and T. F. Davies: Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. *J Clin Endocrinol Metabol* 87, 404-407 (2002)
26. S. Shirasawa, H. Harada, K. Furugaki, T. Akamizu, N. Ishikawa, K. Ito, K. Ito, H. Tamai, K. Kuma, S. Kubota, H. Hiratani, T. Tsuchiya, I. Baba, M. Ishikawa, M. Tanaka, K. Sakai, M. Aoki, K. Yamamoto and T. Sasazuki: SNPs in the promoter of a B cell-specific antisense transcript, SAS-ZFAT, determine susceptibility to autoimmune thyroid disease. *Hum Mol Genet* 13, 2221-2231 (2004)
27. B. P. Tsao: Update on human systemic lupus erythematosus genetics. *Curr Opin Rheumatol* 16, 513-521 (2004)
28. F. Capon, S. Semprini, S. Chimenti, G. Fabrizi, G. Zambruno, S. Murgia, C. Carcassi, M. Fazio, R. Mingarelli, B. Dallapiccola and G. Novelli: Fine mapping of the PSORS4 psoriasis susceptibility region on chromosome 1q21. *J Invest Dermatol* 116, 728-730 (2001)
29. T. Muta, T. Kurosaki, Z. Misulovin, M. Sanchez, M. C. Nussenzweig and J. V. Ravetch: A 13-amino-acid motif in the cytoplasmic domain of Fc-gamma-RIIb modulates B-cell receptor signaling. *Nature* 368, 70-73 (1994)
30. M. C. Blank, R. N. Stefanescu, E. Masuda, F. Marti, P. D. King, P. B. Redecha, R. J. Wurzburger, M. G. E. Peterson, S. Tanaka and L. Pricop: Decreased transcription of the human FCGR2B gene mediated by the-343 G/C promoter polymorphism and association with systemic lupus erythematosus. *Human Genetics* 117, 220-227 (2005)
31. Y. Kochi, R. Yamada, A. Suzuki, J. B. Harley, S. Shirasawa, T. Sawada, S. C. Bae, S. Tokuhiro, X. T. Chang, A. Sekine, A. Takahashi, T. Tsunoda, Y. Ohnishi,

K. M. Kaufman, C. S. P. Kang, C. W. Kang, S. Otsubo, W. Yumura, A. Mimori, T. Koike, Y. Nakamura, T. Sasazuki and K. Yamamoto: A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. *Nature Genetics* 37, 478-485 (2005)
32. K. Okkenhaug and B. Vanhaesebroeck: PI3K in lymphocyte development, differentiation and activation. *Nature Rev Immunol* 3, 317-330 (2003)
33. N. Li, K. Nakamura, Y. Jiang, H. Tsurui, S. Matsuoka, M. Abe, M. Ohtsuji, H. Nishimura, K. Kato, T. Kawai, T. Atsumi, T. Koike, T. Shirai, H. Ueno and S. Hirose: Gain-of-function polymorphism in mouse and human Ltk: implications for the pathogenesis of systemic lupus erythematosus. *Hum Mol Genet* 13, 171-179 (2004)
34. S. Hirose, H. Tsurui, H. Nishimura, Y. Jiang and T. Shirai: Mapping of a gene for hypergammaglobulinemia to the distal region of chromosome 4 in NZB mice and its contribution to systemic lupus erythematosus in (NZB X NZW)F1 mice. *Int Immunol* 6, 1857-1864 (1994)
35. Y. Jiang, S. Hirose, Y. Hamano, S. Kodera, H. Tsurui, M. Abe, K. Terashima, S. Ishikawa and T. Shirai: Mapping of a gene for the increased susceptibility of B1 cells to Mott cell formation in murine autoimmune disease. *J Immunol* 158, 992-997 (1997)
36. J. Rolf, V. Motta, N. Duarte, M. Lundholm, E. Berntman, M. L. Bergman, L. Sorokin, S. L. Cardell and D. Holmberg: The enlarged population of marginal zone/CD1dhigh B lymphocytes in Nonobese Diabetic Mice maps to diabetes susceptibility region Idd11. *J Immunol* 174, 4821-4827 (2005)
37. K. Attanavanich and J. F. Kearney: Marginal zone, but not follicular B cells, are potent activators of naive CD4 T cells. *J Immunol* 172, 803-811 (2004)
38. T. Akashi, S. Nagafuchi, K. Anzai, S. Kondo, D. Kitamura, S. Wakana, J. Ono, M. Kikuchi, Y. Niho and T. Watanabe: Direct evidence for the contribution of B cells to the progression of insulitis and the development of diabetes in non-obese diabetic mice. *Int Immunol* 9, 1159-1164 (1997)
39. G. Morahan, P. McClive, D. Huang, P. Little and A. Baxter: Genetic and physiological association of diabetes susceptibility with raised Na+/H+ exchange activity. *Proc Natl Acad Sci U S A* 91, 5898-5902 (1994)
40. P. A. Lyons, W. W. Hancock, P. Denny, C. J. Lord, N. J. Hill, N. Armitage, T. Siegmund, J. A. Todd, M. S. Phillips, J. F. Hess, S. L. Chen, P. A. Fischer, L. B. Peterson and L. S. Wicker: The NOD Idd9 genetic interval influences the pathogenicity of insulitis and contains molecular variants of Cd30, Tnfr2, and Cd137. *Immunity* 13, 107-115 (2000)
41. T. C. Brodnicki, K. O'Donnell, F. Quirk and D. M. Tarlinton: Congenic nonobese diabetic mouse strains fail to confirm linkage of a marginal zone B lymphocyte phenotype to the Idd11 locus on chromosome 4. *J Immunol* 176, 701-702 (2006)
42. Y. Hamano, S. Hirose, A. Ida, M. Abe, D. Q. Zhang, S. Kodera, Y. Jiang, J. Shirai, Y. Miura, H. Nishimura and T. Shirai: Susceptibility alleles for aberrant B-1 cell proliferation involved in spontaneously occurring B-cell chronic lymphocytic leukemia in a model of New Zealand white mice. *Blood* 92, 3772-3779 (1998)
43. R. Berland and H. H. Wortis: Origins and functions of B-1 cells with notes on the role of CD5. *Ann Rev Immunol* 20, 253-300 (2002)
44. B. Duan and L. Morel: Role of B-1a cells in autoimmunity. *Autoimmun Rev* (2006)
45. B. L. Kotzin and E. Palmer: The contribution of NZW genes to lupus-like disease in (NZB X NZW)F1 Mice. *J Exp Med* 165, 1237-1251 (1987)
46. L. Morel, X. H. Tian, B. P. Croker and E. K. Wakeland: Epistatic modifiers of autoimmunity in a murine model of lupus nephritis. *Immunity* 11, 131-139 (1999)
47. L. Morel, Y. Yu, K. R. Blenman, R. A. Caldwell and E. K. Wakeland: Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mammalian Genome* 7, 335-339 (1996)
48. E. K. Wakeland, A. E. Wandstrat, K. Liu and L. Morel: Genetic dissection of systemic lupus erythematosus. *Curr Opin Immunol* 11, 701-707 (1999)
49. L. Morel, U. H. Rudofsky, J. A. Longmate, J. Schiffenbauer and E. K. Wakeland: Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1, 219-229 (1994)
50. C. Mohan, L. Morel, P. Yang and E. K. Wakeland: Genetic dissection of systemic lupus erythematosus pathogenesis - Sle2 on murine chromosome 4 leads to B cell hyperactivity. *J Immunol* 159, 454-465 (1997)
51. C. Mohan, E. Alas, L. Morel, P. Yang and E. K. Wakeland: Genetic dissection of SLE pathogenesis - Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J Clin Invest* 101, 1362-1372 (1998)
52. C. Mohan, Y. Yu, L. Morel, P. Yang and E. K. Wakeland: Genetic dissection of Sle pathogenesis: Sle3 on murine chromosome 7 impacts T cell activation, differentiation, and cell death. *J Immunol* 162, 6492-6502 (1999)
53. L. Morel, C. Mohan, Y. Yu, B. P. Croker, N. Tian, A. Deng and E. K. Wakeland: Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J Immunol* 158, 6019-6028 (1997)
54. E. S. Sobel, C. Mohan, L. Morel, J. Schiffenbauer and E. K. Wakeland: Genetic dissection of SLE pathogenesis: Adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. *J Immunol* 162, 2415-2421 (1999)
55. E. S. Sobel, L. Morel, R. Baert, C. Mohan, J. Schiffenbauer and E. K. Wakeland: Genetic dissection of systemic lupus erythematosus pathogenesis: Evidence for functional expression of Sle3/5 by non-T cells. *J Immunol* 169, 4025-4032 (2002)
56. E. S. Sobel, M. Satoh, W. F. Chen, E. K. Wakeland and L. Morel: The major murine systemic lupus erythematosus susceptibility locus Sle1 results in abnormal functions of both B and T cells. *J Immunol* 169, 2694-2700 (2002)
57. E. S. Sobel, C. Mohan, L. Morel, J. Schiffenbauer and E. K. Wakeland: Genetic dissection of SLE pathogenesis: Adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. *J Immunol* 162, 2415-2421 (1999)
58. E. K. Wakeland, K. Liu, R. R. Graham and T. W. Behrens: Delineating the genetic basis of systemic lupus erythematosus. *Immunity* 15, 397-408 (2001)

59. R. W. Burlingame and R. L. Rubin: Autoantibody to the nucleosome subunit (H2A-H2B)-DNA is an early and ubiquitous feature of lupus-like conditions. *Mol Biol Rep* 23, 159-166 (1996)
60. L. Morel, B. P. Croker, K. R. Blenman, C. Mohan, G. Huang, G. Gilkeson and E. K. Wakeland: Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci U S A* 97, 6670-6675 (2000)
61. X. Y. Shi, C. Xie, D. Kreska, J. A. Richardson and C. Mohan: Genetic dissection of SLE: SLE1 and FAS impact alternate pathways leading to lymphoproliferative autoimmunity. *J Exp Med* 196, 281-292 (2002)
62. S. Bolland, Y. S. Yim, K. Tus, E. K. Wakeland and J. V. Ravetch: Genetic modifiers of systemic lupus erythematosus in Fc gamma RIIB-/- mice. *J Exp Med* 195, 1167-1174 (2002)
63. C. Mohan, L. Morel, P. Yang, H. Watanabe, B. Croker, G. Gilkeson and E. K. Wakeland: Genetic dissection of lupus pathogenesis: a recipe for nephrophilic autoantibodies. *J Clin Invest* 103, 1685-1695 (1999)
64. L. Morel, K. R. Blenman, B. P. Croker and E. K. Wakeland: The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc Natl Acad Sci U S A* 98, 1787-1792 (2001)
65. Y. Chen, C. Cuda and L. Morel: Genetic determination of T cell help in loss of tolerance to nuclear antigens. *J Immunol* 174, 7692-7702 (2005)
66. A. E. Wandstrat, C. Nguyen, N. Limaye, A. Y. Chan, S. Subramanian, X. H. Tian, Y. S. Yim, A. Pertsemlidis, H. R. Garner, L. Morel and E. K. Wakeland: Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* 21, 769-780 (2004)
67. P. Engel, M. J. Eck and C. Terhorst: The SAP and SLAM families in immune responses and X-linked lymphoproliferative disease. *Nat Rev Immunol* 3, 813-821 (2003)
68. N. Gao, P. Schwartzberg, J. A. Wilder, B. R. Blazar and D. Yuan: B Cell Induction of IL-13 Expression in NK Cells: Role of CD244 and SLAM-Associated Protein. *J Immunol* 176, 2758-2764 (2006)
69. S. V. Mikhalap, L. M. Shlapatska, O. V. Yurchenko, M. Y. Yurchenko, G. G. Berdova, K. E. Nichols, E. A. Clark and S. P. Sidorenko: The adaptor protein SH2D1A regulates signaling through CD150 (SLAM) in B cells. *Blood* 104, 4063-4070 (2004)
70. K. Liu, C. Liang, Z. Liang, K. Tus and E. K. Wakeland: Sle1ab mediates the aberrant activation of STAT3 and Ras-ERK signaling pathways in B lymphocytes. *J Immunol* 174, 1630-1637 (2005)
71. S. G. Tangye, B. C. M. van de Weerdt, D. T. Avery and P. D. Hodgkin: CD84 is up-regulated on a major population of human memory B cells and recruits the SH2 domain containing proteins SAP and EAT-2. *Eur J Immunol* 32, 1640-1649 (2002)
72. K. R. Kumar, L. Li, M. Yan, M. Bhaskarabhatla, A. B. Mobley, C. Nguyen, J. M. Mooney, J. D. Schatzle, E. K. Wakeland and C. Mohan: Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science* 312, 1665-1669 (2006)
73. S. A. Boackle, V. M. Holers, X. J. Chen, G. Szakonyi, D. R. Karp, E. K. Wakeland and L. Morel: Cr2, a candidate gene in the murine Sle1c lupus susceptibility locus, encodes a dysfunctional protein. *Immunity* 15, 775-785 (2001)
74. R. H. Carter and R. A. Barrington: Signaling by the CD19/CD21 complex on B cells. In: Complement in Autoimmunity. Eds: Tsokos G C, *Karger*, Basel 4-32 (2004)
75. A. P. Prodeus, S. Goerg, L. M. Shen, O. O. Pozdnyakova, L. Chu, E. M. Alicot, C. C. Goodnow and M. C. Carroll: A critical role for complement in maintenance of self-tolerance. *Immunity* 9, 721-731 (1998)
76. X. B. Wu, N. Jiang, C. Deppong, J. Singh, G. Dolecki, D. L. Mao, L. Morel and H. D. Molina: A role for the Cr2 gene in modifying autoantibody production in systemic lupus erythematosus. *J Immunol* 169, 1587-1592 (2002)
77. E. Levy, J. Ambrus, L. Kahl, H. Molina, K. Tung and V. M. Holers: Lymphocyte-T expression of complement receptor-2 (Cr-2 Cd21) - A Role in adhesive cell cell-interactions and dysregulation in a patient with systemic lupus-erythematosus (SLE). *Clin Exp Immunol* 90, 235-244 (1992)
78. J. G. Wilson, W. D. Ratnoff, P. H. Schur and D. T. Fearon: Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. *Arthritis and Rheumatism* 29, 739-747 (1986)
79. H. V. Marquart, A. Svendsen, J. M. Rasmussen, C. H. Nielsen, P. Junker, S. E. Svehag and R. G. Q. Leslie: Complement Receptor Expression and Activation of the Complement Cascade on B-Lymphocytes from Patients with Systemic Lupus-Erythematosus (Sle). *Clin Exp Immunol* 101, 60-65 (1995)
80. K. Takahashi, Y. Kozono, T. J. Waldschmidt, D. Berthiaume, R. J. Quigg, A. Baron and V. M. Holers: Mouse complement receptors type 1 (CR1;CD35) and type 2 (CR2;CD21): expression on normal B cell subpopulations and decreased levels during the development of autoimmunity in MRL/lpr mice. *J Immunol* 159, 1557-1569 (1997)
81. N. Feuerstein, F. Q. Chen, M. Madaio, M. Maldonado and R. A. Eisenberg: Induction of autoimmunity in a transgenic model of B cell receptor peripheral tolerance: Changes in coreceptors and B cell receptor-induced tyrosine-phosphoproteins. *J Immunol* 163, 5287-5297 (1999)
82. Y. Chen, D. Perry, S. A. Boackle, E. S. Sobel, H. Molina, B. P. Croker and L. Morel: Several genes contribute to the production of autoreactive B and T cells in the murine lupus susceptibility locus Sle1c. *J Immunol* 175, 1080-1089 (2005)
83. Z. Xu, E. J. Butfiloski, E. S. Sobel and L. Morel: Mechanisms of peritoneal B-1a cells accumulation induced by murine lupus susceptibility locus Sle2. *J Immunol* 173, 6050-6058 (2004)
84. Z. Xu, B. Duan, B. P. Croker, E. K. Wakeland and L. Morel: Genetic dissection of the murine lupus susceptibility locus *Sle2*: contributions to increased peritoneal B-1a cells and lupus nephritis map to different loci. *J Immunol* 175, 936-943 (2005)
85. J. K. Zhu, X. B. Liu, C. Xie, M. Yan, Y. Yu, E. S. Sobel, E. K. Wakeland and C. Mohan: T cell hyperactivity

in lupus as a consequence of hyperstimulatory antigen-presenting cells. *J Clin Invest* 115, 1869-1878 (2005)
86. M. Wakui, J. Kim, E. J. Butfiloski, L. Morel and E. S. Sobel: Genetic dissection of lupus pathogenesis: Sle3/5 impacts IgH CDR3 sequences, somatic mutations, and receptor editing. *J Immunol* 173, 7368-7376 (2004)
87. S. J. Rozzo, J. D. Allard, D. Choubey, T. J. Vyse, S. Izui, G. Peltz and B. L. Kotzin: Evidence for an interferon-inducible gene, Ifi202, in the susceptibility to systemic lupus. *Immunity* 15, 435-443 (2001)
88. J. E. Wither, A. D. Paterson and B. Vukusic: Genetic dissection of B cell traits in New Zealand black mice. The expanded population of B cells expressing up-regulated costimulatory molecular shows linkage to *Nba2*. *Eur J Immunol* 30, 356-365 (2000)
89. S. Atencio, H. Amano, S. Izui and B. L. Kotzin: Separation of the New Zealand black genetic contribution to lupus from New Zealand black determined expansions of marginal zone B and B1a cells. *J Immunol* 172, 4159-4166 (2004)
90. J. E. Wither, G. Lajoie, S. Heinrichs, Y. C. Cai, N. Chang, A. Ciofani, Y. H. Cheung and R. MacLeod: Functional dissection of lupus susceptibility loci on the New Zealand Black mouse chromosome 1: Evidence for independent genetic loci affecting T and B cell activation. *J Immunol* 171, 1697-1706 (2003)
91. Y. H. Cheung, N. H. Chang, Y. C. Cai, G. Bonventi, R. MacLeod and J. E. Wither: Functional interplay between intrinsic B and T cell defects leads to amplification of autoimmune disease in New Zealand black chromosome 1 congenic mice. *J Immunol* 175, 8154-8164 (2005)
92. M. R. Gubbels, T. N. Jorgensen, T. E. Metzger, K. Menze, H. Steele, S. A. Flannery, S. J. Rozzo and B. L. Kotzin: Effects of MHC and gender on lupus-like autoimmunity in Nba2 congenic mice. *J Immunol* 175, 6190-6196 (2005)
93. S. Kikuchi, M. L. Santiago-Raber, H. Amano, E. Amano, L. Fossati-Jimack, T. Moll, B. L. Kotzin and S. Izui: Contribution of NZB autoimmunity 2 to Y-linked autoimmune acceleration-induced monocytosis in association with murine systemic lupus. *J Immunol* 176, 3240-3247 (2006)
94. W. Stohl, D. Xu, T. E. Metzger, K. S. Kim, L. Morel and B. L. Kotzin: Dichotomous effects of complete versus partial class II major histocompatibility complex deficiency on circulating autoantibody levels in autoimmune-prone mice. *Arthritis and Rheumatism* 50, 2227-2239 (2004)
95. W. Stohl, D. Xu, K. S. Kim, M. N. Koss, T. N. Jorgensen, B. Deocharan, T. E. Metzger, S. A. Bixler, Y. S. Hong, C. Ambrose, F. Mackay, L. Morel, C. Putterman, B. L. Kotzin and S. L. Kalled: BAFF overexpression accelerates glomerular disease inmice with an imcomplete genetic predisposition to systemic lupus erythematosus. *Arthritis and Rheumatism* (2005)
96. D. H. Kono, R. W. Burlingame, D. G. Owens, A. Kuramochi, R. S. Balderas, D. Balomenos and A. N. Theofilopoulos: Lupus susceptibility loci in New Zealand mice. *Proc Natl Acad Sci USA* 91, 10168-10172 (1994)
97. M. K. Haraldsson, N. G. dela Paz, J. G. Kuan, G. S. Gilkeson, A. N. Theofilopoulos and D. H. Kono: Autoimmune alterations induced by the New Zealand black Lbw2 locus in BWF1 mice. *J Immunol* 174, 5065-5073 (2005)
98. S. T. Waters, S. M. Fu, F. Gaskin, U. S. Deshmukh, S. S. J. Sung, C. C. Kannapell, K. S. K. Tung, S. B. Mcewen and M. McDuffie: NZM2328: A new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin Immunol* 100, 372-383 (2001)
99. S. T. Waters, M. McDuffie, H. Bagavant, U. S. Deshmukh, F. Gaskin, C. Jiang, K. S. K. Tung and S. M. Fu: Breaking tolerance to double stranded DNA, nucleosome, and other nuclear antigens is not required for the pathogenesis of lupus glomerulonephritis. *J Exp Med* 199, 255-264 (2004)
100. C. G. Drake, S. J. Rozzo, H. F. Hirschfeld, N. P. Smarnworawong and B. L. Kotzin: The NZB contribution to lupus-like renal-disease - multiple genes that operate in a threshold manner. *J Cell Biochem*140-140 (1995)
101. L. Morel, C. Mohan, Y. Yu, J. Schiffenbauer, U. H. Rudofsky, N. Tian, J. A. Longmate and E. K. Wakeland: Multiplex inheritance of component phenotypes in a murine model of lupus. *Mammalian Genome* 10, 176-181 (1999)
102. J. E. Wither, C. Loh, G. Lajoie, S. Heinrichs, Y. C. Cai, G. Bonventi and R. MacLeod: Co-localization of expansion of the splenic marginal zone population with abnormal B cell activation and autoantibody production in B6 mice with an introgressed New Zealand black chromosome 13 interval. *J Immunol* 175, 4309-4319 (2005)
103. K. Raman and C. Mohan: Genetics underpinnings of autoimmunity - lessons from studies in arthritis, diabetes, lupus and multiple sclerosis. *Curr Opin Immunol* 15, 651-659 (2003)
104. D. H. Kono, R. Baccala and A. N. Theofilopoulos: Genes and genetics of murine lupus. In: Systemic Lupus Erythematosus. Eds: Lahita R G, *Elsevier Academic Press*, Oxford, UK 225-263 (2004)
105. E. J. Woodward and J. W. Thomas: Multiple germline κ light chains generate anti-insulin B cells in nonobese diabetic mice. *J Immunol* 175, 1073-1079 (2005)
106. A. E. Wandstrat and E. K. Wakeland: The genetics of complex autoimmune diseases: non-MHC susceptibility genes. *Nat Immunol* 2, 802-809 (2001)
107. S. Sato, M. Hasegawa, M. Fujimoto, T. F. Tedder and K. Takehara: Quantitative genetic variation in CD19 expression correlates with autoimmunity. *J Immunol* 165, 6635-6643 (2000)
108. T. L. O'Keefe, G. T. Williams, S. L. Davies and M. S. Neuberger: Hyper-responsive B cells in CD22-deficient mice. *Science* 274, 798-801 (1996)
109. T. L. O'Keefe, G. T. Williams, F. D. Batista and M. S. Neuberger: Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity sutoantibodies. *J Exp Med* 189, 1307-1313 (1999)
110. J. O. Pers, C. Daridon, V. Devauchelle, S. Jousse, A. Saraux, C. Jamin and P. Youinou: BAFF overexpression is associated with autoantibody production in autoimmune diseases. *Ann N Y Acad Sci* 1050, 34-39 (2005)
111. E. Toubi and Y. Shoenfeld: The role of CD40-CD154 interactions in autoimmunity and the benefit of disrupting this pathway. *Autoimmunity* 37, 457-464 (2004)
112. R. J. Cornall, J. G. Cyster, M. L. Hibbs, A. R. Dunn, K. L. Otipoby, E. A. Clark and C. C. Goodnow: Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* 8, 497-508 (1998)

113. T. Muta, T. Kurosaki, Z. Misulovin, M. Sanchez, M. C. Nussenzweig and J. V. Ravetch: A 13-amino-acid motif in the cytoplasmic domain of Fc-Gamma-RIIb modulates B-Cell receptor signaling. *Nature* 369, 340-340 (1994)
114. S. Bolland and J. V. Ravetch: Inhibitory pathways triggered by ITIM-containing receptors. *Adv Immunol* 72, 149-177 (1999)
115. Y. Jiang, S. Hirose, R. Sanokawa-Akakura, M. Abe, X. Mi, N. Li, Y. Miura, J. Shirai, D. Zhang, Y. Hamano and T. Shirai: Genetically determined aberrant down-regulation of FcγRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. *Int Immunol* 11, 1685-1691 (1999)
116. N. R. Pritchard, A. J. Cutler, S. Uribe, S. J. Chadban, B. J. Morley and K. G. C. Smith: Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcγRII. *Current Biology* 10, 227-230 (2000)
117. Y. Xiu, K. Nakamura, M. Abe, N. Li, X. S. Wen, Y. Jiang, D. Zhang, H. Tsurui, S. Matsuoka, Y. Hamano, H. Fujii, M. Ono, T. Takai, T. Shimokawa, C. Ra, T. Shirai and S. Hirose: Transcriptional regulation of Fcgr2b gene by polymorphic promoter region and its contribution to humoral immune responses. *J Immunol* 169, 4340-4346 (2002)
118. Z. S. M. Rahman and T. Manser: Failed up-regulation of the inhibitory IgG Fc receptor FcγRIIB on germinal center B cells in autoimmune-prone mice is not associated with deletion polymorphisms in the promoter region of the FcγRIIB gene. *J Immunol* 175, 1440-1449 (2005)
119. T. L. Mcgaha, B. Sorrentino and J. V. Ravetch: Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science* 307, 590-593 (2005)
120. H. Fukuyama, F. Nimmerjahn and J. V. Ravetch: The inhibitory Fc receptor modulates autoimmunity by limiting the accumulation of immunoglobulin G+ anti-DNA plasma cells. *Nature Immunol* 6, 99-106 (2005)
121. A. E. Bygrave, K. L. Rose, J. Cortes-Hernandez, J. Warren, R. J. Rigby, H. T. Cook, M. J. Walport, T. J. Vyse and M. Botto: Spontaneous autoimmunity in 129 and C57BL/6 mice - Implications for autoimmunity described in gene-targeted mice. *Plos Biol* 2, 1081-1090 (2004)
122. L. Morel: Mouse models of human autoimmune diseases: Essential tools that require the proper controls. *PloS Biol* 2, 3-6 (2004)
123. R. Merino, L. Fossati, M. Lacour and S. Izui: Selective autoantibody production by Yaa+ B-cells in autoimmune Yaa+-Yaa- bone-marrow chimeric mice. *J Exp Med* 174, 1023-1029 (1991)
124. P. Pisitkun, J. A. Deane, M. J. Difilippantonio, T. Tarasenko, A. B. Satterthwaite and S. Bolland: Autoreactive B cell responses to RNA-related antigens due to TLR7 gene duplication. *Science* 312, 1669-1672 (2006)
125. S. Subramanian, K. Tus, Q. Z. Li, A. Wang, X. H. Tian, J. Zhou, C. Liang, G. Bartov, L. D. McDaniel, X. J. Zhou, R. A. Schultz and E. K. Wakeland: A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. *Proc Natl Acad Sci USA* 10.1073/pnas.0603912103 (2006)
126. C. C. Goodnow: Balancing immunity and tolerance: Deleting and tuning lymphocyte repertoires. *Proc Natl Acad Sci U S A* 93, 2264-2271 (1996)
127. M. L. Fields and J. Erikson: The regulation of lupus-associated autoantibodies: immunoglobulin transgenic models. *Cur Opin Immunol* 15, 709-717 (2003)
128. E. S. Sobel, V. N. Kakkanaiah, J. Schiffenbauer, E. A. Reap, P. L. Cohen and R. A. Eisenberg: Novel immunoregulatory B cell pathways revealed by lpr-+ mixed chimeras. *J Immunol* 160, 1497-1503 (1998)
129. M. Chen, Y. H. Wang, Y. Wang, L. Huang, H. Sandoval, Y. J. Liu and J. Wang: Dendritic cell apoptosis in the maintenance of immune tolerance. *Science* 311, 1160-1164 (2006)
130. L. Mandik-Nayak, S. j. Seo, C. Sokol, K. M. Potts, A. Bui and J. Erikson: MRL-lpr/lpr mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J Exp Med* 189, 1799-1814 (1999)
131. N. H. Chang, R. MacLeod and J. E. Wither: Autoreactive B cells in lupus-prone New Zealand black mice exhibit aberrant survival and proliferation in the presence of self-antigen in vivo. *J Immunol* 172, 1553-1560 (2004)
132. K. L. Hippen, L. E. Tze and T. W. Behrens: CD5 maintains tolerance in anergic B cells. *J Exp Med* 191, 883-889 (2000)
133. Y. Liu, L. N. Li, C. Xie, D. Kreska, K. Hsu, S. Lightfoot, X. J. Zhou, M. Weigert and C. Mohan: Genetic dissection of Sle: Sle2 breaches B-Cell tolerance by impairing receptor revision of nuclear antigen reactive B-cells. *Faseb J* 19, A910-A911 (2005)
134. C. A. Acevedo-Suarez, C. Hulbert, E. J. Woodward and J. W. Thomas: Uncoupling of anergy from developmental arrest in anti-insulin B cells supports the development of autoimmune diabetes. *J Immunol* 174, 827-833 (2005)
135. C. G. Vinuesa and C. C. Goodnow: Illuminating autoimmune regulators through controlled variation of the mouse genome sequence. *Immunity* 20, 669-679 (2004)
136. P. Papathanasiou and C. C. Goodnow: Connecting mammalian genome with phenome by ENU mouse mutagenesis: Gene combinations specifying the immune system. *Ann Rev Genet* 39, 241-262 (2005)
137. L. A. Miosge, J. Blasioli, M. Blery and C. C. Goodnow: Analysis of an ethylnitrosourea-generated mouse mutation defines a cell intrinsic role of nuclear factor kappa B2 in regulating circulating B cell numbers. *J Exp Med* 196, 1113-1119 (2002)
138. P. Yu, R. Constien, N. Dear, M. Katan, P. Hanke, T. D. Bunney, S. Kunder, L. Quintanilla-Martinez, U. Huffstadt and A. Schroder: Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase Cγ2 that specifically increases external Ca2+ entry. *Immunity* 22, 451-465 (2005)
139. J. E. Jun, L. E. Wilson, C. G. Vinuesa, S. Lesage, M. Blery, L. A. Miosge, M. C. Cook, E. M. Kucharska, H. Hara, J. M. Penninger, H. Domashenz, N. A. Hong, R. J. Glynne, K. A. Nelms and C. C. Goodnow: Identifying the MAGUK protein CARMA-1 as a central regulator of humoral immune responses and atopy by genome-wide mouse mutagenesis. *Immunity* 18, 751-762 (2003)
140. C. G. Vinuesa, M. C. Cook, C. Angelucci, V. Athanasopoulos, L. Rui, K. M. Hill, D. Yu, H. Domaschenz, B. Whittle, T. Lambe, I. S. Roberts, R. R.

Copley, J. I. Bell, R. J. Cornall and C. C. Goodnow: A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435, 452-458 (2005)
141. Y. Shen, J. Iqbal, L. Xiao, R. Lynch, A. Rosenwald, L. Staudt, S. Sherman, K. Dybkaer, G. Zhou, J. Eudy, J. Delabie, T. McKeithan and W. Chan: Distinct gene expression profiles in different B-cell compartments in human peripheral lymphoid organs. *BMC Immunol* 5, 20-2004)
142. C. G. Vinuesa, M. C. Cook, M. P. Cooke, I. C. M. MacLennan and C. C. Goodnow: Analysis of B cell memory formation using DNA microarrays. *Microarrays, Immune Responses and Vaccines* 975, 33-45 (2002)
143. L. J. Palmer and L. R. Cardon: Shaking the tree: mapping complex disease genes with linkage disequilibrium. *The Lancet* 366, 1223-1234 (2005)
144. M. K. Haraldsson, N. G. dela Paz, J. G. Kuan, G. S. Gilkeson, A. N. Theofilopoulos and D. H. Kono: Autoimmune alterations induced by the New Zealand black Lbw2 locus in BWF1 Mice. *J Immunol* 174, 5065-5073 (2005)
145. M. Wakui, J. Kim, E. J. Butfiloski, L. Morel and E. S. Sobel: Genetic dissection of lupus pathogenesis: Sle3/5 Impacts IgH CDR3 sequences, somatic mutations, and receptor editing. *J Immunol* 173, 7368-7376 (2004)
146. J. E. Wither, C. Loh, G. Lajoie, S. Heinrichs, Y. C. Cai, G. Bonventi and R. MacLeod: Colocalization of expansion of the splenic marginal zone population with abnormal B cell activation and autoantibody Production in B6 mice with an introgressed New Zealand black chromosome 13 interval. *J Immunol* 175, 4309-4319 (2005)
147. A. Strasser, S. Whittingham, D. L. Vaux, M. L. Bath, J. M. Adams, S. Cory and A. W. Harris: Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc Natl Acad Sci U S A* 88, 8661-8665 (1991)
148. R. Majeti, Z. Xu, T. G. Parslow, J. L. Olson, D. I. Daikh, N. Killeen and A. Weiss: An inactivating point mutation in the inhibitory wedge of CD45 causes lymphoproliferation and autoimmunity. *Cell* 103, 1059-1070 (2000)
149. T. Takai, M. Ono, M. Hikida, H. Ohmori and J. V. Ravetch: Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* 379, 346-349 (1996)
150. Y. Jiang, S. Hirose, R. Sanokawa-Akakura, M. Abe, X. Mi, N. Li, Y. Miura, J. Shirai, D. Zhang, Y. Hamano and T. Shirai: Genetically determined aberrant down-regulation of FcγRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. *Int Immunol* 11, 1685-1691 (1999)
151. N. R. Pritchard, A. J. Cutler, S. Uribe, S. J. Chadban, B. J. Morley and K. G. C. Smith: Autoimmune-prone mice share a promoter haplotype associated with reduced expression and function of the Fc receptor FcγRII. *Current Biology* 10, 227-230 (2000)
152. T. L. Mcgaha, B. Sorrentino and J. V. Ravetch: Restoration of tolerance in lupus by targeted inhibitory receptor expression. *Science* 307, 590-593 (2005)
153. Z. S. M. Rahman and T. Manser: Failed Up-Regulation of the Inhibitory IgG Fc Receptor FcγRIIB on Germinal Center B Cells in Autoimmune-Prone Mice Is Not Associated with Deletion Polymorphisms in the Promoter Region of the FcγRIIB Gene. *J Immunol* 175, 1440-1449 (2005)
154. M. J. Marin, I. Garcia, M. Pena, A. H. Bolivar, A. Zubiaga and M. Loez-Hoyos: E2F1-/- C57BL/6 Mice overexpressing a human Bcl-2 transgene in B cells develop a mild autoimmune syndrome. *Ann NY Acad Sci* 1051, 156-165 (2005)
155. P. Yu, R. Constien, N. Dear, M. Katan, P. Hanke, T. D. Bunney, S. Kunder, L. Quintanilla-Martinez, U. Huffstadt and A. Schroder: Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase Cγ2 that specifically increases external Ca2+ entry. *Immunity* 22, 451-465 (2005)
156. M. L. Hibbs, D. M. Tarlinton, J. Armes, D. Grail, G. Hodgson, R. Maglitto, S. A. Stacker and A. R. R. Dunn: Multiple defects in the immune-system of Lyn-deficient mice, culminating in autoimmune-disease. *Cell* 83, 301-311 (1995)
157. H. Nishizumi, I. Taniuchi, Y. Yamanashi, D. Kitamura, D. Ilic, S. Mori, T. Watanabe and T. Yamamoto: Impaired proliferation of peripheral B-cells and indication of autoimmune-disease in Lyn-deficient Mice. *Immunity* 3, 549-560 (1995)
158. M. L. Hibbs, K. W. Harder, J. Armes, N. Kountouri, C. Quilici, F. Casagranda, A. R. Dunn and D. M. Tarlinton: Sustained activation of Lyn tyrosine kinase in vivo leads to autoimmunity. *J Exp Med* 196, 1593-1604 (2002)
159. E. J. Woodward and J. W. Thomas: Multiple germline κ light chains generate anti-insulin B cells in nonobese diabetic mice. *J Immunol* 175, 1073-1079 (2005)
160. L. D. Shultz, P. A. Schweitzer, T. V. Rajan, T. L. Yi, J. N. Ihle, R. J. Matthews, M. L. Thomas and D. R. Beier: Mutations at the murine moth-eaten locus are within the hematopoietic-cell protein-tyrosine-phosphatase (Hcph) gene. *Cell* 73, 1445-1454 (1993)
161. H. W. Tsui, K. A. Siminovitch, L. Desouza and F. W. L. Tsui: Moth-eaten and viable moth-eaten mice have mutations in the hematopoietic-cell phosphatase gene. *Nature Genetics* 4, 124-129 (1993)
162. T. L. O'Keefe, G. T. Williams, S. L. Davies and M. S. Neuberger: Hyperresponsive B cells in CD22-deficient mice. *Science* 274, 798-801 (1996)
163. T. L. O'Keefe, G. T. Williams, F. D. Batista and M. S. Neuberger: Deficiency in CD22, a B Cell-specific Inhibitory Receptor, Is Sufficient to Predispose to Development of High Affinity Autoantibodies. *J Exp Med* 189, 1307-1313 (1999)
164. F. Mackay, S. A. Woodcock, P. Lawton, C. Ambrose, M. Baetscher, P. Schneider, J. Tschopp and J. L. Browning: Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 190, 1697-1710 (1999)
165. L. Zhang, A. Eddy, Y. T. Teng, M. Fritzler, M. Kluppel, F. Melet and A. Bernstein: An immunological renal disease in transgenic mice that overexpress Fli- 1, a member of the ets family of transcription factor genes. *Mol Cell Biol* 15, 6961-6970 (1995)

166. C. C. K. Yu, T. S. B. Yen, C. A. Lowell and A. L. DeFranco: Lupus-like kidney disease in mice deficient in the Src family tyrosine kinases Lyn and Fyn. *Curr Biol* 11, 34-38 (2001)
167. J. H. Wang, N. Avitahl, A. Cariappa, C. Friedrich, T. Ikeda, A. Renold, K. Andrikopoulos, L. B. Liang, S. Pillai, B. A. Morgan and K. Georgopoulos: Aiolos regulates B cell activation and maturation to effector state. *Immunity* 9, 543-553 (1998)
168. I. Mecklenbrauker, K. Saijo, N. Y. Zheng, M. Leitges and A. Tarakhovsky: Protein kinase C delta controls self-antigen-induced B-cell tolerance. *Nature* 416, 860-865 (2002)
169. A. Miyamoto, K. Nakayama, H. Imaki, S. Hirose, Y. Jiang, M. Abe, T. Tsukiyama, H. Nagahama, S. Ohno, S. Hatakeyama and K. I. Nakayama: Increased proliferation of B cells and auto-immunity in mice lacking protein kinase C delta. *Nature* 416, 865-869 (2002)
170. Y. Zhang, S. F. Schlossman, R. A. Edwards, C. N. Ou, J. Gu and M. X. Wu: Impaired apoptosis, extended duration of immune responses, and a lupus-like autoimmune disease in IEX-1-transgenic mice. *Proc Natl Acad Sci U S A* 99, 878-883 (2002)
171. T. Higuchi, Y. Aiba, T. Nomura, J. Matsuda, K. Mochida, M. Suzuki, H. Kikutani, T. Honjo, K. Nishioka and T. Tsubata: Cutting Edge: Ectopic Expression of CD40 Ligand on B Cells Induces Lupus-Like Autoimmune Disease. *J Immunol* 168, 9-12 (2002)
172. H. W. Wang and M. J. Shlomchik: Autoantigen-specific B cell activation in Fas-deficient rheumatoid factor immunoglobulin transgenic mice. *J Exp Med* 190, 639-649 (1999)
173. J. William, C. Euler, S. Christensen and M. J. Shlomchik: Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* 297, 2066-2070 (2002)
174. D. Friedmann, N. Yachimovich, G. Mostoslavsky, Y. Pewzner-Jung, A. Ben Yehuda, K. Rajewsky and D. Eilat: Production of high affinity autoantibodies in autoimmune New Zealand Black, New Zealand White F-1 mice targeted with an anti-DNA heavy chain. *J Immunol* 162, 4406-4416 (1999)
175. U. Wellmann, A. Werner and T. H. Winkler: Altered selection processes of B lymphocytes in autoimmune NZB/W mice, despite intact central tolerance against DNA. *Eur J Immunol* 31, 2800-2810 (2001)
176. L. Mandik-Nayak, S. j. Seo, C. Sokol, K. M. Potts, A. Bui and J. Erikson: MRL-lpr/lpr Mice Exhibit a Defect in Maintaining Developmental Arrest and Follicular Exclusion of Anti-double-stranded DNA B Cells. *J Exp Med* 189, 1799-1814 (1999)
177. M. L. Fields, C. L. Sokol, A. Eaton-Bassiri, S. j. Seo, M. P. Madaio and J. Erikson: Fas/Fas ligand deficiency results in altered localization of anti-double-stranded DNA B cells and dendritic cells. *J Immunol* 167, 2370-2378 (2001)
178. Y. Li, H. Li, D. Ni and M. Weigert: Anti-DNA B cells in MRL/lpr mice show altered differentiation and editing pattern. *J Exp Med* 196, 1543-1552 (2002)
179. S. Santulli-Marotto, Y. Qian, S. Ferguson and S. H. Clarke: Anti-Sm B cell differentiation in Ig transgenic MRL/Mp-lpr/lpr mice: altered differentiation and an accelerated response. *J Immunol* 166, 5292-5299 (2001)

Key Words: Immune System, Genetics, Autoimmunity, B cells, Autoantibodies, Review

Send correspondence to: Laurence Morel, Ph.D., Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, Florida, 32610, Tel: 352-392-3790, Fax: 352-392-3053, E-mail: morel@ufl.edu

http://www.bioscience.org/current/vol12.htm