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

<table>
<thead>
<tr>
<th>QTL</th>
<th>Position (chr, cM)</th>
<th>Strain</th>
<th>Phenotype</th>
<th>Disease model</th>
<th>References</th>
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<tbody>
<tr>
<td>Sle1</td>
<td>1 (76-110)</td>
<td>NZM2410/NZW</td>
<td>ANA, B cell hyperactivity</td>
<td>SLE</td>
<td>51,57,56</td>
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<td>Nha2</td>
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<td>SLE</td>
<td>81</td>
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<tr>
<td>NZh1</td>
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<td>NZB</td>
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<td>90</td>
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<tr>
<td>Lk4</td>
<td>2 (65-569)</td>
<td>NZB</td>
<td>High B-1 cell numbers</td>
<td>SLE</td>
<td>33</td>
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<tr>
<td>Sle2</td>
<td>2 (21-57)</td>
<td>NZM2410/NZW/NZB</td>
<td>Increased B-1a cell numbers, lower threshold for B cell activation</td>
<td>SLE</td>
<td>50,83</td>
</tr>
<tr>
<td>Imh-1, Mott-1</td>
<td>4 (62-69)</td>
<td>NZB</td>
<td>Hypergamma globulinemia, Mott cell formation</td>
<td>SLE</td>
<td>34,35</td>
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<tr>
<td>Lhb2</td>
<td>4 (59-62)</td>
<td>NZB</td>
<td>B cell hyperactivity, IgM AAbs</td>
<td>SLE</td>
<td>144</td>
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<tr>
<td>Idd11</td>
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<td>NZD</td>
<td>Increased MZ B-cell numbers</td>
<td>TID</td>
<td>36,4</td>
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<td>Sle3</td>
<td>7 (23-33)</td>
<td>NZM2410/NZW</td>
<td>Ig repertoire</td>
<td>SLE</td>
<td>145</td>
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<tr>
<td>Bpal-2</td>
<td>13 (8-35)</td>
<td>NZW</td>
<td>High B-1 cell numbers</td>
<td>SLE, B-CLL</td>
<td>42</td>
</tr>
<tr>
<td>NZBc13</td>
<td>13 (24-73)</td>
<td>NZB</td>
<td>B cell hyperactivity, autoAbs</td>
<td>SLE</td>
<td>146</td>
</tr>
<tr>
<td>Bpal-1</td>
<td>17 (H-2' linked)</td>
<td>NZW</td>
<td>High B-1 cell numbers</td>
<td>SLE, B-CLL</td>
<td>42</td>
</tr>
</tbody>
</table>

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 Iq23 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 AUTOACTIVITY 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 Llk 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 Llk polymorphism was also found in humans and its frequency, albeit low, is significantly higher in SLE patients than in controls (33).

Hypergamma globulinemia 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 naive 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
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lupus prone strain NZW. This study linked this phenotype to the H-2\(^d\) 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\(^d\) 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 iddc11 and the size of the MZB compartment, congenic analysis has shown that the H-2\(^d\) 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 H-2 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.NZMSle1, 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 FcRRIIB\(^-\) (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\(^+\) and Ly108\(^-\), 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 germline center efficiency through antigen trapping by the follicular dendritic cells (74). Cr2 deficiency has been associated with loss of B cell tolerance and autoimmune 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.

Sle3 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 VH DJH junctional diversity and VH 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-2^q locus (46,92,93). On the other hand, detailed side by side characterization of the Sle1 and Nba2 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 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, underscores 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.
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Table 2. B-cell expressed genes whose targeting is associated with autoreactivity in rodent models

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Position (chr, cM)</th>
<th>Method</th>
<th>Allele type</th>
<th>Function</th>
<th>Associated disease</th>
<th>References</th>
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</thead>
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<tr>
<td>bcl2</td>
<td>Bcl-2</td>
<td>1 (59.8)</td>
<td>Eu-hbcl2 tg</td>
<td>over-expression</td>
<td>in B cells</td>
<td>apoptosis</td>
<td>Lupus 147</td>
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<td>purc</td>
<td>CD45</td>
<td>1 (74)</td>
<td>KO</td>
<td>null allele</td>
<td>signaling</td>
<td>Lupus 148</td>
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<tr>
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<td>FcγRIIB</td>
<td>1 (93)</td>
<td>KO on B6</td>
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<td>signaling</td>
<td>Lupus 149,150</td>
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<tr>
<td>Fcg2b</td>
<td>FcγRIIB</td>
<td>1 (93)</td>
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<td>NZB, BXS8, MRL,</td>
<td>and NOD allele</td>
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<tr>
<td>Fcg2b</td>
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<td>1 (93)</td>
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<td>Cr2</td>
<td>CD21/CD35</td>
<td>1 (106.6)</td>
<td>KO</td>
<td>Null on lpr</td>
<td>background</td>
<td>BCR co-receptor</td>
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<td>pleg2</td>
<td>phospholipase C, gamma 2</td>
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<td>CD22</td>
<td>CD22</td>
<td>7 (9)</td>
<td>KO</td>
<td>null allele</td>
<td>signaling</td>
<td>Lupus 162,163</td>
<td></td>
</tr>
<tr>
<td>CD19</td>
<td>CD19</td>
<td>7 (59)</td>
<td>Tg</td>
<td>hypermorph</td>
<td>signaling</td>
<td>Lupus 107</td>
<td></td>
</tr>
<tr>
<td>Tnfsf15b</td>
<td>BAFF</td>
<td>8 (3)</td>
<td>Tg</td>
<td>over-expression</td>
<td>growth factor</td>
<td>Lupus 164</td>
<td></td>
</tr>
<tr>
<td>Tnfsf15b</td>
<td>BAFF</td>
<td>8 (3)</td>
<td>Tg on B6.Sle1 or B6.Nba2</td>
<td>over-expression</td>
<td>growth factor</td>
<td>Accelerated lupus 95</td>
<td></td>
</tr>
<tr>
<td>Fli-1</td>
<td>Fli-1</td>
<td>9 (16)</td>
<td>Tg (class 1 promoter)</td>
<td>over-expression</td>
<td>apoptosis</td>
<td>Lupus 165</td>
<td></td>
</tr>
</tbody>
</table>

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, FcγRIIB, has crystallized more attention than others in the recent years. FcγRIIB 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 FcγRIIB 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 FcγRIIB allele in the bone marrow cells of lupus-prone NZM2410 mice, which carry an NZW FcγRIIB allele (119). This result suggested that restoration of FcγRIIB 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 FcγRIIB 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...
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-deficient FcγRIIB-/- mice. As for most KO strains, 129/SV alleles of flanking genes may also contribute to the strong autoimmunity phenotype displayed by B6.FcγRIIB-/- mice. 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 AUTOACTIVE 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 autoreactive 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.
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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 spontaneous 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 throughput engineering. This high-tech revamping of vetted concepts is showing promises. The high-throughput put screen of ethylNitosourea (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 Pleg2 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.

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