Review

Genetics of T Cell Defects in Lupus

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by anti-nuclear autoantibodies that cause damage to multiple organs and tissues. Intrinsic defects have been demonstrated in the lymphoid and myeloid cellular compartments, including T cells. Lupus susceptibility is mediated through the interplay of a large number of genes, most of which are still unidentified. Most of the genetic studies in both human patients and mouse models have addressed lupus susceptibility as a whole. More recently however, more attention has been paid to the inheritance of specific lupus-associated phenotypes. In this review, we summarized our results obtained with the *Sle1* locus in the NZM2410 mouse model, which mediates the generation of anti-histone autoreactive T cells. *Sle1*, which is constituted of multiple genes, is the only known genomic region that is sufficient for the generation of autoreactive T cells. The identification of the corresponding genes will constitute a landmark for our understanding of the mechanisms of autoimmunity. Our results are discussed in the context of candidate genes and the role of T cells in systemic autoimmunity. *Cellular & Molecular Immunology*. 2005;2(6):403-409.

Key Words: autoimmunity, T cell, genetics, lupus, mouse model

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder, characterized by the production of large amounts of autoantibodies directed against nuclear-protein complexes. Clinical disease developing from immune complexes deposits in target organs such as the kidney. It is now widely acknowledged that the etiology of this disease combines multiple genetic and environmental factors. Considerable progress has been made in determining the genomic location and, to a lesser extent, the identity of the lupus susceptibility loci in humans (1) and mice (2). These studies have largely addressed lupus susceptibility as a whole, although more recent analyses have stratified lupus patients according to the targeted organs, and specific loci have now been identified for nephritis (3, 4) and thrombocytopenia (5). A voluminous body of data has shown again both in human patients and mouse models that lupus is sustained by various immunological abnormalities affecting B cells (6) and T cells (see below). The congenic dissection of the NZM2410 murine model has shown that these defects have a genetic basis, as specific genetic loci segregate with specific immunological defects (7). To briefly summarize these results, we have mapped the position of four NZM2410 lupus nephritis-susceptibility QTL loci, *Sle1-4* (8), 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 (9). The phenotypes contributed by each locus have been determined *via* detailed analysis of the immunological properties of each congenic strain in comparison to B6 (10-16). Briefly, *Sle1* mediates the loss of tolerance to nuclear antigens leading to the production of autoreactive B and T cells culminating in the secretion of anti-nuclear autoantibodies (11). *Sle2* leads to B cell hyperactivity and elevated B-1 cell numbers (10, 17). *Sle*3 leads to increased T cell activation and decreased activation-induced cell death in $CD4^+$ T cells, with evidence that this phenotype is induced by defects in the myeloid compartment (18). Finally, *Sle4* corresponds to a resistance locus (19) that maps within 1 Mb of the $H\text{-}2^z$ allele (20). This review will focus on the genetic evidence for T cell defects in lupus.

Autoreactive T cells are required for the development of systemic autoimmunity (21). Specifically, nucleosome-specific T cells provide help to anti-DNA specific B cells in murine models (22) and human lupus (23). SLE patients show abnormal T cell receptor signaling, at least partially due to decreased levels of the CD3ζ chain, and aberrant proliferation, cytokine production, and apoptosis have been described in lupus T cells in response to stimuli (24). Help

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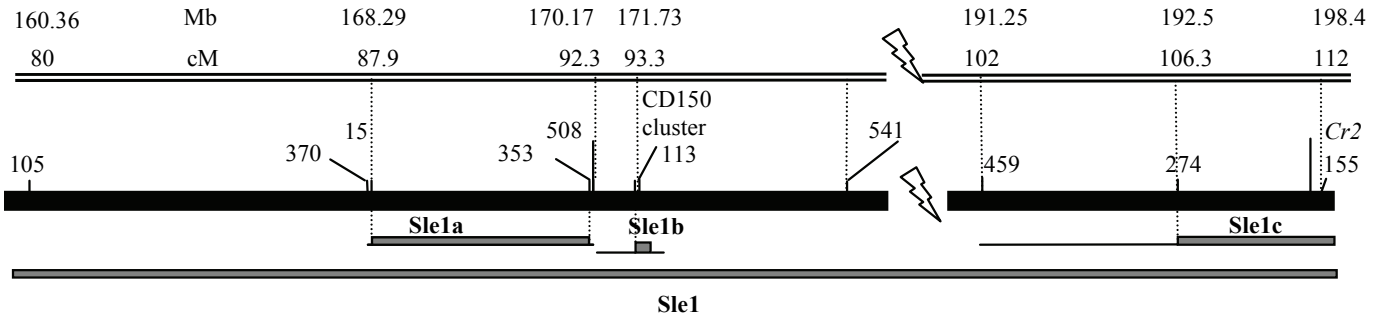

Figure 1. Genetic map of the *Sle1* locus showing the position of the *Sle1a*, *Sle1b*, and *Sle1c* intervals. The telomeric end of chromosome 1 is represented with, from top to bottom, the position in Mb and cM of D1MIT markers and candidate genes for the *Sle1* loci. The positions of the NZM2410-derived congenic intervals for B6.*Sle1a*, B6.*Sle1b*, B6.*Sle1c*, and B6.*Sle1* are shown as boxes linked to the flanking homozygous NZM2410 markers indicated on the chromosome. Horizontal lines extended on each side on these boxes indicate the area of recombination between the NZM2410 and B6 markers. For clarity, the centromeric end of the *Sle1* interval is not shown, and a break between D1MIT541 and D1MIT407 has been introduced.

from cognate αβ T cells is required for disease induction in the MRL/lpr mouse model (25). Lupus-prone mice carry an increased number of activated T cells, and the use of TCR transgenic mice has shown that MRL T cells have a lower threshold of activation (26). Reverse genetic experiments have demonstrated that the lowered T cell activation thresholds found in Cbl-b (27), Gadd45 (28) or E2F2 (29) knock-out mice result in severe systemic autoimmunity. However, such complete genetic deficiencies are rare in SLE patients, and T cell defects in spontaneous murine models of lupus are most likely to mirror the human genetic defects. As lupus is a polygenic disease with multiple phenotypic defects, including T cell defects, congenic lines are necessary to conduct the genetic analysis and reduce the genetic and phenotypic complexity (30). We have used the NZM2410 model and congenic strains that we have produced from it to characterize the genetics of T cell contribution to lupus pathogenesis.

The NZM2410 *Sle1* locus

The NZM2410 strain is derived from (NZB X NZW)F_1 mice. Linkage analysis has identified the major genetic loci responsible for SLE susceptibility (8). The locus with the strongest linkage was *Sle1* on telomeric chromosome 1, a region that has been independently linked to SLE in several mouse models and human patients (31). Functional analyses of the B6.*Sle1* congenic mice carrying *Sle1* on a non-autoimmune B6 background have shown that this locus induces a loss of tolerance to nuclear antigens (11, 13), primarily directed to the H2A/H2B/DNA sub-nucleosomal particles, which are the primary targets in the early production of anti-nuclear antibodies (32). Genetic experiments have demonstrated that *Sle1* was necessary for the development of nephritis in the NZM2410 model (19, 33). Moreover, co-expression of *Sle1* with a number of single mutations such as Yaa (33), Lpr (34), or FcrγRIIb$^{-/-}$ (35), or other SLE-susceptibility locus such as *Sle3* produced a highly penetrant clinical pathology (36). By using mixed bone-marrow chimeras or breeding *Sle1* on mice genetically deficient in B or T cells, we have determined that *Sle1* independently affects B and T cells (14, 16). Mixed bone marrow chimeras have determined that *Sle2* is functionally expressed in B cells (17) and *Sle3* is functionally expressed in macrophages and myeloid dendritic cells (15, 18). As a consequence, *Sle1* is the only major susceptibility locus in the NZM2410 model that is functionally expressed in T cells. Therefore, the functional and genetic characterization of this locus will provide unique insights into the mechanisms by which lupus T cells are dysregulated.

The B6.*Sle1* congenic strain contains a 53 Mb, 39-cM NZM2410-derived interval defined by the D1MIT101 (145.29 Mb, 73 cM) and D1MIT155 (198.4 Mb, 112 cM) markers (Figure 1). The analysis of *Sle1* congenic recombinants showed that production of anti-nuclear Abs corresponds to at least three independent loci, *Sle1a*, *Sle1b* and *Sle1c*, with overlapping but distinct phenotypes (37). The *Sle1a* locus represents a 2.5-Mb segment that is relatively gene-poor, with 14 reported known genes or predicted transcripts. No candidate gene has been identified yet for *Sle1a*. The *Sle1b* locus has been narrowed down to a less than 1 Mb segment, and is allelic with polymorphisms in a cluster of seven genes from the CD150/SLAM family (38). 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 (39), as we have shown to be the case for *Sle1*. Furthermore, SAP, the adaptor molecule that links the CD150 family member to FynT, regulates Th2 cytokine production by T cells (40) and SAP-deficiency protects mice from experimental lupus by targeting T-dependent humoral immune responses (41). Further work will be necessary to determine which of the genes in the CD150 family is responsible for the *Sle1b* phenotypes and by which mechanism it occurs. A candidate

gene approach has proposed that polymorphisms in the complement receptor 2 (*Cr2*) gene were responsible for *Sle1c* phenotypes (42). The CR1 and CR2 proteins, which are alternatively spliced 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 (43). *Cr2* deficiency has been associated with loss of B cell tolerance and autoantibody production, especially in the absence of FAS expression (44, 45). Finally, CR1/CR2 levels are significantly decreased in lupus patients (46, 47), and in certain cases, inversely related to disease activity (48). Similarly, decreased CR2 expression has been reported on B cells before the onset of disease in the MRL/lpr (49) and chronic graft versus host disease (cGVHD) models of SLE (50).

Each of the *Sle1* loci has a relatively modest phenotype, leading to the production of anti-nuclear antibodies with a penetrance varying from 80% for *Sle1b* to about 30% for *Sle1a* and *Sle1c* (37). The production of autoreactive lymphocytes by these loci is kept in check by a FAS-dependent pathway, as illustrated by a dramatic increase in pathogenicity when each of the loci is expressed on a FAS-deficient background (51). The pathogenic impact of each locus is greatly dependent on genetic interactions, which play a crucial role in lupus susceptibility (52). Specifically, within the *Sle1* locus, *Sle1a* and *Sle1b* synergistic expression results in phenotypes that are absent in either single congenics (51), and the combination of these two loci results in an aberrant activation of the Ras-ERK pathway in B lymphocytes (53). The respective contribution of *Sle1a* and *Sle1b* to this differentiation pathway is still to be characterized. The rest of the review will discuss the specific impact of each of the three loci *Sle1a*, *Sle1b*, and *Sle1c*, on T cell phenotypes.

Genetic of T cell defects within the *Sle1* locus

Mixed bone marrow chimeras have shown that *Sle1* was functionally expressed in T cells, where it resulted in an up-regulation of activation markers, cytokine production, and generation of histone-specific autoreactive IFN-γ-secreting $CD4^+$ T cells. By breeding the *Sle1* locus with the *Igh6* mutation, which prevents the development of mature B cells, we demonstrated that the *Sle1* T cell defects are intrinsic and independent of the presence of B cells (16). We have performed experiments to further define the nature of the T cell phenotypes mediated by *Sle1*, and to map out the genes responsible (54, 55).

Sle1-expressing T cells are hyper-activated, and show increased proliferation and decreased apoptosis

A large panel of activation markers showed that *Sle1*-expressing $CD4^+$ T cells are hyper-activated. The *Sle1*-induced up-regulation of CD154 (CD40L) is of particular interest since abnormal expression of CD154 has been associated with lupus (23, 56). Furthermore, blocking the CD40/CD154 interactions significantly delayed disease onset and severity in murine lupus (57). These results suggest that up-regulation of CD154 might be one of the mechanisms by which *Sle1* T cells participate in SLE pathogenesis. We also showed that *Sle1* was associated with a greater proliferation in response to receptor cross-linking, or even in medium alone. In addition, *Sle1* is associated with a lower activation-induced cell death in $CD4^+$ T cells, which is a phenotype that has been demonstrated in MRL helper T cells (58). Our results showed that this phenotype is under the genetic control of *Sle1* in the NZM2410 model. Furthermore, we have shown that *Sle1* $CD4^+$ T cells produce higher amounts of IL-2, IL-4 and IFN-γ, suggesting that *Sle1* does not mediate lupus through cytokine polarization, but through a greater effector capability.

When these phenotypes were examined in the B6.*Sle1* sub-congenic strains, it showed that *Sle1a* expression alone resulted in the same phenotypes as the whole *Sle1* interval, identifying this locus as the strongest T cell phenotype modifier in the NZM2410 model. *Sle1c* produced intermediate phenotypes, indicating that this interval contains alleles impacting on T cell phenotypes, but not as strongly as *Sle1a*. Interestingly, *Sle1b* expressing $CD4^+$ T cells were identical to that of B6. This result is in apparent conflict with the *Sle1b* gene being a member of the CD150 family, which is expressed on T cells. Furthermore, *Sle1b* T cells have shown increased calcium flux in response to receptor cross-linking (38). Our data does not exclude an impact of *Sle1b* on T cells, but shows that, when compared side by side, *Sle1a* and *Sle1c* have a stronger effect on $CD4^+$ T cell proliferation, activation, and cytokine production than *Sle1b*.

Sle1 and Sle1a T cells induce activation and plasmablast formation in B cells

To assess the respective contribution of *Sle1a* and *Sle1c* to T cell phenotypes and their indirect effect on B cells, we performed adoptive transfers of *Sle1*, *Sle1a*, *Sle1c*, or B6 T cells in mice genetically deficient in conventional αβ T cells, either expressing *Sle1* (B6.*Sle1.Tcrα*$^{-/-}$) or not (B6.*Tcrα*$^{-/-}$). A significantly higher number of *Sle1*-expressing $CD4^+$ T cells were recovered after transfer into either B6.*Sle1.Tcrα*$^{-/-}$ or B6.*Tcrα*$^{-/-}$ mice, compared with transfers from the other strains, reflecting a higher proliferation and survival of these cells in the chimeric mice. Significantly higher numbers of *Sle1* and *Sle1a* $CD4^+$ T cells expressed CD69 after transfer into B6.*Sle1.Tcrα*$^{-/-}$ or B6.*Tcrα*$^{-/-}$ mice, which confirmed the $CD4^+$ T cell activation induced by these loci.

Transfers of *Sle1* or *Sle1a* T cells resulted in a significantly higher expression of MHC class II I-Ab molecules by B cells in B6.*Sle1.Tcrα*$^{-/-}$ or B6.*Tcrα*$^{-/-}$ mice, compared with transfer of B6 T cells in the same strains. As the result of *Sle1* expression, I-Ab levels on B cells was already higher in B6.*Sle1.Tcrα*$^{-/-}$ than in B6.*Tcrα*$^{-/-}$ mice, and the addition of *Sle1* or *Sle1a*, but not B6 T cells increased this level by 40% in both recipient strains. *Sle1c* T cells induced a significantly higher I-Ab expression only in *Sle1*-expressing B cells. Transfers of *Sle1* or *Sle1a* T cells resulted in significantly higher numbers of $B220^+$ $CD138^+$ plasmablasts in the spleens of either B6.*Sle1.Tcrα*$^{-/-}$ or B6.*Tcrα*$^{-/-}$ mice,

compared with transfer of B6 T cells. Similar to the B cell activation markers, transfers of *Sle1c* T cells induced a significantly higher number of plasmablasts only in B6.*Sle1.Tcrα*$^{-/-}$ spleens. These results showed that *Sle1* heightens T cell help to B cells by increasing B cell activation and plasma cell differentiation, independently of *Sle1* expression in B cells for *Sle1a*, but not for *Sle1c*.

Sle1a T cells induce anti-chromatin IgG antibody production from both Sle1-expressing and non-autoimmune B cells, but Sle1c T cells induce anti-chromatin IgG antibodies only from Sle1-expressing B cells

Adoptively transferred T cells expressing either the entire *Sle1* locus or only *Sle1a* provided help to chromatin-specific B cells, whether or not these B cells expressed *Sle1*. These results demonstrate that *Sle1a* is necessary and sufficient to induce nuclear antigen-specific autoreactive T cells, and these T cells are able to provide help to anti-chromatin-specific B cells to produce autoantibodies. No difference was obtained between the levels of anti-chromatin antibodies or the number of antibody secreting cells induced by *Sle1* or *Sle1a* T cells, indicating that *Sle1a* accounts for the entire *Sle1* phenotype. *Sle1c* T cells provided help only to *Sle1*-expressing but not to B6-derived chromatin-specific B cells.

B cells from B6.*Sle1.Tcrα*$^{-/-}$ mice were induced to produce anti-chromatin IgG antibodies by T cells expressing *Sle1*, *Sle1a*, or *Sle1c*, but not by T cells obtained from B6. Interestingly, significantly higher amounts of anti-chromatin IgG and numbers of antibody secreting cells were obtained with transfers of *Sle1*, *Sle1a*, or *Sle1c* T cells to B6.*Sle1.Tcrα*$^{-/-}$ mice than the same transfers to B6.*Tcrα*$^{-/-}$ mice. This clearly demonstrates that interactions between *Sle1* T and B cells are responsible for the high level of anti-nuclear antibodies produced by B6.*Sle1* mice. Finally, *Sle1* B cells, which contain a high number of autoreactive anti-nuclear clones, cannot be induced to produce anti-chromatin IgG antibodies by B6 T cells. These results also confirm that T cell tolerance is controlled more tightly than B cell tolerance since chromatin-reactive B cells, but not T cells, can be easily found and functionally activated in non-autoimmune B6 mice.

As implied by the *in vivo* T cell transfers, *Sle1a* and *Sle1c* resulted in the production of chromatin-specific T cells. *Sle1*, *Sle1a* and *Sle1c* T cells proliferated significantly more *in vitro* and produced significantly more cytokines than B6 T cells in response to a mixture of histone and dsDNA. These T cells did not respond to non-nuclear self or non-self antigens，confirming the specificity of *Sle1* T cells for nuclear antigens.

The requirement for cognate T cell help for anti-nuclear antibody production has been demonstrated in a double transgenic system in which cognate h-LA specific T cell help was sufficient to trigger anti-nuclear auto-antibodies in h-LA transgenic naïve B cells (59). Our results show that anti-nuclear cognate T cell help is produced by *Sle1a* and, to a lesser extent, *Sle1c* expression, and that these loci constitute an essential component of the production of class-switched anti-nuclear antibodies in the NZM2410 model.

The *Sle1* locus affects the number of CD4$^+$CD25$^+$ Treg cells

CD4$^+$CD25$^+$ regulatory T (Treg) cells undoubtedly play a role in preventing organ-specific autoimmune diseases such as type 1 diabetes (60) and experimental autoimmune encephalomyelitis (61). The contribution of Treg cells to the prevention of systemic humoral autoimmunity has been shown with double transgenic models expressing a TCR and its cognate antigen (59, 62, 63). Tolerance induction to nuclear antigen with histone peptides in the (SWR X NZB)F_1 model is dependent on the generation of antigen-specific Treg cells (64). Results have been less clear in spontaneous models. Neonatal thymectomies have shown mixed results in lupus models (65, 66). In the NZM2328 model, which is closely related to NZM2410, Treg cells clearly play a role in humoral autoimmunity, but do not seem to play a major role in end-organ damage (67). Treg cells have been shown, however, to play a protective role in experimental glomerulonephritis by migrating to the kidneys and inhibiting cell-mediated autoimmunity (68). This result is in full agreement with the intra-islet location of the Treg cell protective effect in the NOD model of type 1 diabetes (69). Overall, these results strongly suggest that Treg cells play a significant role in protection against systemic autoimmunity, and suggest that defects in the number or function of these cells may be involved in the etiology of spontaneous SLE.

The percentage of CD4$^+$CD25$^+$CD62L$^+$ splenocytes was significantly decreased in B6.*Sle1*, B6.*Sle1a*, and B6.*Sle1c* mice as compared with B6.*Sle1b* or B6 (54). This reduction was not secondary to the autoimmune process and overall immune dysregulation, because the *Sle1a*- and *Sle1c*-associated decrease in Treg cell population was found in young mice before the apparition of anti-chromatin antibodies. *Foxp3* is a transcription factor that is necessary and sufficient to confer the suppressive Treg cell phenotype to CD4$^+$ T cells (70). *Foxp3* expression was reduced by 4-fold in B6.*Sle1* and B6.*Sle1a* and by 2.5-fold in B6.*Sle1c* CD4$^+$CD25$^+$ cells, as compared with B6.*Sle1b* and B6. A similar decreased *Foxp3* expression pattern was found in both *Sle1* spleen and thymus, suggesting that these loci are not simply involved in the peripheral maintenance of Treg cells but may control their production from the thymus. A similar decreased Foxp3 protein expression was found in CD4$^+$CD25$^+$ cells in B6.*Sle1*, B6.*Sle1a*, and B6.*Sle1c* mice. Our results thus show for the first time a significant reduction of Treg cells associated with the generation of autoreactive T cells in a spontaneous model of lupus, and associate two specific genetic loci with this phenotype.

The mechanism by which a reduction in Treg results into the production of anti-nuclear autoreactive T cells is currently not known. Specifically, it is not known whether *Sle1* affects only nuclear antigen-specific Treg cells or the entire regulatory subset. This issue will be resolved by

characterizing the specificity of *Sle1* T cell clones and the effect of *Sle1* on T cells with defined specificities.

Fine-mapping of the *Sle1c* locus

By crossing B6.*Sle1c* to NZB and comparing these mice to (NZB X B6)F1 controls, we have established that this locus significantly contributes to anti-dsDNA antibody production and aggravation of renal lesions (71). We have proposed that *Cr2* was a candidate gene for *Sle1c* based on the functional polymorphisms found in the NZM2410 strain that affect B cell responses to sub-optimal receptor cross-linking, and the autoimmune phenotype of the *Cr2* deficient mice (42). *Sle1c* expression also contributes to the production of activated autoreactive T cells, and mixed bone marrow chimeras demonstrated that this phenotype was intrinsic to *Sle1c*-expressing T cells (55). Expression of CR1/CR2 has been reported on activated T cells in A/J mice (72), and is also expressed on a subset of activated *Sle1c* and B6 T cells. Whether this expression directly affects T cell functions is unknown. Several lines of evidence suggest that *Cr2* may indirectly affect T cell phenotypes, either through antigen presentation or B cell regulation (73). Alternatively, another gene in the *Sle1c* interval besides *Cr2* may contribute to the T cell phenotype. Indeed, examination of T cells in *Sle1c* recombinants excluded *Cr2* from playing a major role in their activation. Instead, two more centromeric loci, *Sle1c-1* and *Sle1c-2* are most likely to account for the *Sle1c*-activated T cell phenotypes. Both of these loci play a role in increasing activation and a reducing Treg cells population, and *Sle1c-2* plays an additional role in increasing proliferation. These results reveal a complex situation in which at least three genes are responsible for the *Sle1c* phenotypes. Additional recombinants will be necessary for fine-mapping and to eliminate candidate genes. A similar type of congenic recombinant is currently being conducted with *Sle1a*, and initial results suggest that more than one gene may also be involved.

In conclusion, the phenotypic characterization of *Sle1*-expressing T cells has identified *Sle1a* and *Sle1c* as being responsible for the production of autoreactive T cells and decreased number of regulatory T cells. The on-going efforts to identify the corresponding genes should lead to elucidation of the pathways responsible for this integral part of systemic autoimmune pathogenesis.

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