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Murine Lupus Susceptibility Locus Sle1c2 Mediates CD4+ T Cell Activation and Maps to Estrogen-Related Receptor γ

Daniel J. Perry,* Yiming Yin,† Tiffany Telarico,‡,§ Henry V. Baker,¶ Igor Dozmorov,‖ Andras Perl,†‡ and Laurence Morel*†

Sle1c is a sublocus of the NZM2410-derived Sle1 major lupus susceptibility locus. We have shown previously that Sle1c contributes to lupus pathogenesis by conferring increased CD4+ T cell activation and increased susceptibility to chronic graft-versus-host disease (cGVHD), which mapped to the centromeric portion of the locus. In this study, we have refined the centromeric sublocus to a 675-kb interval, termed Sle1c2. Mice from recombinant congenic strains expressing Sle1c2 exhibited increased CD4+ T cell intrinsic activation and cGVHD susceptibility, similar to mice with the parental Sle1c. In addition, B6.Sle1c2 mice displayed a robust expansion of IFN-γ–expressing T cells. NZB complementation studies showed that Sle1c2 expression exacerbated B cell activation, autoantibody production, and renal pathology, verifying that Sle1c2 contributes to lupus pathogenesis. The Sle1c2 interval contains two genes, only one of which, Esrrg, is expressed in T cells. B6.Sle1c2 CD4+ T cells expressed less Esrrg than B6 CD4+ T cells, and Esrrg expression was correlated negatively with CD4+ T cell activation. Esrrg encodes an orphan nuclear receptor that regulates oxidative metabolism and mitochondrial functions. In accordance with reduced Esrrg expression, B6. Sle1c2 CD4+ T cells present reduced mitochondrial mass and altered mitochondrial functions as well as altered metabolic pathway utilization when compared with B6 CD4+ T cells. Taken together, we propose Esrrg as a novel lupus susceptibility gene regulating CD4+ T cell function through their mitochondrial metabolism.


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Abbreviations used in this article: aTRA, all-trans retinoic acid; BM, bone marrow; cGVHD, chronic graft-versus-host disease; DAR-4M, diaminorhodamine-4M; ERR, estrogen-related receptor; Esrrg, estrogen-related receptor γ; Fluo-3AM, fluo-3-acetoxyethyl ester; GN, glomerulonephritis; HE, hydroethidine; MFI, mean fluorescence intensity; MTG, MitoTracker Green-FM; PI, propidium iodide; ROI, reactive oxygen intermediate; SLAM, signaling lymphocytic activation molecule; SLE, systemic lupus erythematosus; SNP, single-nucleotide polymorphism; Tem, T effector memory cell; TMRM, tetramethylrhodamine methyl ester; Treg, regulatory T cell; VDAC, voltage-dependent anion channel.
We have previously reported that Slelc2 is associated with increased activation and proliferation of CD4⁺ T cells (12). In the current study, we mapped Slelc2 to estrogen-related receptor γ (Esrrγ), which encodes the orphan nuclear receptor ERR-γ. The expression of this gene, which regulates oxidative metabolism (20), has not been reported previously in T cells. We showed that the NZW allele is associated with reduced Esrrγ expression in CD4⁺ T cells, which strongly correlates with increased cell activation, and the expansion of IFN-γ–secreting T cells. In addition, B6.Slelc2 CD4⁺ T cells showed reduced mitochondrial mass and hyperpolarization consistent with their reduced Esrrγ expression.

Finally, we demonstrated that Slelc2 contributes to lupus phenotypes in two disease models. These results suggest that Esrrγ is a novel lupus susceptibility gene that regulates CD4⁺ T cell function and activation through their mitochondrial metabolism.

### Materials and Methods

#### Mouse

B6.Slelc mice that contain a NZW-derived interval at the telomeric end of chromosome 1 have been described previously (9). The loci previously referred to as Slelc.Cr2-1 on the telomeric end and Slelc.Cr2-2 on the centromeric end (12) have been renamed Slelc1 and Slelc2, respectively, to be more consistent with the terminology for the other loci. To generate additional recombinant subcongenic strains, (B6 × B6.Slelc)F1 × B6 backcross progeny were genotyped for recombination in the Slelc interval with microsatellites that are polymorphic between NZW and B6 mice. Recombinants were bred to B6 mice, and the progeny of this expansion backcross then were bred to homozygosity. Th17 polarization, CD4⁺ T cells were cultured with anti-CD3e and anti-CD28 (37.51) (BD Biosciences) or with 0.5 μM all-trans retinoic acid (Sigma-Aldrich).

Calcium stores were measured by fluorescence of fluo-3-acetoxymethyl ester (Fluo-3AM) (1 μM). Hydroethidine (HE), dihydrohydorobenzamide 123 (DHR), and 2',7'-dichlorofluorescin were used as indicators for superoxide production. Apoptosis and necrosis were evaluated by costaining with FITC-conjugated Annexin V and propidium iodide (PI). TMRM, MTG, HF, Fluo-3AM, and PI were obtained from Invitrogen-Molecular Probes, DAR-4M from Calbiochem, and FITC-conjugated Annexin V from BD Biosciences. The metabolic profiling was evaluated on a LSR-II flow cytometer (BD Biosciences), with 50,000 events per sample collected. Data were analyzed using FlowJo cytometry analysis software (Tree Star, Ashland, OR). Mean fluorescence intensity (MFI) values of B6.Slelc2 mice were normalized to the means obtained for B6 mice studied in parallel and set at 1.0.

### Gene expression analyses

Total RNA was isolated using RNeasy mini kits, QIAshredders, and RINase-free DNase sets (Qiagen). cDNA then was synthesized using the ImProm-II Reverse Transcription System (Promega). Primers for ERRγ target genes (Supplemental Table I) were designed with the Primer3 software (http://frodo.wi.mit.edu/primer3) and used in Sybr Green (Applied Biosystems)-based quantitative RT-PCR. Taqman Gene Expression Assays (Applied Biosystems) were used to measure Esrrγ (Mm00516269_mH, spans exons 3a and 3b of transcript 1) and GAPDH expression in 4 of 6 transconjugant lines. The quantity of gene expression was calculated using the comparative Cₜ method (RQ = 2⁻ΔΔCₜ) normalized to the average ΔCₜ of the B6 samples. Global gene expression was compared between negatively bead-selected CD4⁺ T cells from 6-mo-old B6 and B6.Slelc2 mice (n = 5 per strain). Their cDNA was synthesized, fragmented, and biotin-labeled using the Ovation Biotin RNA Amplification and Labeling System (NuGEN Technologies), then hybridized to Affymetrix Mouse Genome 430 2.0 arrays. Data analysis was based on the use of "internal standards" and generalization of the "Error Model" (24) as presented elsewhere (25).

### ELISA

Anti-dsDNA and anti-chromatin IgG were measured by ELISAs as described previously (4). Sera were tested in duplicate in a 1:100 dilution. Relative units were standardized using serial dilutions of a positive serum from a B6.129P2-Sle2.2eGFP/NeomLo/LmoJ mouse, arbitrarily setting the 1:100 dilution reactivity to 100 U.

### Western blot analysis

Cell lysates prepared from splenic CD4⁺ T cells purified by negative selection were probed with rabbit polyclonal ERRγ Ab (Z-21; Santa Cruz Biotechnology) and revealed with HRP-conjugated anti-rabbit IgG (Sigma Aldrich). HRP-conjugated anti-GAPDH Ab (Sigma Aldrich) was used as a control. The membranes were developed by enhanced SuperSignal West Pico Chemiluminescent substrate (Thermo Scientific, Rockford, IL). The bands were quantified using AlphaVei software (Alpha Innotech).

For voltage-dependent anion channel (VDAC) expression, protein lysates were prepared from splenocytes and negatively isolated T cells (Dynal Biotech).
mixed mouse T cell negative isolation kit (Invitrogen) through lysis in radio-immunoprecipitation assay buffer [150 mM NaCl, 2% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris (pH 8.0), 1 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml pepstatin, 1 mM NaF, 1 mM sodium orthovanadate, 0.1 mM sodium molybdate, and 10 mM sodium pyrophosphate] at a density of 4 × 10⁶ cells per milliliter on ice, followed by the addition of equal volumes of Laemmli buffer and were heated to 95°C for 5 min before separation via SDS-PAGE and transferred to 0.45-μm nitrocellulose membranes. VDAC/porin was detected with rabbit polyclonal Ab H+L chains (Jackson ImmunoResearch). Staining intensity was evaluated in a blind manner on a semiquantitative 0–3 scale and averaged in two separate sets of cGVHD induction were performed with at least five mice per strain, yielding similar results.

**Mixed bone marrow chimera**

Chimeras were prepared as described previously (5). In brief, 4- to 5-mo-old B6.Tcvrb1 mice were lethally irradiated with two doses of 252 rad 4–6 h apart the day before reconstitution. Bone marrow (BM) cell suspensions from B6.Thp1b and B6.Sle1c2 were mixed 1:1 after depleting T cells using anti-CD5 magnetic beads (Miltenyi Biotec). Recipients received 10⁷ BM cells from sex-matched donors by i.v. injections, and grafts were allowed to reconstitute for 8 wk.

**Chronic graft-versus-host disease**

Chronic graft-versus-host disease (cGVHD) was induced as described previously (26). In brief, B6 and B6.Sle1c2 hosts received 8 × 10⁶ B6, bml2 splenocytes via i.p. injections. Sera were collected weekly after addition of equal volumes of Laemmli buffer and were heated to 95°C for 5 min before separation via SDS-PAGE and transferred to 0.45-μm nitrocellulose membranes. VDAC/porin was detected with rabbit polyclonal Ab H+L chains (Jackson ImmunoResearch). Staining intensity was evaluated in a blind manner on a semiquantitative 0–3 scale and averaged on at least 10 glomeruli per section. Two separate sets of cGVHD induction were performed with at least five mice per strain, yielding similar results.

**Experimental autoimmune encephalomyelitis**

Experimental autoimmune encephalomyelitis was induced in 4-mo-old male B6 or B6.Sle1c2 mice. On day 0, mice received an emulsion of 50 μg myelin oligodendrocyte glycoprotein peptide sequence 35–55 and 500 μg Mycobacterium tuberculosis glycolipid (Difco) in IFA (Sigma-Aldrich) via s.c. injection. Clinical scores were assessed by the following criteria: 0, no disease, 1, flaccid tail, 2, hind limb paralysis, 3, hind limb paraparesis, 4, quadriplegia. Mice were euthanized at a score of 4 or 40 d after induction. Because the strain with the shortest interval necessary for increased activation is the REC5 subcongenic and REC1, REC2, REC3, and REC8 were B6-like, Sle1c2 then was defined as the 675-kb region between SNPs rs30920616 and rs32528185 (Fig. 1). Except where noted, the REC5 strain was used as B6.Sle1c2 for the remainder of this study.

As opposed to the splenomegaly phenotype, which is age-dependent, CD4⁺ T cell increased activation was detectable as early as 2–3 mo of age (Supplemental Fig. 1A), suggesting that it is a primary consequence of Sle1c2 expression. Importantly, as shown previously for the entire Sle1c interval (12), a mixed BM chimera experiment showed that increased CD4⁺ T cell activation was intrinsic to Sle1c2-expressing T cells (Supplemental Fig. 1B). Because we have reported previously an increased proliferation of B6.Sle1c CD4⁺ T cells (12), we screened the congenic recombinants to discern whether this phenotype also mapped to the refined Sle1c2 locus. To assess whether the increased proliferation was Ag-specific, B6.Sle1c2.OTII double-congenic mice were generated for comparison with B6.OTII mice, which carry a transgenic...
TCR that is specific for the OVA323–339 peptide. Increased proliferation was observed with Ag-specific (B), polyclonal (C), and PMA-induced (D) proliferation of CD4+ T cells from 3- to 5-mo-old mice was measured by [3H]thymidine incorporation, and stimulation indexes were calculated relative to cultures with medium only. Both REC2b.OTII and REC5.OTII strains were used as Sle1c2.OTII. Two-way ANOVA (‡‡) was used to measure strain effect on Ag-specific proliferation with Bonferroni’s posttest indicating significance at each concentration in (B). The Mann–Whitney test was used in (C), and the t test was used in (D). (E and F) Absolute numbers of splenic CD4+ T cells (E) and of B and CD8+ T cells (F) were compared between B6 and B6.Sle1c2 in 5- to 8-mo-old and 10- to 14-mo-old cohorts. Naive T cells were defined as CD4+CD44loCD62L+, and Tems were defined as CD4+CD44hiCD62L-. Significance levels indicate Mann–Whitney comparisons to B6 mice of the same age group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ‡‡p ≤ 0.001.

**FIGURE 2.** Phenotypic mapping of Sle1c2. (A) Comparison of spleen weights, CD4/CD8 ratios, CD69 expression, and Tem percentages of CD4+ T cells from spleens of 10- to 14-mo-old mice. Significance levels indicate ANOVA with Dunn’s multiple comparison analysis to B6. (B–D) Ag-specific (B), polyclonal (C), and PMA-induced (D) proliferation of CD4+ T cells from 3- to 5-mo-old mice was measured by [3H]thymidine incorporation, and stimulation indexes were calculated relative to cultures with medium only. Both REC2b.OTII and REC5.OTII strains were used as Sle1c2.OTII. Two-way ANOVA (‡‡) was used to measure strain effect on Ag-specific proliferation with Bonferroni’s posttest indicating significance at each concentration in (B). The Mann–Whitney test was used in (C), and the t test was used in (D). (E and F) Absolute numbers of splenic CD4+ T cells (E) and of B and CD8+ T cells (F) were compared between B6 and B6.Sle1c2 in 5- to 8-mo-old and 10- to 14-mo-old cohorts. Naive T cells were defined as CD4+CD44loCD62L+, and Tems were defined as CD4+CD44hiCD62L-. Significance levels indicate Mann–Whitney comparisons to B6 mice of the same age group. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ‡‡p ≤ 0.001.

B6.Sle1c2 CD4+ T cells exhibit marked Th1 skewing
A gene expression and pathway analysis of CD4+ splenocytes revealed that a large number of genes related to IFN-γ expression were upregulated in B6.Sle1c2 mice, suggesting Th1 skewing (Supplemental Fig. 2A, 2B). Intracellular staining confirmed that a larger percentage of Sle1c2 CD4+ splenocytes expressed IFN-γ as compared with B6 (Fig. 3A). Importantly, CD4+ T cells from the parental B6.Sle1c strain showed a similar percentage of IFN-γ+ T cells as the B6.Sle1c2 subcongenic strain. No difference was observed for IL-4 production (data not shown).

The microarray analysis also revealed that several genes involved in Th17/Treg homeostasis were upregulated in B6.Sle1c2 CD4+ T cells (Supplemental Fig. 2C). These included Foxp3, Il2ra, and Il10 for Tregs and Ifnb, Rora, Il17a, Il21, and Il22 for Th17 cells. In addition, the Ahr and Hif1α pathways, which have been implicated in Th17/Treg homeostasis (22, 27, 28), also were upregulated (Supplemental Fig. 2B, 2C). Given this altered gene expression profile, we examined whether Sle1c2 expression augmented Treg and Th17 differentiation. Using Foxp3eGFP reporter mice, we showed that TGF-β with or without all-trans retinoic acid equally induced Foxp3 expression in B6 and B6.Sle1c2 CD4+ GFP+ T cells (Supplemental Fig. 1C). Moreover, contrary to our previous reports that described a decrease in the percentage of Tregs defined as CD4+CD62L−CD25+ (10, 12), the size of the
CD4\(^+\)Foxp3\(^+\) Treg compartment was similar between B6 and B6.Sle1c2 mice, with an expansion of Foxp3\(^+\) Tregs in older B6.Sle1c2 mice (Supplemental Fig. 1D). Interestingly, in vitro Treg polarization produced significantly more Foxp3\(^+\)IFN-\(\gamma\)\(^+\) cells in B6.Sle1c2 mice at 8–10 mo of age, but not 1–3 mo of age (Fig. 3B, 3D). This not only confirms the strong Th1 skewing associated with Sle1c2 but also suggests that B6.Sle1c2 mice may accumulate Tregs corresponding to a plastic population of IFN-\(\gamma\)\(^+\) adaptive Tregs that have been linked to autoimmunity (23).

Th17 in vitro polarization did not reveal differences in IL-17 production between the two strains (Fig. 3C, 3D). However, as with Treg polarization, IFN-\(\gamma\) production was increased in the B6.Sle1c2 Th17-polarized CD4\(^+\) T cells (Fig. 3C, 3D), highlighting again the Th1 skewing induced by Sle1c2 expression. Finally, experimental autoimmune encephalomyelitis was used as a model to test for differences in Th17/Treg homeostasis in vivo, and no differences were observed in day of onset or severity between the two strains (Supplemental Fig. 1E). Hence, although the global gene expression analysis predicted increased commitment to Th1, Treg, and Th17 lineages, cellular assays only revealed strong Th1 skewing.

Sle1c2 exacerbates lupus in the induced cGVHD model and via epistatic interactions with the NZB genome

To determine whether the CD4\(^+\) T cell phenotypes that segregate with Sle1c2 are relevant to lupus pathogenesis, we used the cGVHD-induced lupus model (29). Preliminary results mapped increased...
cGVHD susceptibility to the centromeric portion of Sle1c that includes Sle1c2 (12). In this study, we showed that CD4+ T cells were significantly more activated in B6.Sle1c2 recipients of B6. bm12 splenocytes as compared with B6 recipients (Fig. 4A). In addition, B6.Sle1c2 CD11c+ dendritic cells and B cells displayed altered activation (Fig. 4B, 4C) that was concurrent with increased serum autoantibody production (Fig. 4D). Finally, the kidneys of cGVHD-induced B6.Sle1c2 mice showed increased deposition of IgG2a immune complexes as compared with those of B6 mice (Fig. 4E). These results demonstrate that the activated phenotype of Sle1c2 CD4+ T cells contributes to induced humoral autoimmunity.

We have shown previously that Sle1c expression increases lupus phenotypes presented by (NZB × B6)F1 hybrids (30). Using the same model, we compared the phenotypes of (NZB × B6.Sle1c2)F1 mice, in which the NZB hemigenome interacts with either the B6 or the NZW Sle1c2 alleles, respectively. Importantly, the NZB and NZW strains share the same haplotype at the chromosome 1 189.80–190.050 Mb interval that includes the Sle1c2 portion of Esrrg, as determined for all 228 published SNPs that are polymorphic between B6 and NZW, with an NZB allele known or imputed with confidence ≥0.9 (data not shown). Therefore, the Sle1c locus is predicted to be homozygous for the NZB/NZW allele in (NZB × B6.Sle1c2)F1 mice and heterozygous in (NZB × B6)F1 mice. This is relevant because the Sle1c2 CD4+ T cell phenotype is recessive (data not shown). Our findings showed that, in 12-mo-old NZB hybrids, splenomegaly, CD4/CD8 T cell ratio, percentage of Tems, and B7-2 expression on B cells were increased significantly in mice with the homozygous NZW Sle1c2 allele (Fig. 5A). Moreover, the expression of Sle1c2 significantly enhanced the production of anti-dsDNA IgG triggered by the NZB hemigenome as the mice aged (Fig. 5B). Finally, IgG immune complex deposition was exacerbated greatly by Sle1c2 (Fig. 5C, 5D) but did not result in clinical disease. Taken together, these data establish that Sle1c2 can contribute to lupus through interactions with the NZB genome.

**Esrrg expression is downregulated in B6.Sle1c2 CD4+ T cells**

The 675-kb Sle1c2 interval contains two protein-coding genes, Esrrg and Ush2a (Fig. 1B), with the latter having no detectable expression in CD4+ cells (data not shown). Esrrg message expression was detected in CD4+ splenocytes and at a lower level in CD4− splenocytes and in thymocytes (Fig. 6A). Furthermore, Esrrg expression was significantly lower in the B6.Sle1c2 CD4+ T cell fraction as compared with that in the B6 CD4+ T cell fraction (40.6 ± 11.4% less). No difference between strains was observed in CD4− splenocytes. Esrrg expression in CD4+ T cells is low compared with that in liver, heart, brain, and kidney, highly metabolic tissues in which Esrrg expression has been reported.
Esrrg negatively regulates CD4+ T cell activation. In 12-mo-old (NZB × B6)F1 and (NZB × B6.Sle1c2)F1 mice (n = 14 and 25, respectively). (B) Serum anti-dsDNA IgG is shown as unit values at 5 and 12 mo of age and as relative individual increase between these two ages for each strain. (C and D) Glomerular IgG and C3 deposition was detected in frozen kidney sections with FITC-conjugated anti-C3 and anti-IgG Abs. Sections were scored separately for each Ab, and additive values (IgG score + C3 score) are shown in (C). Representative sections (original magnification ×100) are shown in (D). Mann–Whitney tests: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.

To determine if decreased Esrrg expression in CD4+ T cells had transcriptional consequences on its putative targets, we selected 12 genes (Supplemental Table I) that were differentially expressed by B6.Sle1c2 CD4+ T cells in the microarray analysis and that also were known to have ERR-γ bound to their promoters by chip-on-chip analyses (34, 35). The expression of five of these genes, transcriptional regulators c-myc binding protein (Mycbp) and retinoic acid receptor α (Rara), a subunit of electron transport complex I (Ndufs1), a mitochondrial protein modifier (Ppif), and a mitochondrial oxidoreductase (Rrn4ip1), was decreased significantly in Sle1c2 CD4+ T cells (Supplemental Fig. 3C). These data suggest that the decreased Esrrg expression induced by Sle1c2 affects Esrrg target gene expression.

Because Esrrg is known to regulate glycolytic and oxidative metabolic programs (34), we analyzed the expression of several genes involved in these pathways by quantitative PCR. Hif1α and Slc16a3, encoding monocarboxylic acid transporter 4, which are both mediators of glycolysis (22), were expressed at a higher level in B6.Sle1c2 CD4+ T cells (Fig. 6D). In addition to glycolysis, a microarray pathway analysis revealed an influence of Sle1c2 in several other metabolic pathways, including glutaminolysis, glycosogenesis, and fatty acid oxidation (Fig. 6E). Furthermore, genes regulating the electron transport chain also were differentially regulated, indicating altered mitochondrial function. Finally, out of the seven metabolic genes that were analyzed in both the Esrrg−/− heart (34) and the Sle1c2 CD4+ T cells in this study, five of them (Eno2, Gsk3, Cox6a2, Ndufa10, and Ndufa1) showed an expression change in the same direction. Overall, these data provide evidence that Sle1c2 results in an altered metabolism.
metabolic profile in CD4+ T cells, due to decreased Esrrg expression.

B6.Sle1c2 CD4+ T cells exhibit mitochondrial dysfunction

Esrrg expression has been reported to directly correlate with mitochondrial biogenesis and functions (33). Accordingly, decreased Esrrg expression is predicted to reduce mitochondrial mass and function in Sle1c2 CD4+ T cells. This indeed was verified in the spleens of 2- to 3-mo-old mice, in which the difference in T cell activation is still minimal and therefore not likely to induce secondary metabolic changes. The mitochondrial membrane potential and mass were decreased significantly in Sle1c2 CD4+ T cells as compared with those in B6 T cells (Fig. 7A, 7B), and this was associated with decreased Ca2+ and NO levels (Fig. 7D, 7E). In turn, the production of reactive oxygen intermediates (ROIs), as measured by HE fluorescence, was increased in Sle1c2 CD4+ T cells. However, no difference in ROIs was found between the two strains when measured with the 2′,7′-dichlorofluorescin or dihydrorhodamine 123 dyes (data not shown). These findings are suggestive of mitochondrial dysfunction, characterized by an inability to maintain transmembrane potential while leaking ROIs. Consistent with impaired mitochondrial function, a significant increase of necrosis was observed in Sle1c2 splenocytes (Fig. 7F). This corresponds to a predisposition to proinflammatory death by necrosis that has been reported previously in lupus T cells (36).

Interestingly, VDAC levels were increased significantly in Sle1c2 CD4+ T cells (Fig. 7G), which may correspond to a compensatory mechanism to maintain mitochondrial membrane potential despite reduced Esrrg expression. A reduced mitochondrial mass was not observed in other Sle1c2 immune cell populations, except CD11c+ cells (Table I). This may be related to the fact that Esrrg expression is very low in CD4 splenocytes (Fig. 6A), therefore not affecting mitochondria in these cell types. Other parameters showed significant differences between the two strains in non-CD4+ cell types, such as NO levels, which were reduced significantly in CD8+ T cells, B cells, and CD11b+ and CD11c+ splenocytes (Table I), indicating that the metabolic consequences of Esrrg deficiency may not be confined to T cells.

Discussion

Within the major NZM2410 lupus susceptibility Sle1 locus, increased CD4+ T cell activation mapped to both Sle1a and Sle1c (10), with each of these loci corresponding to two subloci (12, 37). The current study focused on the functional characterization and mapping of the CD4+ T cell phenotypes associated with Sle1c. Originally defined as the 7.72 Mb at the telomeric end of chromosome 1, Sle1c contains 48 protein-coding genes and 4 microRNAs.
Previous analyses have identified Crl2 on the telomeric end of Sle1c as the candidate gene associated with impaired B cell responses (3). In this study, we confirmed that centromeric Sle1c2 enhances CD4+ T cell activation and have established that Sle1c2 CD4+ T cells are characterized by strong Th1 skewing. In addition, we have demonstrated the contribution of Sle1c2 to autoimmune pathology by showing that its expression enhances both induced and spontaneous lupus. Furthermore, congenic mapping and gene expression analysis have identified Esrrg, one of the two genes located in the critical interval, as the candidate gene for Sle1c2. Finally, consistent with published works that have established that Esrrg is a potent regulator of mitochondrial metabolism (33), the decreased expression of Esrrg in Sle1c2 CD4+ T cells is associated with decreased mitochondrial mass and with significant alterations of mitochondrial function.

ERRs belong to a family of nuclear receptors that are transcription factors whose activities are mediated by endogenous ligands and other coregulatory proteins. Several nuclear receptors already have been implicated in T cell biology, including retinoic acid receptor α, retinoic acid-related orphan receptor γ, PPARγ, the vitamin D and the glucocorticoid receptors, and the estrogen receptors (38-40). ERR-γ, belonging to the ERR family, is expressed at low levels in human spleen and thymus (41) but does not yet have an established role in T cell biology. ERRs are structurally related to estrogen receptors but do not bind natural estrogens. In fact, ERR endogenous ligands have not been identified yet, and they thus are referred to as orphan nuclear receptors. It has been shown, however, that ERRs are constitutively active due to the properties of their ligand-binding domains (42). Regardless, several synthetic compounds augment ERR-γ-mediated transactivation of its target genes, demonstrating its potential as a therapeutic target (43). Interestingly, bisphenol A, an industrial component known to be an endocrine disruptor, binds to and alters the activity of ERR-γ (44). Bisphenol A exposure has been linked to autoimmunity, and relevant to this study, it has been shown to strongly downregulate IFN-γ production (45). This raises the possibility that ERR-γ may mediate environmental triggers of autoimmunity.

ERRs are important regulators of metabolism and energy homeostasis, and Esrrg expression is highly restricted to metabolically active tissues (42). Esrrg transactivates genes involved in mitochondrial biogenesis, lipid transport and metabolism, tricarboxylic acid cycle, electron transport chain, and oxidative phosphorylation, allowing for energy production by efficient fatty acid oxidation. This vital function is demonstrated in Esrrg null mice, where the lack of a critical switch from glycolytic to lipid-based metabolism in the myocardium results in perinatal lethality (34). Conversely, Esrrg overexpression in skeletal muscle resulted in increased mitochondrial biogenesis and function as well as exercise capacity (33). Activation and proliferation by T cells also is metabolically demanding (46). However, contrary to other metabolically demanding tissues, activated T cells use aerobic glycolysis to meet energy requirements. Known as the Warburg effect, this form of metabolism generates glucose metabolites at the expense of ATP production (47). Remarkably, when ERR-γ expression is suppressed by microRNA, the Warburg effect is induced (48). The rate of glycolysis has direct consequences on CD4+ T cell activation and differentiation. GLUT1 transgenic mice, which have increased glucose uptake in their T cells, show T cell activation phenotypes very similar to B6.Sle1c2 mice, namely, age-dependent accumulation of CD69+ T cells and Tems, increased proliferation, and increased IFN-γ production (49). Consequently, aged mice suffered from hypergammaglobulinemia and GN, exhibiting a direct connection between T cell metabolism and autoimmunity. Conversely, as reported recently in Mhc-deficient mice, T cells display impaired proliferation with an altered activation phenotype when the switch to aerobic glycolysis is obstructed (50).
We hypothesize that Sle1c2 contributes to the Warburg effect in CD4+ T cells because decreased Esrrg expression would limit the transcription of target genes that regulate oxidative lipid-based metabolism, skewing toward glucose-based programs. This hypothesis is supported first by the decreased expression of Esrrg target genes, *Ndufs1, Ppif*, and *Rtn4p1*, which regulate metabolism and mitochondrial function (51–53), and second by the decreased mitochondrial biogenesis and functions in Sle1c2 CD4+ T cells. Recently, Michalek et al. (54) reported that ERR-α functions as a regulator of T cell metabolism. This isoform, encoded by a separate gene, *Esrra*, binds to the same consensus DNA sequence as ERR-γ and shares the same general function as a metabolic regulator. However, contrary to the increased T cell activation observed in B6.Sle1c2 mice, which have reduced Esrrg expression in CD4+ T cells, *Esrra−/−* mice lacked an expansion of Tems as they aged. Interestingly, *Esrra−/−* CD4+ T cells were not defective in their proliferative and glycolytic capacities in response to TCR activation, whereas wild-type T cells in which ERR-α was acutely inhibited were deficient in these capacities. This suggested that a developmental compensatory mechanism was offsetting the effect of *Esrra* deficiency, and indeed the authors observed alterations in mTOR and AMPK pathways. At this point, it is not clear why chronic ERR-γ deficiency leads to increased activation, whereas ERR-α deficiency results in a more naive phenotype. It is conceivable that these two isoforms use different cofactors to dictate their function. Overall, these findings validate our observations that deficiencies in ERR-γ cause globally augmented metabolism in CD4+ T cells and result in altered T cell function.

Dysregulated T cells are a major contributor to SLE pathogenesis, and targeting them is an important focus for therapeutic intervention (55). Mitochondrial dysfunctions have been identified specifically as a characteristic of human lupus T cells, which may offer additional therapeutic targets (56). Recent studies have highlighted the regulation of metabolism as an important checkpoint of T cell activation and effector functions. Our study associates, for the first time, to our knowledge, a gene known as a major regulator of metabolism, mitochondrial dysfunction, and CD4+ T cell activation Th1 polarization in a lupus model. A better understanding of the role of ERR-γ in CD4+ T cells will provide a better understanding of how metabolism regulates immune functions in the context of autoimmunity.

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### References


murine systemic lupus erythematosus susceptibility locus Slel results in ab-