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T-bet-expressing B cells contribute to the autoreactive plasma cell pool in Lyn−/− mice

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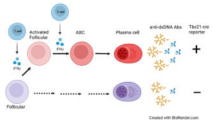
Abstract

Systemic Lupus Erythematosus (SLE) is characterized by pathogenic autoantibodies against nucleic acid-containing antigens. Understanding which B cell subsets give rise to these autoantibodies may reveal therapeutic approaches for SLE that spare protective responses. Mice lacking the tyrosine kinase Lyn, which limits B and myeloid cell activation, develop lupus-like autoimmune disease characterized by increased autoreactive plasma cells (PCs). We used a fate-mapping strategy to determine the contribution of T-bet+ B cells, a subset thought to be pathogenic in lupus, to the accumulation of PCs and autoantibodies in Lyn−/− mice. Approximately 50% of splenic PCs in Lyn−/− mice originated from T-bet+ cells, a significant increase compared to wild type mice. *In vitro*, splenic PCs derived from T-bet+ B cells secreted both IgM and IgG anti-dsDNA antibodies. To determine the role of these cells in autoantibody production *in vivo*, we prevented T-bet+ B cells from differentiating into PCs or class switching in Lyn−/− mice. This resulted in a partial reduction in splenic PCs and anti-dsDNA IgM and complete abrogation of anti-dsDNA IgG. Thus, T-bet+ B cells make an important contribution to the autoreactive PC pool in Lyn−/− mice.

Graphical Abstract



Lyn−/− mice, a model of lupus, have increased T-bet+ follicular B cells and age associated B cells. Plasma cells derived from T-bet expressing B cells (marked by a Tbx21-cre.tomato reporter) accumulate in the spleens of Lyn−/− mice and give rise to some IgM, and the majority of IgG, anti-dsDNA autoantibodies.

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Author Contributions

K.O. performed experiments and analyzed data, J.S. performed experiments, and A.S. obtained funding, designed experiments, analyzed data, and wrote the manuscript.

Conflict of Interest Disclosure

The authors declare no financial or commercial conflicts of interest.

Ethics Approval Statement

All mouse experiments were approved by the UT Southwestern Institutional Animal Care and Use Committee

Keywords

autoantibody; plasma cell; lupus; T-bet; Lyn

Introduction

The autoimmune disease systemic lupus erythematosus (SLE) is characterized by pathogenic autoantibodies against nucleic acid-containing antigens. In combination with innate immune system hyperactivity, this results in feed forward proinflammatory loops and damage to multiple tissues (1). B cell-targeted therapy for SLE has had surprisingly mixed results, perhaps due to the presence of both protective and pathogenic B cell subsets (2). Thus, defining which B cell populations contribute to lupus pathogenesis is important for the development of more targeted and efficacious therapies.

The Src family kinase Lyn is expressed in B and myeloid cells. While it plays both activating and inhibitory roles, its net effect is to limit the activation of both cell types via the phosphorylation of ITIM motifs in inhibitory receptors (3). In its absence, mice develop a lupus-like autoimmune disease characterized by an accumulation of plasma cells (PCs) in the periphery, the production of lupus-associated autoantibodies, and kidney damage (4–8). B cell-specific loss of Lyn is sufficient for these phenotypes (9). Reduced levels and altered subcellular localization of Lyn have been observed in B cells from SLE patients (10, 11) and polymorphisms in Lyn and its regulator CSK are associated with SLE (12–14). Understanding mechanisms of autoantibody production in Lyn−/− mice thus has important therapeutic implications.

PC accumulation and autoantibody production in Lyn−/− mice occurs in two genetically separable stages. The first involves accumulation of splenic, IgM-secreting PCs with a wide range of autoreactive specificities (7). This requires the tyrosine kinase Btk and is associated with inappropriate downregulation of the transcription factor Ets1, which normally inhibits PC differentiation (7, 15, 16). The second involves class switching of B cells reactive with lupus-associated, nucleic acid-containing antigens and depends on Btk, IL-6, and IL-21 (7, 8, 16, 17). However, it is unknown which B cell subsets give rise to the autoreactive PC pool in Lyn−/− mice.

A subset of B cells expressing CD11c and the transcription factor T-bet and lacking CD21 and CD23 is expanded in, and implicated in the pathogenesis of, both human and murine lupus (18). These cells are known as age associated B cells (ABCs) in mice and DN2 cells in humans. They are induced by combinations of stimuli elevated in lupus including TLR7 ligands, IFNγ, and IL-21 (19–25). Once developed, they are highly sensitive to TLR7 engagement and prone to differentiate into PCs that secrete autoantibodies with specificities commonly associated with lupus (19, 23, 24). However, the degree to which ABCs/DN2s and the transcription factor T-bet contribute to autoantibody production and disease manifestations is unclear. In some cases, B cell-specific deletion of T-bet or depletion of CD11c+ B cells reduces autoantibodies and limits kidney damage (26–28). However, this is not the case in all lupus models, and in other situations B cell-expressed T-bet amplifies, but is not required for, autoantibody production (25, 26, 29–31).

TLR signaling, IFNγ, and IL-21 are elevated in Lyn−/− mice (9, 17, 32), indicative of an ABC-supportive environment. Here, we use complimentary strategies to determine the degree to which T-bet+ B cells participate in the accumulation of PCs and production of autoantibodies in Lyn−/− mice. These include fate mapping to identify PCs derived from T-bet-expressing B cells and a genetic approach to limit their differentiation. These studies indicate that T-bet+ B cells contribute to the autoreactive peripheral PC pool and are the main producers of IgG autoantibodies in Lyn−/− mice.

Results

Characterization of Tbx21-cre.tomato reporter mice

T-bet-expressing B cells are elevated in lupus and have been implicated in disease pathogenesis (18). However, B cell-specific deletion of T-bet in lupus models has given mixed results (26–30). T-bet may be a marker of pathogenic B cells rather than a requirement for them. To identify T-bet-expressing B cells and their progeny, we crossed Tbx21-cre mice (Tbx21 encodes T-bet) (33) to a Rosa26-lox-stop-lox-tdtomato reporter strain (34). The reporter was not expressed in developing B lineage cells in the bone marrow (BM) or in transitional B cells, follicular (FO) or marginal zone (MZ) B cells in the spleen of Tbx21-cre.tomato mice (Figure 1a–d). A subset of CD21-CD23- and CD11c+ B cells were reporter+, consistent with an ABC phenotype (Figure 1c,d,g,h). To further validate the system, Tbx21-cre.tomato splenic B cells were stimulated *in vitro* with anti-IgM, anti-CD40, LPS, R848 (TLR7 ligand), IFNγ, or R848 + IFNγ. Only R848 + IFNγ induced tomato expression (Supplemental Figure 1a), as expected based on their synergy in upregulating T-bet expression in B cells (19–25).

Increased frequency of Tbx21-cre labeled FO B cells and ABCs in Lyn−/− mice

We hypothesized that T-bet+ B cells contribute to the autoreactive PC pool in Lyn−/− mice since these animals have increased T cell-derived IFNγ (32). We crossed Lyn−/− mice to the Tbx21-cre.tomato reporter system. As in wt mice, tomato was not expressed during B cell development in the BM or in splenic transitional B cells (Figure 1a,b). However, a larger percentage of splenic B cells expressed the reporter in Lyn-deficient compared to wt mice (Figure 1c,d). Most reporter+ B cells in Tbx21-cre.tomato.Lyn−/− mice were CD21lo/-CD23lo/- (Figure 1c–f) and CD11c+ (Figure 1g,h), consistent with an ABC phenotype. Furthermore, CD11c+tomato+ cells comprised a greater fraction of B cells in Lyn−/− mice than in wt mice (Figure 1g,h). However, 10–15% of the B220+tomato+ cells in Lyn−/− mice were FO B cells (Figure 1e, f). These were more activated than their reporter-negative counterparts as measured by CD69 and CD86 expression (Supplemental Figure 1b). MZ B cells, rare in Lyn−/− mice, made a negligible contribution to the overall population of tomato-expressing B cells (Figure 1e,f). T-bet expression is thus predominant in, but not limited to, ABCs among B cells in Lyn−/− mice.

Autoreactive PCs derived from T-bet+ B cells accumulate in Lyn−/− spleens

A greater frequency of splenic PCs (B220lo/-CD138+) were tomato+ in Lyn−/− mice compared to wt mice (Figure 2a,b). In contrast, the percentage of BM PCs expressing the reporter was unaffected by Lyn-deficiency (Figure 2a,b). To determine whether PCs

derived from T-bet-expressing cells were enriched in autoreactivity, we sorted tomato+ and tomato- CD138+ cells from pools of Tbx21-cre.tomato.Lyn−/− spleens and cultured them for 24 hours. Supernatants were subjected to anti-dsDNA ELISA. In three independent experiments, tomato+ PCs secreted more anti-dsDNA antibodies than tomato- cells from the same mice (Figure 2c). Consistent with the equal distribution of tomato+ and tomato- cells among splenic PCs, the level of anti-dsDNA antibodies secreted by CD138+ cells from tomato.Lyn−/− mice (no cre or reporter expression) was approximately the average of that produced by tomato+ and tomato- cells from Tbx21-cre.tomato.Lyn−/− mice (Figure 2d). Tomato+ PCs secreted slightly less total IgM and more total IgG than their tomato- counterparts (Figure 2e,f), consistent with T-bet's role in promoting class switching (35). T-bet+ B cells thus make a significant contribution to the autoreactive PC pool that accumulates in the spleens of Lyn−/− mice. However, they are not the only source as tomato- CD138+ cells also produced some anti-dsDNA antibodies *in vitro*.

Targeting IRF4 with Tbx21-cre reduces PCs and autoantibodies in Lyn−/− mice

To define the contribution of T-bet-expressing B cells to autoantibody production *in vivo*, we crossed Tbx21-cre.Lyn−/− mice to IRF4f/f mice. The transcription factor IRF4 is required for PC differentiation, germinal center formation, and class switching (36, 37). T-bet-expressing B cells should therefore be unable to produce IgM or IgG autoantibodies in Tbx21-cre.IRF4f/f.Lyn−/− mice. GFP marks cre-mediated recombination at the IRF4 locus in IRF4f/f mice (36). GFP+ B cells expressed less IRF4 protein than GFP- cells from the same Tbx21-cre.IRF4f/f.Lyn−/− mouse (Supplemental Figure 2a). However, some cells did not completely eliminate IRF4. This could be due to recent deletion such that previously expressed IRF4 protein remains, or recombination of only one IRF4 allele. In the former case residual IRF4 protein should eventually degrade, and B cells heterozygous for IRF4 demonstrate impaired PC differentiation *in vivo*, particularly in competition with wt cells (38). Thus, even partial reduction of IRF4 in T-bet+ B cells should limit their contribution to the PC pool. Indeed, GFP+ cells were significantly underrepresented among PCs relative to B220+ cells (Supplemental Figure 2b). This is in contrast to tomato+ cells in Tbx21-cre.tomato.Lyn−/− mice, which are enriched among PCs relative to total B cells (Figures 1c,d and 2a,b). Targeting IRF4 with Tbx21-cre therefore impairs the development or survival of PCs derived from T-bet-expressing B cells.

Tbx21-cre.IRF4f/f.Lyn−/− mice had a reduced, but not completely normalized, frequency of splenic PCs (Figure 3a,b). Consistent with this observation, the increase in total IgM characteristic of Lyn−/− mice was partially prevented by loss of IRF4 in T-bet-expressing cells (Figure 3c). Total IgG2c, but not IgG1, was also decreased (Figure 3c), as expected given the expression of T-bet in B cells induced to switch to IgG2c (35). anti-dsDNA IgM was present, but reduced, and anti-dsDNA IgG was not detected in Tbx21-cre.IRF4f/f. Lyn−/− mice (Figure 3d).

Targeting IRF4 with Tbx21-cre does not affect IFNγ expression by CD4+ T cells

T-bet is also expressed in IFNγ-producing CD4+ T cells (39), which are elevated in Lyn−/− mice (32). Tbx21-cre.tomato mice expressed tomato in a subset of CD4+ cells, particularly activated (CD69+) cells (Figure 4a,b). A larger fraction of CD4+ T cells expressed tomato

in Tbx21-cre.tomato.Lyn−/− mice (Figure 4a,b). IFNγ induces T-bet expression (19–25) and class switching to IgG2c (35) by B cells. It is also required for anti-dsDNA IgG in Lyn−/− mice (32). If reducing IRF4 expression in T-bet+ CD4+ T-cells (Supplemental Figure 2a) impairs their expression of IFNγ, this could contribute to the reduction in total IgG2c and loss of anti-dsDNA IgG in Tbx21-cre.IRF4f/f.Lyn−/− mice. This was not the case, however, as IFNγ-expressing CD4+ cells remained elevated in Tbx21-cre.IRF4f/f.Lyn−/− mice (Figure 4c,d). Taken together, our observations suggest that T-bet-expressing B cells are the major source of IgG autoantibodies, and contribute to some degree to IgM autoantibody production, in Lyn−/− mice.

Discussion

Using a Tbx21-cre reporter, we demonstrate that about half of splenic PCs in Lyn−/− mice are derived from T-bet-expressing B cells, a significant increase relative to wt mice. These PCs produce more anti-dsDNA antibodies than those derived from B cells that have not expressed T-bet. Given the general increase in IFNγ in Lyn−/− mice (32) and our characterization of reporter expression, it is likely that multiple T-bet+ B cell subsets including ABCs and FO B cells contribute to this PC pool. We hypothesize that upon encounter with antigen, TLR ligands, and IFNγ, autoreactive FO B cells upregulate T-bet and differentiate into ABCs and subsequently PCs. Consistent with this idea, tomato+ FO B cells appear more activated than their tomato- counterparts in Lyn−/− mice. MZ B cells are likely not involved as they are dramatically reduced in Lyn−/− mice (9, 40) and do not contribute appreciably to the tomato+ B cell population.

The activation and differentiation of autoreactive T-bet+ B cells in Lyn−/− mice likely occurs primarily, although not exclusively, in an extrafollicular response. BM PCs, which are predominantly derived from germinal centers, did not differ in reporter expression between wt and Lyn−/− mice. Furthermore, we previously found that the expansion of PCs in Lyn−/− mice predominates in the spleen (7) and that Lyn−/− mice have increased Tfh-like cells that lack CXCR5 (17), consistent with the activation of B cells outside of germinal centers. Extrafollicular activation of CD11c+T-bet+ DN2 cells is also thought to occur in SLE patients (23, 41). Unlike in the spleen, tomato+ PCs were observed at similar frequencies wt and Lyn−/− BM. These may derive from germinal center responses to environmental antigens activated in a Th1 environment. Indeed, T-bet+ B cells are a major source of influenza-induced long lived IgG+ PCs in the BM (42). Recent studies have revealed dramatic heterogeneity among BM PCs with respect to phenotype and origin (43). It will be interesting to determine how tomato+ cells map to these newly identified PC populations.

To determine the contribution of T-bet-expressing B cells to autoantibodies *in vivo*, we targeted IRF4 with Tbx21-cre. This reduced IRF4 expression and impaired the differentiation or survival of PCs originating from T-bet-expressing B cells. Tbx21-cre.IRF4f/f.Lyn−/− mice had anti-dsDNA IgM levels between those of Lyn−/− and Lyn+/+ controls. A similar effect was observed for splenic PCs and total IgM. Thus, T-bet expression is not a prerequisite for the inappropriate differentiation of splenic PCs or their production of anti-DNA IgM in Lyn−/− mice. However, cells that have encountered

T-bet-inducing stimuli do contribute to this pool of autoreactive PCs. This supports our previously proposed model in which a widespread increase in PC differentiation (7) due to increased BCR signaling and reduced levels of the transcription factor Ets1 (15) underlies the increase in IgM+ autoantibodies in Lyn−/− mice.

In contrast to the partial decrease in anti-dsDNA IgM, anti-dsDNA IgG was completely absent in Tbx21-cre.IRF4f/f.Lyn−/− mice. This suggests that T-bet-expressing B cells are the major producers of IgG autoantibodies in Lyn−/− mice. One caveat is that in addition to ABCs, Tbx21-cre is expressed in IFNγ+ T cells (39). Previous studies have shown that IRF4 is not required for IFNγ production by Th1 cells polarized *in vitro* (44, 45). However, naïve CD4+ T cells lacking IRF4 generated IFNγ+ expressing cells poorly upon adoptive transfer, likely due to reduced proliferation (45). It was therefore possible that the dramatic loss of anti-dsDNA IgG we observed was due to a requirement for IRF4 in the expression of IFNγ by CD4+ T cells in our system. This was not the case, however, as Tbx21-cre.IRF4f/f.Lyn−/− mice had similarly elevated frequencies of IFNγ-expressing CD4+ T cells as Lyn−/− controls. However, we cannot rule out the possibility that another dose-sensitive function of IRF4 in T-bet+ T cells facilitates autoantibody production in Lyn−/− mice.

There are mixed results regarding the contribution of B cell-expressed T-bet to IgG autoantibody production in lupus models. In the WAS chimera, imiquimod-induced, and Sle1.Sle2.Sle3 lupus models, deletion of T-bet in all B cells did not prevent the production of anti-dsDNA or anti-chromatin IgG, although IgG2c autoantibodies were reduced (26, 29, 31). In contrast, we found that blocking differentiation of T-bet-expressing B cells did abrogate the production of anti-dsDNA IgG in Lyn−/− mice. T-bet may serve as a marker of, rather than a requirement for, pathogenic autoantibody-producing B cells. Alternatively, the autoantibody response in Lyn−/− mice may be particularly skewed towards IgG2c, class switching to which requires T-bet (35), because of the elevated expression of IFNγ by T cells in this model (32). Finally, different pathways of, or requirements for, B cell activation may dominate in different lupus models. Consistent with this idea, DN2 cells accumulate more frequently and to a greater degree in African American SLE patients compared to those of other ethnicities (23). It will be interesting to characterize DN2 cells in patients with impaired Lyn-mediated inhibitory pathways (10, 11, 14).

Materials and Methods

Mice

To identify Tbx21+ cells and their progeny, Tbx21-cre mice (33) (Jackson Labs #024507) were crossed to Ai14 mice which carry cre-inducible td-tomato in the Rosa26 locus (34) (Jackson Labs #007914). Tbx21-cre.tomato mice were then crossed to Lyn−/− mice (6). Lyn−/−, Tbx21-cre, and IRF4f/f mice (36) (Jackson Labs #009380) were crossed together to delete IRF4 in T-bet-expressing cells. Mice were housed in a specific pathogen free barrier facility and analyzed at 4–6 months of age. Mice were sex matched and littermate controls used whenever possible. All mouse experiments were approved by the UT Southwestern Institutional Animal Care and Use Committee.

Flow cytometry

Single cell suspensions of spleen and BM were depleted of red blood cells and stained with combinations of the following antibodies coupled to FITC, Alexa 488, PE, PerCP-Cy5.5, APC, Alexa 647, or biotin. Biotinylated antibodies were detected with Streptavidin-APC (Tonbo Biosciences). Spleen: CD4 (BD Pharmingen), CD69 (BD Pharmingen), B220 (Invitrogen), CD19 (BioLegend), CD21 (BD Pharmingen), CD23 (BD Pharmingen), CD93 (Invitrogen), CD138 (BD Pharmingen), CD11b (BD Pharmingen), CD11c (BD Pharmingen), CD86 (Biolegend). BM: B220, IgM (BD Pharmingen), CD93, CD138. For IFNγ expression, splenocytes were stimulated with eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) (Thermofisher) for 5 hours and then stained extracellularly with anti-CD4-PerCP-Cy5.5 (BD Pharmingen) and intracellularly with anti-IFNγ-PE (eBioscience). For IRF4 expression in GFP+ cells, splenocytes were stained extracellularly with antibodies against B220 or CD4 and intracellularly with antibodies against GFP (Biolegend) and IRF4 (Invitrogen/eBioscience) or isotype control (Invitrogen/eBioscience) as in (46). Samples were run on a FACS Calibur (Becton Dickinson) and analyzed with FlowJo Software (TreeStar).

Cell purification and culture

B cells: Splenic B cells were depleted of red blood cells and purified by negative selection using anti-CD43 magnetic beads (Miltenyi Biotech) according to the manufacturer's instructions. Cells were stimulated for 48 hours at 10^6/ml with complete media (RPMI 1640 + 10 % FBS + L-glut + pen/strep + β-ME) alone, 10 ug/ml anti-IgM F(ab)'$_2$ fragments (Jackson Immunoresearch), 0.35 ug/ml CD40L (R&D Systems), 5 ug/ml LPS (Sigma), 1 uM ODN 1826 (Invivogen), 1 ug/ml R848 (Invivogen), 10 ng/ml IFNγ (R&D Systems), or 1 ug/ml R848 + 10 ng/ml IFNγ. Stimulated cells were subjected to flow cytometry for tomato expression.

Plasma cells: Pooled splenocytes from 2–3 mice per group were depleted of red blood cells and enriched for CD138+ cells using anti-CD138 magnetic beads (Miltenyi Biotech) according to the manufacturer's instructions. CD138+ cells were stained with antibodies against B220 (Invitrogen) and CD138 (BD Pharmigen) and tomato+, tomato-, and total B220lo CD138+ cells sorted on a FACS Aria. Cells were then cultured at 2×10^5/ml for 24 hrs in complete media and supernatants collected for ELISA.

ELISAs

Anti-dsDNA: 1:100, 1:400, and 1:1600 dilutions of serum and undiluted, 1:4, and 1:16 dilutions of culture supernatant were subjected to anti-dsDNA IgM and IgG ELISA as described in (16). *Total Ig*: 1:1000, 1:4000, and 1:16,000 dilutions of serum and 1:10, 1:40, and 1:160 dilutions of culture supernatant were subjected to total IgM, IgG, IgG1, and IgG2c ELISA as described in (16, 47).

Statistical Analysis

Statistical analysis was performed with Graph Pad Prism using Student's t-test, Mann-Whitney test, one way ANOVA, or Kruskal-Wallis test depending on the number of groups

being compared and whether the data was distributed normally. $p<0.05$ was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Data Sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

ABC	age-associated B cell
BM	bone marrow
FO	follicular
MZ	marginal zone
PC	plasma cell
SLE	Systemic Lupus Erythematosus
wt	wild type

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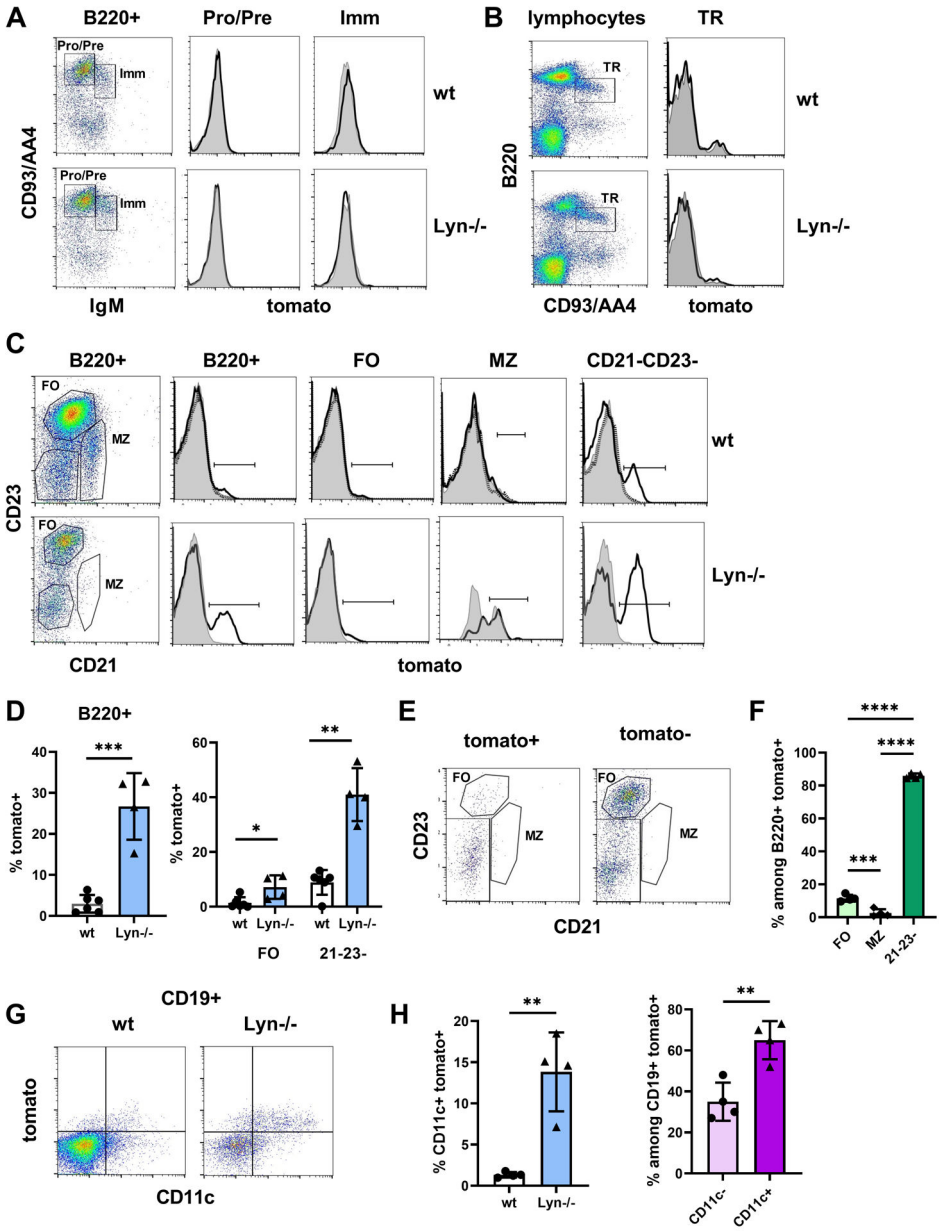

Figure 1: Increased Tbx21-cre.tomato reporter expression in Lyn−/− B cells.

A-D) Tomato expression in tomato-negative (dotted), tomato (gray), and Tbx21-cre.tomato (open) wt or Lyn−/− mice was assessed in the following BM (A) and spleen (B-D) populations. A) Pro and pre-B cells (B220+CD93+IgM-) and immature B cells (B220+CD93+IgM+). B) Transitional B cells (B220+CD93+). C) FO (B220+CD23+CD21+), MZ (B220+CD23lo/-CD21hi), and CD21-CD23- B cells. A-C) are representative of 3–6 mice per group, pooled from 3–6 experiments with one mouse/group/experiment. D) The frequency of tomato+ cells among the indicated populations from Tbx21-cre.tomato (wt) and Tbx21-cre.tomato.Lyn−/− (Lyn−/−) mice (gated as in C). n = 4–6, pooled from 4–6 experiments with one mouse/group/experiment. The bar shows mean +/− SD. *p<0.05, **p<0.01 by Mann-Whitney test. ***p<0.001 by unpaired

Student's t-test. E) CD21 and CD23 expression among B220+tomato+ and B220+tomato- cells from a representative Tbx21-cre.tomato.Lyn−/− mouse. F) Frequency of FO, MZ, and CD21-CD23- cells among B220+tomato+ cells in Tbx21-cre.tomato.Lyn−/− mice, gating as in (C, E). n = 4, pooled from 4 experiments with one mouse/group/experiment. The bar shows mean +/− SD. ***p<0.001, ****p<0.0001 by one way ANOVA. G) Representative CD11c vs tomato expression in CD19+ splenocytes from Tbx21-cre.tomato (wt) and Tbx21-cre.tomato.Lyn−/− (Lyn−/−) mice. H) Left: Frequency of CD11c+tomato+ cells among CD19+ splenocytes from Tbx21-cre.tomato (wt) and Tbx21-cre.tomato.Lyn−/− (Lyn−/−) mice. Right: Frequency of CD11c- and CD11c+ cells among CD19+tomato+ splenocytes from Tbx21-cre.tomato.Lyn−/− mice. n = 4, pooled from four experiments with one mouse/group/experiment. The bar shows the mean +/− SD. **p<0.01 by unpaired Student's t-test. Gating as in (G).

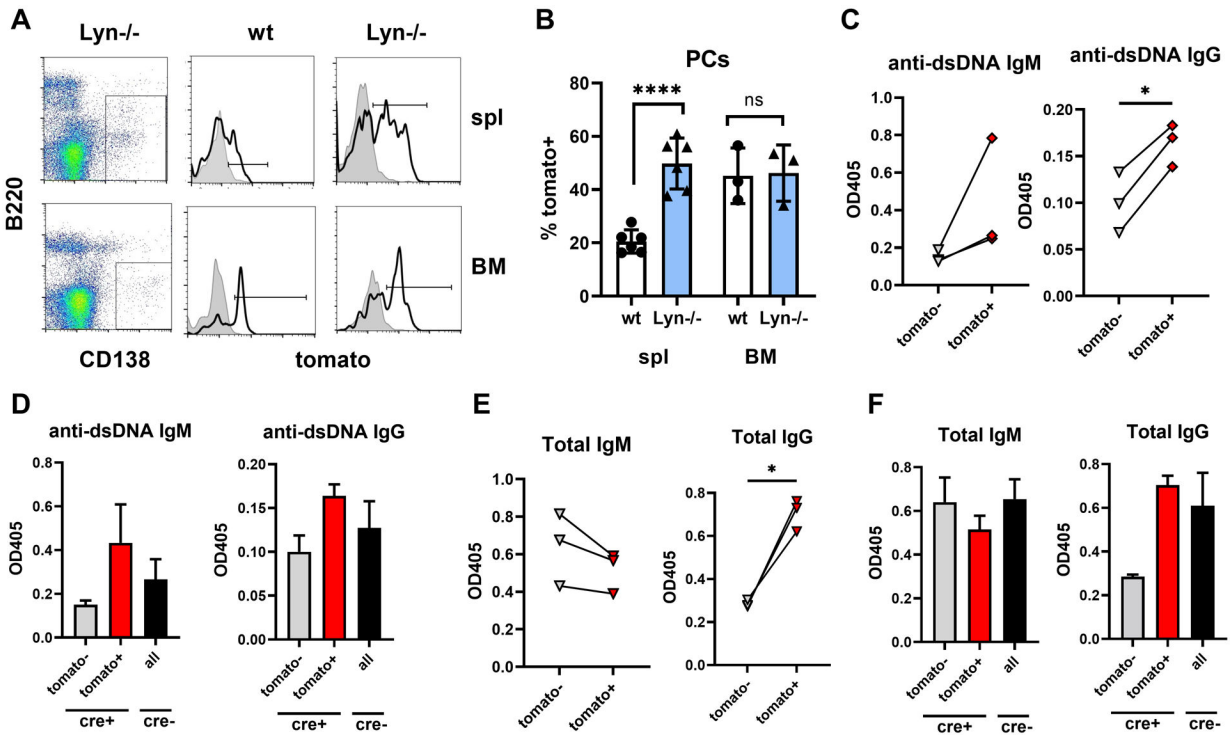

Figure 2: Autoreactive PCs derived from T-bet-expressing B cells are expanded in Lyn−/− spleens.

A, B) Splenocytes (spl) and BM cells were stained with anti-B220 and anti-CD138. (A) Histograms show tomato expression in B220lo/-CD138+ cells (gated as on the left) from representative tomato (gray) and Tbx21-cre.tomato (open) mice on the wt or Lyn−/− background. (B) Frequency of tomato+ cells among CD138+ cells in spleen and BM from Tbx21.cre-tomato (wt) and Tbx21-cre.tomato.Lyn−/− (Lyn−/−) mice. n = 3–6, pooled from 3–6 experiments with one mouse/group/experiment). The bar shows mean +/− SD. ****p<0.0001 by unpaired Student's test. C-F) (C,E) Tomato+ (red) and Tomato- (gray) CD138+ plasma cells (gated as in A) were sorted from pooled spleens of 2–3 Tbx21-cre.tomato.Lyn−/− mice per experiment (n = 3 experiments). Sorted cells were cultured for 24 hrs and supernatants subjected to ELISA for anti-dsDNA (C) or total (E) IgM and IgG. Lines connect tomato- and tomato+ from the same pool of mice in an individual experiment. *p<0.05 by paired Student's t-test. (D, F) Results from (C, E) are compared to those from total CD138+ cells from tomato.Lyn−/− mice (cre-, black bars). Mean +/− SEM, n = 3.

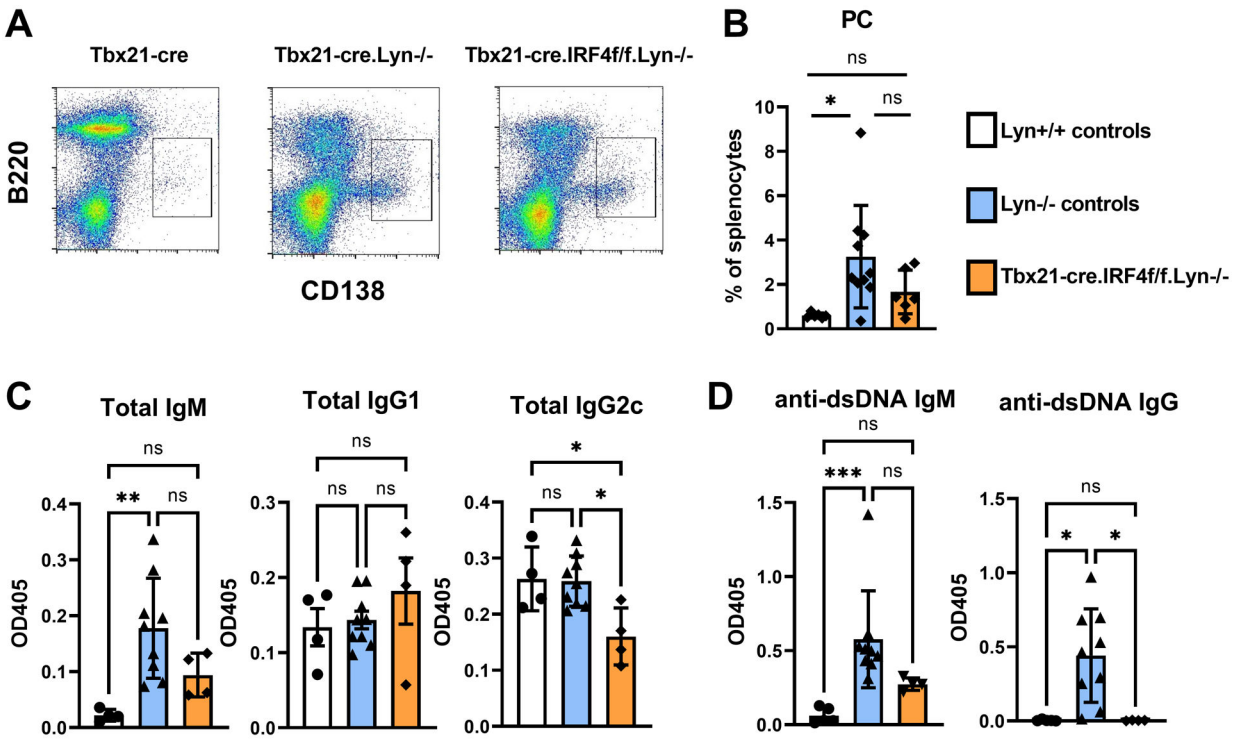

Figure 3: Reduced autoantibodies in Tbx21-cre.IRF4f/f.Lyn−/− mice.

A) Representative dot plots of splenocytes stained with anti-B220 and anti-CD138. B) The frequency of B220lo/-CD138+ PCs (gated as in A) in Lyn+/+ controls (Tbx21-cre and wt, open), Lyn−/− controls (Tbx21-cre.Lyn−/− and Lyn−/−, blue), and Tbx21-cre.IRF4f/f.Lyn−/− mice (orange). n = 4–7, pooled from 4 experiments with one to two mice/group/experiment. The bar shows mean +/− SD. *p<0.05 by one way ANOVA. C) ELISA for total Ig levels in 1:16,000 (IgM, IgG1) or 1:4000 (IgG2c) dilutions of serum from Lyn+/+ controls (Tbx21-cre and wt, open), Lyn−/− controls (Tbx21-cre.Lyn−/− and Lyn−/−, blue), and Tbx21-cre.IRF4f/f.Lyn−/− mice (orange). n = 4–9, pooled from 2 experiments. The bar shows mean +/− SD. *p<0.05, **p<0.01 by one way ANOVA. D) ELISA for anti-dsDNA antibodies in 1:100 dilution of serum samples from (C). *p<0.05 by Kruskal-Wallis test, ***p<0.001 by one way ANOVA.

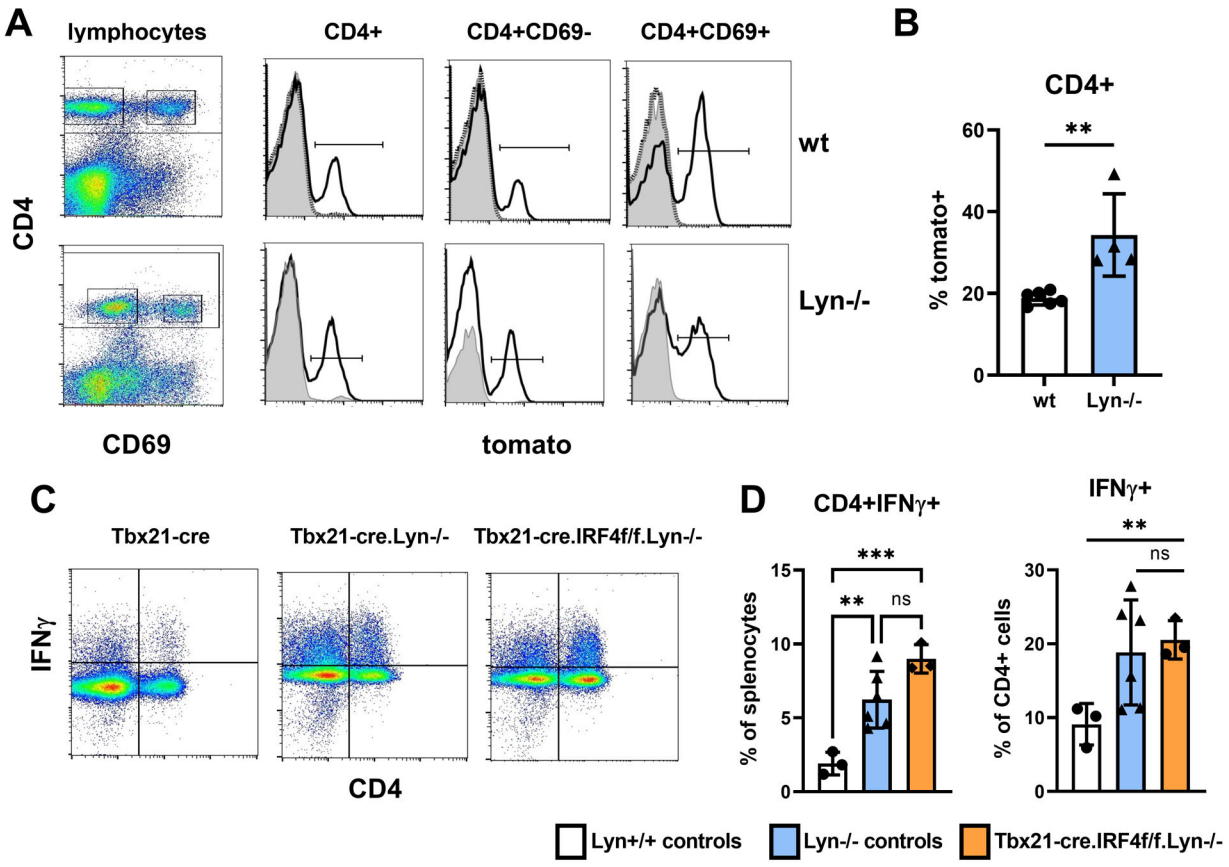

Figure 4: Unimpaired IFNγ production by CD4+ T cells in Tbx21-cre.IRF4f/f.Lyn−/− mice.

A,B) Tomato expression in splenic CD4+, CD4+CD69-, and CD4+CD69+ cells from tomato-negative (dotted), tomato (gray), and Tbx21-cre.tomato (open) wt or Lyn−/− mice. A) Representative flow cytometry plots. B) The frequency of tomato+ cells among CD4+ cells. n = 4–6, pooled from 4–6 experiments with one mouse/group/experiment. The bar shows mean +/− SD. **p<0.01 by Student's t-test. C,D) Splenocytes were stimulated for 5 hours with PMA, ionomycin, monensin, and brefeldin A and stained with antibodies against CD4 (extracellular) and IFNγ (intracellular). C) Representative flow cytometry plots. D) The frequency of CD4+IFNγ+ cells among splenocytes (left) and IFNγ+ cells among CD4+ cells (right) in Lyn+/+ controls (Tbx21-cre and wt, open), Lyn−/− controls (Tbx21-cre.Lyn−/− and Lyn−/−, blue), and Tbx21-cre.IRF4f/f.Lyn−/− mice (orange). n = 3–6, pooled from 3 experiments with one to two mice/group/experiment). The bar shows the mean +/− SD. Left: **p<0.01, ***p<0.001 by one way ANOVA. Right: **p<0.01 by unpaired Student's t-test.