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Yaa-autoimmune phenotypes are conferred by an overexpression of TLR7

Anna-Marie Fairhurst[1], **Sun-hee Hwang**[1], **Andrew Wang**[1], **Xiang-Hong Tian**[1], **Christopher Boudreaux**[1], **Xin J Zhou**[2], **Jose Casco**[1], **Quan-Zhen Li**[1], **John E Connolly**[3], and **Edward K. Wakeland**[1,*]

[1]Department of Immunology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

[2]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

[3]Baylor Institute for Immunology Research, Dallas, TX 75204

Summary

The y-linked autoimmune accelerating (*Yaa*) locus drives the transition to fatal lupus nephritis when combined with B6.*Sle1* in our B6-congenic model of systemic autoimmunity. We and others recently demonstrated that the translocation of a cluster of X-linked genes onto the Y chromosome is the genetic lesion underlying *Yaa (*Subramanian, S. et al., *Proc Natl Acad Sci USA* 2006. 103: 9970–9975; Pisitkun, P. et al., *Science* 2006. 312: 1669–1672). In male mice carrying *Yaa*, the transcription of several genes within the translocated segment is increased roughly 2-fold. Although the translocated X chromosome segment in *Yaa* may contain as many as 16 genes, the major candidate gene for causation of the *Yaa*-associated autoimmune phenotypes has been TLR7. To confirm the role of TLR7 in *Yaa*-mediated autoimmune phenotypes, we introgressed a targeted disruption of TLR7 (TLR7^{-}) onto B6.*Sle1Yaa* to produce B6.*Sle1Yaa*TLR7^{-} and examined evidence of disease at 6 and 9 months of age. Our results demonstrate that the upregulation of TLR7 in the B6.*Sle1Yaa* strain is responsible for splenomegaly, glomerular nephritis and the majority of the cellular abnormalities of B, T and myeloid cells. The upregulation of TLR7 was also responsible for driving the infiltration and activation of leukocytes into the kidney, in which activated T cellswere a primary component. However, the resolution of TLR7 upregulation did not eliminate the enhanced humoral autoimmunity observed in B6.*SleYaa,* suggesting that additional elements in the translocation may contribute to the disease phenotype.

Keywords

Autoimmunity; TLR7; genetics; SLE; congenic

*Corresponding Author: Edward.wakeland@utsouthwestern.edu, 6000 Harry Hines, UT Southwestern Medical Center at Dallas, TX, 75390-9093, Fax: 214 6487331.

Conflict of Interest

The authors declare that they have no conflict of interest.

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease classically associated with a loss in immune tolerance that leads to the production of a complex array of autoantibodies that predominantly recognize nuclear material[1]. In severe forms of disease, immune complex deposition can affect multiple end organs and initiate a chronic inflammation that can culminate in severe pathology and fatal disease, as exemplified in lupus nephritis. Genetic predisposition plays a dominant role in SLE susceptibility and a variety of genetic studies in both humans and animal models have identified multiple susceptibility alleles that interact to cause a profound dysregulation of the immune system.

We have utilized a congenic dissection strategy to characterize the individual genetic components that interact to drive the development of severe pathology in murine models of SLE.[2] Our initial studies focused on the NZM2410 strain, which is a congenic recombinant inbred strain derived from a backcross of NZB onto NZW.[3, 4] In an extensive series of investigations, we produced a collection of B6-congenic strains carrying individual susceptibility loci derived from NZM2410 (reviewed in ref [2]). Detailed analyses of the component phenotypes expressed in these strains have defined three separate pathways that interact to mediate severe disease (reviewed in ref [1]). Extensive characterizations of the B6.*Sle1*strain have identified polymorphisms in the SLAM/CD2 gene cluster as causative for a loss in immune tolerance in both B and T lymphocytes.[5] When isolated on the B6 background, *Sle1* confers a mild autoimmune phenotype, with antinuclear antibodies developing as early as 3 months of age, together with hypergammaglobinemia and a mild splenomegaly chararacterised by an expansion of the CD4 T and B cell populations [6, 7]. However, the addition of a second susceptibility locus, such as *Sle3* or *Yaa*, causes the transition of this benign autoimmune phenotype into severe disease [8, 9]. Thus, our congenic dissection strategy has provided a model system with which to characterize the genetic interactions that drive the development of severe disease pathology.

The Y-linked autoimmune accelerating (*Yaa*) locus is derived from the lupus-prone BXSB mouse[10]. Although *Yaa* mediates only minimal autoimmune consequences in isolation on the normal B6 background, it is highly epistatic with the *Sle1* region, accelerating the rapid development of a highly penetrant and fatal form of disease at a relatively young age[11]. We and others recently demonstrated that the genetic lesion underlying *Yaa* is a X to Y translocation in which a telomeric segment (> 1 mBase) of the X chromosome is translocated onto the Y chromosome. This translocation results in a duplication of about 16 genes in male mice carrying *Yaa*, which leads to a roughly 2-fold increase in the transcription of several of the translocated genes. Within the cluster of translocated genes, the major candidate gene for causation of the *Yaa*-associated autoimmune phenotypes has been TLR7.

There is burgeoning evidence for a role of Toll-like receptors in autoimmune disease (TLR). Most interest has focused on TLR3, TLR7 and TLR9, which recognize dsRNA, ssRNA and dsDNA respectively. The association of these genes with lupus arises predominantly from murine models, where stimulation of any of these receptors *in vivo* augments nephritis [12–17]. Furthermore, more recently, it has been demonstrated that inhibitors of TLR7 reduce a

number of lupus associated phenotypes, in the MRL^{lpr} and NZB/W strains [18, 19]. Taken together, these studies provide a compelling argument for the involvement of toll receptors in the development of systemic autoimmunity.

In the present study, we have introgressed a TLR7-deficient allele (TLR7^{-}) onto B6.*Sle1Yaa* to produce B6.S*le1YaaTLR7*$^{-}$. The expression of TLR7 in this strain is limited to the single copy of TLR7 translocated onto the Y chromosome. Expression analyses demonstrate that TLR7 transcription in B6.S*le1YaaTLR7*$^{-}$ mice is indistinguishable from normal B6 levels, although other genes within the translocated segment on Yaa are still over-expressed. Examination of a cohort of B6.S*le1YaaTLR7*$^{-}$ mice aged 9 months revealed that gross splenic pathology, GN and most cellular phenotypes observed in the B6.*Sle1Yaa* strain have returned to phenotypes characteristic of B6.*Sle1*. Surprisingly, the increased levels of humoral autoimmunity in B6.*Sle1Yaa* were not fully resolved in B6.*Sle1YaaTLR7*$^{-}$. Nonetheless, these results indicate that the majority of the autoimmune phenotypes associated with the *Yaa* translocation are conferred by the 2-fold increase in TLR7 expression.

Results

Transition to severe pathology in B6.*Sle1Yaa* is dependent on dysregulation of TLR7

We produced male cohorts of B6, B6.*Sle1,* B6.*Yaa,* B6.*Sle1Yaa,* B6.*YaaTLR7*$^{-}$, and B6.*Sle1YaaTLR7*$^{-}$ mice and assessed the expression of *TLR7*. The upregulation of *TLR7* expression observed in purified B cells from *Yaa*-bearing males was eliminated with deletion of the X-derived copy of *TLR7*, although other translocated genes, such as *Prsp2,* continued to be expressed at 2-fold higher levels (Supplementary figure 1). These results confirm that the *Yaa*-derived copy of TLR7 is expressed in an essentially normal fashion and maintains normal levels of TLR7 when expressed in association with a TLR7-null allele on the X chromosome. As shown in Figure 1, comparisons of B6.*Sle1Yaa* and B6.*Sle1YaaTLR7*$^{-}$ mice demonstrate that restoration of normal levels of expression of *TLR7* eliminates the proteinuria and BUN (Figure 1a), severe GN (Figure 1b), splenomegaly and cellularity (Figure 1c and d) associated with the combination of *Yaa* with *Sle1.* These findings indicate that the gross pathological indications of transition to severe disease that are mediated by *Yaa* are eliminated by returning the expression of TLR7 to normal levels.

Splenomegaly, monocytosis and cellular activation are dependent on dysregulation of TLR7

Flow cytometric analysis of splenocytes revealed that the dysregulated activation and expansion of immune cell lineages that are characteristic of B6.*Sle1Yaa* return to levels seen in B6.*Sle1* in B6.*Sle1YaaTLR7*$^{-}$ mice (Supplementary Table 1 and Supplementary Figure 1). Specifically, the characteristic monocytosis and subsequent cellular composition was restored to a splenic profile similar to B6.*Sle1* (Figure 2a). Analysis of the myeloid lineage indicated that all myeloid populations, including PMNs, inflammatory Gr1+ monocytes and resident Gr1− monocytes, were increased in the B6.*Sle1Yaa* mice and deletion of TLR7 restored the levels to those observed in B6.*Sle1* mice (Figure 2b). The *Yaa*-dependent increased activation of the lymphoid lineages, as exemplified by CD69 expression, was also

abolished (Figure 2c and Supplementary Table 1). The increase in plasma cells observed in B6.*Sle1Yaa* mice was reduced on TLR7 deletion, and although the levels were higher than in B6.*Sle1*, this variation was not significant (Figure 2d and Supplementary figure 1). Furthermore, the follicular T cell phenotype, characteristic of late stage disease development in B6.*Sle1Yaa*, was eliminated on deletion of X-derived TLR7 (Figure 2e).

Incomplete resolution of Serum Autoimmune phenotypes with TLR7 deletion

Analysis of total serum IgG and IgM demonstrated that the high levels typical of the B6.*Sle1Yaa* strain were slightly reduced in the B6.*Sle1YaaTLR7*$^{-}$ mice, suggesting an incomplete resolution of the phenotype (Figures 3a and 3b). Examination of a cohort of 6 month old mice demonstrated that IgM autoantibodies remained high in B6.*Sle1YaaTLR7*- mice, when compared to Sle1 mice, although this difference was not significant. In a separate cohort of 9 month old mice, there was no difference in IgM ANAs, with all mice demonstrating detectable levels (Figure 3c). Furthermore, at 6 and 9 months all B6.*Sle1YaaTLR7*$^{-}$ mice demonstrated significantly higher levels of IgG ANA than in B6.*Sle1*, similar to their B6.*Sle1Yaa* counterparts (Figure 3d). Specific analysis of autoantigens using a protein array in the 9 month old mice showed that the major difference in IgM autoantibodies was that B6.*Sle1Yaa* had comparatively more specificity to subtypes of collagen, histones, sRNP and chromatin when compared to B6, B6.*Sle1* and B6.*Sle1YaaTLR7*$^{-}$ sera (Figure 3e). Similar analysis of IgG ANAs demonstrated that B6.*Sle1Yaa* mice had high levels of IgG antibodies to ssRNA, H2B, ssDNA, dsDNA and glomerular basement membrane (Rat Glom) (Figure 3f). This analysis was repeated on a separate cohort of 9 month old mice, which produced similar results (Supplementary figure 2).

Since TLR7 has been associated with U1_snRNP autoreactivity, we verified the autoantigen array data using an U1_snRNP Elisa (supplemental figure 3)[20–22]. We determined that between 30 and 50% of B6.*Sle1Yaa* mice were statistically positive at 6 and 9 months of age and thus it appears in this model, a high titre of anti-snRNP is not an overwhelming feature of disease in every mouse. Indeed earlier studies in MRL/*lpr* mice suggest that the penetrance of this antibody is less than 50% [21]. Moreover, approximately 30–40% of stimulatory immune complexes from SLE patients contain sn_RNP [23, 24] Taken together, these data suggest that snRNPs are not the only stimulatory factor in human disease or murine models. Elimination of TLR7 prevented this level of penetrance (Supplemental figure 3).

Other autoantibodies, such as those specific to histone and chromatin in the IgG array, detectable in the ANA Elisa, were also detectable in B6.*Sle1* and B6.*Sle1YaaTLR7*$^{-}$ mice. These results suggest that deletion of the X chromosome copy of TLR7 may not fully diminish the increased titers of humoral autoantibodies to chromatin seen in B6.*Sle1Yaa*, however, the transition to production of IgG antibodies specific for pathogenic autoantigens, such as dsDNA and glomerular autoantigens, is eliminated. Serum cytokine analysis using a 22-plex Luminex assay demonstrated detectable increases in the levels of IL-9, IL-10, IL-13, and IL-12p40 in B6.*Sle1Yaa* mice. These increases were eliminated on TLR7 deletion (Figure 3g).

Renal leukocyte infiltration and activation is restored on TLR7 deletion

A detailed flow cytometric analysis of the leukocyte infiltrate in the kidneys of our cohorts revealed that the kidneys of B6.*Sle1Yaa* mice are infiltrated by a characteristic profile of activated inflammatory cells and that these differences are dependent upon the dysregulation of TLR7 (Figure 4a and Supplementary Table 2). The major infiltrating cells into the kidney were CD4+ T cells and inflammatory $Gr1^{high}$ monocytes (Figure 4b and c respectively). The CD4+ T cells infiltrating the kidneys of B6.*Sle1Yaa* mice were activated, demonstrating an increase in surface expression of ICOS and PD-1, although there was no detectable expression of CXCR5 (Figure 4d and Supplementary Table 2). Analysis of the $Gr1^{high}$ monocytes demonstrated that they were activated, with increased expression of CD69 and CXCR4, a chemokine receptor whose upregulation is associated with renal damage (Figure 4e, [25, 26]). B cells were also activated, with increased expression of CD69 and CXCR4 (Figure 4f). The infiltrate and activation was absent in the B6.*Sle1YaaTLR7*$^{-}$ mice. Finally, analysis of cytokines from renal plasma demonstrated that IL-12p40 was upregulated within B6.*Sle1Yaa* mice, and that these levels were restored to levels observed in the mice without severe GN in B6.*Sle1YaaTLR7*$^{-}$ mice (Figure 4g).

Discussion

In the present study, we sought to determine whether TLR7 was responsible for the autoimmune phenotypes conferred by *Yaa* in the B6.*Sle1Yaa* strain. We and others have previously shown that the genetic lesion underlying *Yaa* is an X to Y translocation involving a cluster of tightly linked genes adjacent to the psuedoautosomal region of the X chromosome [8, 27]. Several of the genes in the translocated segment are expressed at a 2-fold higher level in *Yaa*-bearing males, many of these in B cells. Thus, although the presence of TLR7 within the translocated cluster made it a strong candidate for the autoimmune phenotypes associated with *Yaa*, the potential involvement of other translocated genes with autoimmunity could not be excluded.

The results presented in this study indicate that the dysregulated expression of TLR7 is responsible for the bulk of the autoimmune phenotypes elicited by *Yaa* in combination with *Sle1*. Our analyses indicate that the inclusion of a TLR7 deletion in B6.*Sle1Yaa* mice eliminates virtually all of the severe disease phenotypes associated with *Yaa* in this model system. This basic finding is in accordance with a recent publication which eliminated TLR7 in $Fc\gamma RII^{-/-}$ *Yaa* mice [28]. Taken together, these findings firmly establish that a very modest increase in the level of expression of TLR7 can mediate a rapid and highly penetrant transition to severe pathogenesis in mice that are prone to the development of spontaneous humoral autoimmunity.

Our serum analysis of autoantibodies suggests that levels of IgM and IgG ANAs at 6 months remained high in B6.*Sle1YaaTLR7*$^{-}$ mice and were not fully restored to levels demonstrated by their B6.*Sle1* counterparts. However, although the titer of autoantibodies was still somewhat elevated, the transition to pathogenic autoantibodies, such as IgG antibodies to dsRNA, dsDNA and glomerular basement membrane did not occur in B6.*Sle1YaaTLR7*$^{-}$ (Figure 3F). This could reflect the contribution of one of the other translocated genes in the *Yaa* locus, or reflect slight aberrations in the expression of TLR7 when it is dictated solely

by the translocated copy in the *Yaa* locus. In this regard, we did not observe gross abnormalities in the expression of TLR7 in B6.*Sle1YaaTLR7*$^{-}$ mice (Supplementary Fig. 1), although we cannot exclude possible variations in expression following specific cell activation events. Indeed, recent investigations have demonstrated that administration of IFNα to purified B cells increases TLR7 mRNA expression and function [20, 29]. Thus, further work will be needed to identify the causes of these minor phenotypic variations.

The results we report provide important new insights into the impact of TLR7 upregulation on end organ targeting in systemic autoimmunity. In the B6.*Sle1Yaa* model, the impact of upregulating TLR7 is most clearly apparent in CD4+ T cell and myeloid lineages. Thus, the development of CD4+ T_{FH} populations in the spleen is a prominent feature in B6.*Sle1Yaa* mice and these cells are not expanded in B6.*Sle1YaaTLR7*$^{-}$ mice. T_{FH} cells are also a prominent feature of systemic autoimmunity in the *san roquin* mutant mouse, [30] suggesting that the activation of this T cell lineage may be an important element in the development of severe autoimmunity. Interestingly, a significant increase in infiltrating activating CD4+ T cells into the kidney is a dominant feature of B6.*Sle1Yaa* disease that is completely eliminated in B6.*Sle1YaaTLR7*$^{-}$ mice. This suggests that the development of these highly activated infiltrating CD4+ T cells is dependent upon the dysregulation of the innate immune system by TLR7 and that their generation is essential for severe end organ disease. An earlier report demonstrated that TLR7 activation predominantly impacted the development of kidney pathology, suggesting that activation of the innate immune system strongly influenced end organ disease [12]. Taken together, these findings suggest that an upregulation of TLR7 drives end organ disease in our model system, predominantly via the activation of the T cell lineage.

In summary, our analysis of the impact of TLR7 upregulation on disease development in B6.*Sle1Yaa* mice clearly indicates that subtle variations in signaling in the innate immune system are sufficient to drive severe disease pathogenesis in genetically predisposed mice. This firmly establishes that variations in signaling within the innate immune system can profoundly influence lupus development. Although mutations similar to *Yaa* do not appear to be a common feature of human SLE [31], allelic variants of IRF5 are strongly associated with SLE susceptibility [32]. IRF5 is a transcription factor that is induced in response to TLR signaling, most notably via TLR7, and the disease associated allele mediates increased expression of IRF5. Whether this IRF5 polymorphism in humans causes a dysregulation of TLR7 signaling analogous to that of *Yaa,* remains to be determined.

Materials and Methods

Reagents and mice

All mice were bred in the University of Texas Southwestern Medical Center's specific pathogenic free (SPF) facility. The care and use of laboratory animals conformed to the National Institutes of Health guidelines and all experimental procedures conformed to an IACUC approved animal protocol. Breeding pairs for C57BL/6J (B6) mice were originally obtained from the Jackson Laboratory. The derivation of B6.*Sle1Yaa* has been described previously [8, 33]. Gross pathological changes in tissues such as the liver, lung and skin were not observed in the B6.Sle1Yaa mice, therefore further examination of these tissues

was not carried out. Breeding pairs for TLR7−/− mice were obtained from Dr. I Rifkin at Boston University, with Dr. S Akira's permission [34]. Resultant mice were at least 7 generations backcrossed to B6. They were tested for contaminating 129-derived *Sles1* alleles which were confirmed to be of B6 origin.

Assessment of Renal Disease

Mice were caged in metabolic cages and urine was collected over a 24 hr period. Protein was measured using the Coommassie® Plus Protein Assay kit (Pierce Biotechnology Inc. Rockford, IL) as per the manufacturer's instructions. BSA (Pierce) was used as a standard. Blood Urea Nitrogen (BUN) was assessed using the QuantiChrom Urea Assay Kit (BioAssay Systems, CA). Following euthanasia, kidneys were analyzed by an independent pathologist (JZ), as previously described [35] The severity of GN was graded using the following guidelines set by the World Health Organization: 0, normal; 1, mild increase in mesangial cellularity and matrix; 2, moderate increase in mesangial cellularity and matrix, with thickening of the GBM; 3, focal endocapillary hypercellularity with obliteration of capillary lumina and substantial increase in the thickness and irregularity of the GBM; 4, diffuse endocapillary hypercellularity, with segmental necrosis, crescents, and hyalinized end-stage glomeruli). Tubular interstitial nephritis (TIN) was graded on a 0–4 scale (Supplemental figure 4).

Serology

Autoantigen arrays on serum samples were completed at the UT Southwestern Microarray Core facility [36]. Serum ANAs were analyzed using an anti-histone-DNA antibody Elisa as described previously [37].

Cytokine Multiplex Analysis: Following an initial 22-plex screening of B6.*Sle1Yaa* male sera, 9 cytokines were analyzed in sera and supernatants from kidney using the Bio-Plex cytokine assay kit (Bio-Rad) as per manufacturer's protocol at BIIR Luminex Core, Dallas.

Cell Preparation and Flow Cytometry

Peripheral Blood was taken retro-orbitally, or by cardiac puncture. Kidneys were prepared as described previously [25]. Briefly they were minced and resuspended into 0.75mls of PBS. Cells were spun down and the supernatant was kept at −20°C for cytokine analysis. Cells were resuspended in digestion buffer, consisting of Collagenase (1mg/ml) and DNase (1ug/ml) in RPMI Complete Media and incubated at 37°C for 30mins. Cells were centrifuged and filtered through a 70uM mesh and then mixed 1:1 with 40% Percoll solution. This was centrifuged 3000rpm/20mins/RT/brake off. The loose pellet was washed, counted and resuspended in staining buffer. Analysis of myeloid subtypes was based upon analyses described by Geismann and colleagues [38], with the gating strategy shown in Supplementary Figure 5. Acquisition and analysis was completed using a BD LSR II with Diva software, and Flowjo 7.2 for Windows, Treestar.

Statistical Analyses

Results are expressed as the arithmetic mean ± standard error of the mean (SE). Normality was tested using Kolmogorov and Smirnov, followed by 1-way parametric ANOVA & Tukey

post hoc comparison. If normality test failed, 1-way ANOVA and Dunn's multiple comparison test was used to test multiple comparisons. Analyses were completed using InSTAT version 3.0 and Prism 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SLE	Systemic Lupus Erythematosus
ANA	anti-nuclear antibodies
IC	Immune complex
GN	glomerulonephritis
DC	Dendritic Cell

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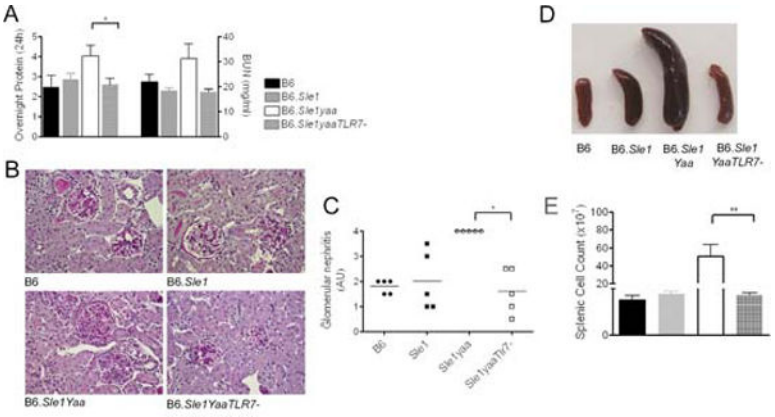

Figure 1. Comparative Pathology of B6Sle1YaaTLR7⁻ and B6Sle1Yaa mice

Proteinuria and BUN in 6month and 9 month cohorts of male mice (a) left and right panels respectively demonstrated that deletion of TLR7 eliminates the increase of B6*Sle1Yaa*. GN was assessed in 9 month mice using H&E staining (b) and graded as described in the methods (c). Splenic weights (d) and counts (e) of 9 month mice showed that the splenomegaly characteristic of B6*Sle1Yaa* mice was abolished with deletion of the additional copy of TLR7.

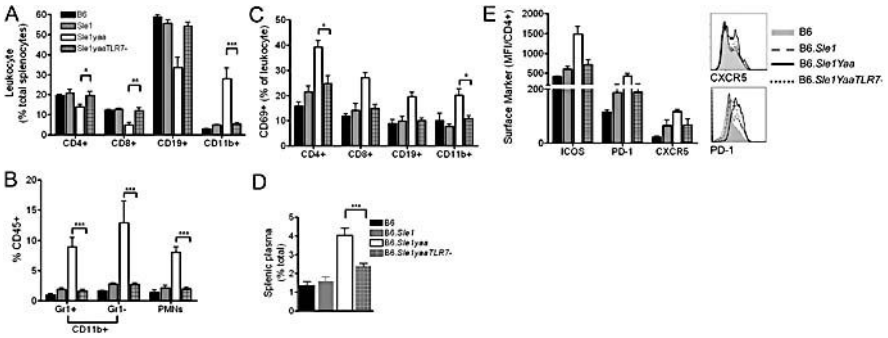

Figure 2. Splenic leukocyte lineage and activation in 9 month mice

Lymphocyte (a) and myeloid (b) lineage and activation (c) were assessed in 9 month mice. The classic B6.*Sle1Yaa* monocytosis was not evident in B6.*Sle1YaaTLR7-* mice. The augmented B6*Sle1Yaa* plasma cell population was also reduced in B6*Sle1YaaTLR7-* mice (d). Increased expression of CXCR5, PD-1 and ICOS on CD4+ T cells, indicates a follicular phenotype in B6*Sle1Yaa* mice which is not present in B6*Sle1YaaTLR7-* counterparts(e).

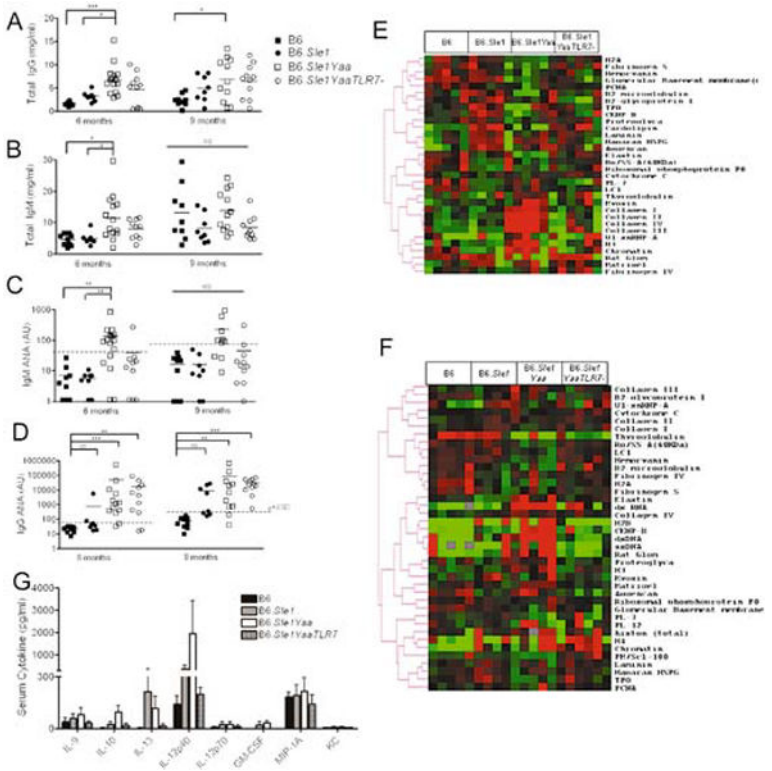

Figure 3. Serum Analysis of Ig in 6 and 9 month mice

Total IgG (a) and IgM (b) were assessed in 9month old mice demonstrating increased levels in all B6*Sle1Yaa* mice. IgM ANAs were high in B6*Sle1Yaa* mice at 6 months compared to B6 and Sle1 control mice (c, left panel). There was no difference at 9 months (c, right panel). IgG ANAs were also determined in 6 and 9 month old mice (d, left and right panels respectively). *NS*: non-significant, *<p0.05, **p<0.01, ***p<0.001. The autoantigen specificity of these antibodies was assessed using a protein array, measuring IgM (e) and IgG (f) autoantigens. The data demonstrates different specificities of B6.*Sle1Yaa* mice compared to B6*Sle1* and B6*Sle1YaaTLR7*$^{-}$. Serum cytokines were determined using luminex which showed an increase in circulating IL-12p40 in B6*Sle1Yaa* mice (g).

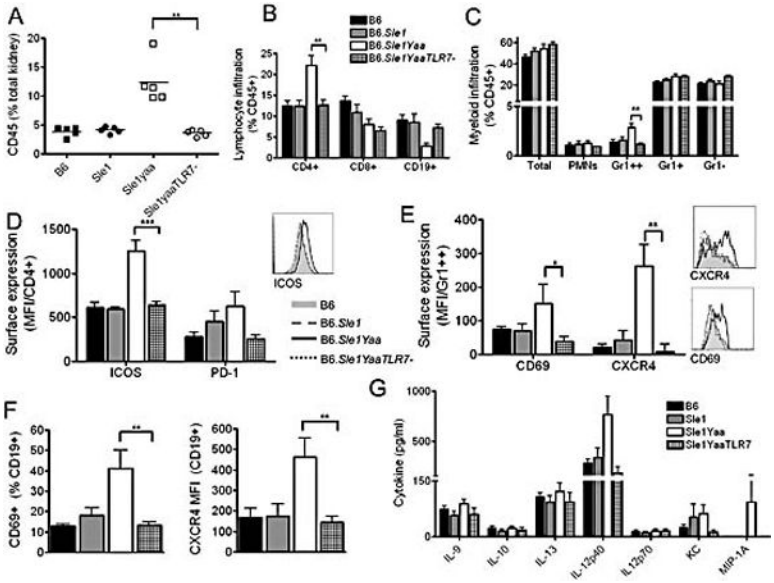

Figure 4. Leukocyte infiltration of the Kidney

Kidneys were perfused and processed as described in the methods and flow cytometry was used to assess leukocyte infiltration and activation. B6.*Sle1Yaa* mice presented increased leukocyte infiltration, which was eliminated on deletion of the additional TLR7 (a). Lineage assessment of the kidney demonstrates that the primary infiltrating cells are CD4+ T cells (b) and Gr1high monocytes (c). Analysis of surface expression markers on the CD4 T cells demonstrated an increase in expression of follicular markers ICOS and PD-1 on B6*Sle1Yaa* mice, which was absent in B6*Sle1YaaTLR7*⁻ mice (d). Analysis of the infiltrating Gr1high population in B6*Sle1Yaa* mice showed increased expression of CD69 and CXCR4 which was not present on deletion of the extra copy of TLR7 (e). B cells were activated in B6*Sle1Yaa* mice as demonstrated by expression of CD69 and CXCR4 and this too was eliminated in B6*Sle1YaaTLR7* mice (f). Analysis of the supernatant in the kidney demonstrated that a primary cytokine in B6*Sle1Yaa* mice within the kidney was IL-12, with some mice producing high levels of MCP-1 (g). This was not evident in B6*Sle1YaaTLR7*⁻ mice.