While T cells are important for the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis, little is known about how T cells function after infiltrating the kidney. The current paradigm suggests that kidney infiltrating T cells (KITs) are activated effector cells contributing to tissue damage and ultimately organ failure. Herein, we demonstrate that the majority of CD4$^+$ and CD8$^+$ KITs in three murine lupus models are not effector cells, as hypothesized, but rather expressed multiple inhibitory receptors and proved highly dysfunctional with reduced cytokine production and proliferative capacity. Mechanistically this was linked directly to metabolic and specifically mitochondrial dysfunction. This was driven by the expression of an “exhausted” transcriptional signature. Our data thus reveal that the tissue parenchyma has the capability to suppress T cell responses and limit damage to self. These findings open novel avenues for the treatment of autoimmunity based on selectively exploiting the exhausted phenotype of tissue-infiltrating T cells.
Kidney-Infiltrating T cells in Murine Lupus Nephritis are Metabolically and Functionally Exhausted

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Abstract:

While T cells are important for the pathogenesis of systemic lupus erythematosus (SLE) and lupus nephritis, little is known about how T cells function after infiltrating the kidney. The current paradigm suggests that kidney-infiltrating T cells (KITs) are activated effector cells contributing to tissue damage and ultimately organ failure. Herein, we demonstrated that the majority of CD4^+ and CD8^+ KITs in three murine lupus models are not effector cells, as hypothesized, but rather expressed multiple inhibitory receptors and were highly dysfunctional, with reduced cytokine production and proliferative capacity. In other systems, this hypofunctional profile is linked directly to metabolic and specifically mitochondrial dysfunction, which we also observed in KITs. The T cell phenotype was driven by the expression of an “exhausted” transcriptional signature. Our data thus revealed that the tissue parenchyma has the capability to suppress T cell responses and limit damage to self. These findings suggest new avenues for the treatment of autoimmunity based on selectively exploiting the exhausted phenotype of tissue-infiltrating T cells.
Introduction:
Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease defined by loss of self-tolerance, autoantibody formation, cellular tissue infiltration and end-organ damage. The most common and severe organ-specific manifestation of SLE is nephritis (LN), with 10% of patients developing end-stage renal disease (1). There are no FDA-approved therapies for LN and current treatments are non-specific, with suboptimal remission rates (1-3). The importance of T cells in SLE has been well-documented in murine models, with a consensus that CD4⁺ T cells propagate the disease (4-7). The role of CD8⁺ T cells in disease pathogenesis is less clear. Several studies have shown that MHC class I is required (8, 9), while others have concluded that disease pathogenesis does not require CD8⁺ T cells (5, 6). Recently the immunosuppressive drug tacrolimus, which targets T cells, has been shown to be efficacious in the treatment of LN (10), further supporting the importance of T cells in LN pathogenesis.

While the nature of kidney-infiltrating cells has been investigated, a much greater focus in murine and human lupus studies has been on the more readily accessible peripheral cell populations, which are relatively well-understood. Comparatively much less is known about organ-specific disease processes, even though infiltrating cells are directly impacting target tissues and hence should be a priority to understand.

T cells comprise the majority of kidney-infiltrating cells in humans (11-13) and murine models of lupus (14). A limited number of prior studies evaluating the role of kidney-infiltrating T cells (KITs) in LN have shown that they undergo clonal expansion (15, 16) and are CD44⁺ (17), suggesting an activated phenotype. KITs from LN samples evaluated using micro-dissection showed expression of IL-2, IL-10, IL-17, IL-4, IL-13 and IFNγ transcripts (18). IL-17 protein has been detected by immunofluorescence staining
in KITs (19); however, genetic deletion of IL-17 did not affect disease in murine models (20).

Based on these studies, the prevailing hypothesis in the field is that KITs are likely to be activated effector T cells (21). This hypothesis has yet to be tested, as the function and metabolic capacity of KITs has not been directly examined. The metabolic status of KITs is of particular interest, given recent work showing that altering metabolic signaling ameliorates disease (22). Here, to address this gap and test whether T cells in target tissues differentiate into highly activated and functional cells, as thought, we directly examined the phenotype, metabolism, and functional capacity of KITs in murine lupus models and compared them to their peripheral T cell counterparts.
Results:
Three murine models of LN were used to determine the role and phenotype of KITs. The first model is the MRL.Fas<sup>br</sup> (MRL/lpr) model of lupus, which recapitulates nearly all features of human disease and is therefore a predominant research model (23-28). Importantly, preclinical therapeutic trials in the MRL/lpr model have accurately predicted responses in human translational studies (29-33). The second model is the Fcgr2b<sup>−/−</sup> Y-chromosome linked autoimmune accelerator (Yaa) model, in which deletion of the inhibitory receptor Fc<sub>γ</sub>RIIB combined with the Yaa mutation results in a proliferative glomerulonephritis in male mice (34). We conducted confirmatory experiments in a third murine model, the MRL. Tlr9<sup>−/−</sup> model, which is sufficient for Fas but uses TLR9 deficiency as a disease accelerator. As non-autoimmune mice have very few T cells in their kidneys, the paradigm we have used in this work is to compare KITs in diseased kidneys with T cells in the spleens of the same mice. However, to determine whether an inflammatory environment was necessary to alter T cell phenotype, we also examined KITs from C57BL/6 (B6) mice.

**T cells comprise the majority of kidney-infiltrating immune cells in lupus prone mice.**

In aged nephritic MRL/lpr mice, T cells comprised 64.2% of the total CD45<sup>+</sup> kidney infiltrate, followed by CD11b<sup>+</sup> populations, which accounted for 25.4% (Figure 1A). We recovered an average of nearly 4 x 10<sup>6</sup> total TCR<sub>β</sub><sup>+</sup> cells from the kidneys of older MRL/lpr mice, and approximately 10-fold fewer cells in younger, 11-12 week old, MRL/lpr mice (Supplemental Figure 1A). The overall composition of T cell populations in the kidney differed from that in the periphery with an increased percentage of CD4<sup>+</sup> and CD8<sup>+</sup> and a lower frequency of double negative (DN) T cells in the kidney (Figure 1B). Consistent with previous reports (17), KITs expressed elevated amounts of activation
markers CD44 and CD69 (Figure 1C and D) and reduced amounts of CD62L (Figure 1C), suggesting a change in migratory status.

In comparison, there were nearly 100-fold fewer TCRβ+ cells isolated from the kidneys of B6 mice compared to MRL/lpr mice of similar age (Supplemental Figure 1A). T cells comprised ~10% and CD11b+ cells accounted for 80% of non-parenchymal cells isolated (Supplemental Figure 1B). Kidney-derived T cells from B6 mice exhibited an increased frequency of T cells with an effector phenotype (CD44^{high}, CD62L^{low}) and increased frequency of CD69+ cells when compared to splenic T cells. Compared to MRL/lpr KITs, B6 KITs had an increased frequency of naïve CD8+ T cells (CD44^{low}, CD62L^{high}) and lower frequency of CD69+ CD4+ and CD8+ T cells (Supplemental Figure 1 C and D).

**KITs are functionally suppressed compared to splenic-derived T cells.**

Because T cell infiltrates have a known role in pathogenesis of SLE in murine models and are the predominant cell in the inflamed kidney, we next evaluated the functional status of KITs. Compared to splenic T cells, a significantly smaller percentage of CD4+ and CD8+ KITs produced inflammatory cytokines after bulk culture stimulation with PMA and ionomycin, in all three murine models of LN (Figure 2A-D, Supplemental Figure 2A). There was also a reduced frequency of IL-10+, CD4+ KITs compared to splenic T cells. In addition to a reduced percentage of cytokine producing cells among KITs compared to splenic T cells, there was also a substantial reduction in MFI of cytokine expression (Figure 2C and D). KITs from B6 mice displayed a similar low frequency of cytokine producing cells (Supplemental Figure 3A). There was no detectable IL-4 or IL-17 staining in KITs or splenic derived T cells in the MRL/lpr model (not shown).
Both CD4$^+$ and CD8$^+$ MRL/lpr derived KITs exhibited reduced proliferation in response to combined anti-CD3/anti-CD28 stimulation compared to splenic T cells, with only a few KITs proliferating past the first division, while splenic T cells underwent up to 6 divisions during the same time frame (Figure 2E). A significant reduction in both proliferative index and division index was observed (Figure 2E). Thus, counter to conventional theory, KITs are functionally suppressed, compared to activated effector splenic T cells.

**KITs exhibit elevated levels of Inhibitory Receptors (IR).**

As functionally impaired KITs may be chronically exposed to self-antigen (15,16), we hypothesized that they would exhibit an exhausted phenotype, as described for TILs and T cells in chronic infection (35, 36). To address this hypothesis, we examined expression of inhibitory receptors (IR) known to be upregulated on exhausted CD8$^+$ T cells, including PD-1, Tim3, Lag3, and 2B4 (35-37). Overall, CD8$^+$ KITs from MRL/lpr and Fcgr2b$^{-/-}$.Yaa LN mouse models exhibited increased IR expression compared to B6 splenocytes (Figure 3). Additionally, IR expression was generally more elevated in KITs than matched splenic comparators. This was most notable in the CD8$^+$ compartment in MRL/lpr and MRL.Tlr9$^{-/-}$ mice, with all IRs being expressed on a significantly higher proportion of KITs compared to splenic counterparts (Figure 3A and, Supplemental Figure 2B). A similar pattern of extensive expression of IRs was noted in the Fcgr2b$^{-/-}$.Yaa KITs when compared to non-lupus control B6 T cells (Figure 3A). However, splenic CD8$^+$ T cells in this model expressed IRs to a similar degree as did KITs, possibly reflecting a high activation status of the Fcgr2b$^{-/-}$.Yaa splenic T cells, as activation per se can also cause increased expression of IRs (36, 37).

Analysis of IR expression on CD4$^+$ T cells is more complex, in part because the association between IR expression and exhaustion versus activation is less clear in
CD4⁺ T cells (38). Nonetheless, we observed a statistically significant increase in the majority of IRs in KITs compared to non-autoimmune comparator T cells (Figure 3B). Notably, there were concomitant increases in the Lag3 and Tim3 expression in the splenic derived MRL/lpr CD4⁺ T cells, which is likely due to the activated nature of these circulating T cells (38). Both CD4⁺ and CD8⁺ KITs isolated from B6 mice exhibited significantly increased expression of PD-1 compared to matched splenic controls. However, these B6-derived KITs expressed significantly less PD-1, Lag3, and Tim3 than KITs derived from lupus prone MRL/lpr mice (Supplemental Figure 4).

Nearly all KITs exhibited suppressed cytokine production (Supplemental Figure 5A and B), independent of PD-1 expression levels, suggesting that there are likely additional mechanisms by which KIT function is suppressed. This hypothesis is further supported by the fact that T cells derived from non-autoimmune kidneys did not produce cytokines after stimulation (Supplemental Figure 3), despite the fact that they expressed a significantly lower frequency of IRs than did KITs from autoimmune mice (Supplemental Figure 4).

**Autoimmune KITs are metabolically quiescent compared to splenic-derived T cells.**

Exhausted T cells are metabolically dysfunctional, as shown by reduced mitochondrial capacity, mitochondrial function and glucose uptake (39). In fact, it has been suggested that metabolic dysfunction may be a mechanistic contributor to the exhaustion phenotype (39, 40). Thus, we subsequently assessed the mitochondrial reserve and metabolic output of KITs using the Seahorse extracellular flux analyzer. Metabolic flux analysis of both CD4⁺ and CD8⁺ KITs from MRL/lpr mice revealed a substantial loss of spare respiratory capacity (SRC), defined as the difference between basal and
uncoupled maximal oxygen consumption (Figure 4A). However, basal oxygen consumption rates were unchanged between cell types.

A reduction in SRC can be explained by loss of mitochondrial membrane potential (Δψm) and/or loss of mitochondrial mass. Δψm is required for production of ATP, thus it has been hypothesized that suppressed Δψm may alter cellular function (40). Indeed, using MitoStatus, a membrane potential-dependent dye, we observed that KITs from MRL/lpr, Fcgr2b−/−.Yaa, MRL.Tlr9−/− mice exhibited reduced Δψm compared to splenic counterpart cells (Figure 4B, Supplemental Figure 2C). Similarly, both CD4+ and CD8+ KIT populations had a significant reduction in MitoTracker Deep Red FM (MitoTracker) staining, which measures mitochondrial mass (39). However, CD8+ KITs had a bimodal distribution of MitoTracker staining (Figure 4C, Supplemental Figure 2D) with some cells retaining substantial staining. Thus, the loss of mitochondrial mass appears to contribute, but alone is insufficient, to account for the almost complete abrogation of SRC.

The extent of glucose utilization depends on T cell function (39, 41), with exhausted T cells exhibiting reduced glucose uptake (39). Indeed, KITs from MRL/lpr mice demonstrated suppressed glucose uptake in vitro, as measured by reduced fluorescent 2-NBD-glucose dye (2NBDG) uptake. In MRL/lpr and MRL.Tlr9−/− mice 25.7% and 30.0% of CD8+ KITs took up glucose, respectively, compared to 86.2% and 67.8% of splenic comparators (Figure 4D, Supplementary Figure 2C). Furthermore, in co-staining studies, very few KITs in either the CD4+ or CD8+ compartment exhibited intact Δψm and glucose uptake (Figure 4D) suggesting that these cells carry out little if any oxidative phosphorylation (Figure 4D). MRL.Tlr9−/− KITs (Supplemental Figure 2C and D) and T cells derived from B6 kidneys (Supplemental Figure 3B) exhibited a similar pattern of
mitochondrial probe and 2-NBDG staining. One notable difference was that CD4<sup>+</sup> cells isolated from kidneys of B6 mice did contain a population of metabolically intact cells (Mitostatus and 2NBDG double positive). Among both KITs and splenic T cells, only those cells that had not upregulated PD-1 demonstrated glucose uptake (Supplemental Figure 5C), but neither mitochondrial parameter had a similar correlation with PD-1 expression (Supplemental Figure 5D, E), again suggesting the PD-1 expression and function alone does not entirely control the metabolic exhaustion phenotype in these cells.

**KITs from younger MRL/lpr mice have a phenotype similar to nephritic MRL/lpr mice.**

To begin to address when the T cell exhaustion phenotype observed in KITs of aged mice first develops, we examined a cohort of 11-12 week MRL/lpr mice, a time when disease is at early stages and prior to clinical proteinuria. KITs derived from 11-12 week old MRL/lpr mice did not differ significantly from those of aged nephritic MRL/lpr mice with regard to activation status, expression of exhaustion markers, cytokine production and metabolic profile (Supplemental Figures 1, 3, and 4). This finding suggests that alteration in T cell profile after kidney infiltration occurs fairly early in the disease process, and may occur relatively rapidly after tissue entry.

**KITs exhibit a transcriptional phenotype that resembles “exhausted” T cells from other models of disease.**

To better understand how KITs and splenic T cells from the same animals differed in function and origin, we performed transcriptional profiling of CD4<sup>+</sup> and CD8<sup>+</sup> KITs and matched splenic T cells. After determining genes that were differentially expressed between splenic T cells and KITs (42), we performed gene set enrichment analysis
Differentially expressed genes were compared to several previously defined gene signatures that are characteristic of CD8\(^+\) and CD4\(^+\) T cell exhaustion in the chronic LCMV infection model (37, 44). Genes from the CD8\(^+\) exhaustion cluster were significantly enriched among genes that were differentially expressed in CD8\(^+\) KITs vs CD8\(^+\) splenocytes (Figure 5A, Supplemental Figure 6). Similarly, genes contained in the CD8\(^+\) LCMV exhaustion-associated gene set were significantly enriched among genes differentially expressed in CD4\(^+\) KITs versus CD4\(^+\) splenocytes (Supplemental Figure 6), and genes characterizing exhausted CD4\(^+\) T cells in LCMV were nearly significantly enriched among CD4\(^+\) KIT differentially expressed genes (p= 0.0508, Figure 5B). These data provide direct and unbiased evidence that CD8\(^+\) KITs are more exhausted than their lymphoid-resident counterparts. The less robust enrichment of exhaustion-related genes among those differentially expressed by CD4\(^+\) KITs could have several explanations: there may be a component of peripheral lymphoid exhaustion in the CD4\(^+\) compartment of older diseased mice we studied; in this case, the differential expression analysis between the kidney and spleen would filter out some exhaustion-related genes. Related to this, highly activated cells are documented to turn on genes associated with exhaustion (36), and our splenic CD4\(^+\) population expressed multiple exhaustion-associated IRs and is CD44\(^+\), CD69\(^+\) (Figures 1 and 3). Additionally, CD4\(^+\) exhaustion is less well-defined than that of CD8\(^+\) cells (35).

We also compared our data sets to the exhaustion profile from a murine model of melanoma (36). Ten gene clusters were identified in this prior study by comparing naïve, effector, and IR-expressing tumor infiltrating CD8\(^+\) cells. Four of these clusters (most predominantly C1, C2, and to a lesser extent C7, C10) were associated with PD-1\(^+\) Tim3\(^+\) TILs (commonly linked with exhaustion) while four clusters (C3, C4, C5, C6) were associated with phenotypically naïve or effector populations, and two clusters showed a
mixed pattern (C8 and C9). A total of 1426 differentially expressed genes between CD4\(^+\) and CD8\(^+\) KITs and splenic T cells from our MRL/\(lpr\) mice were delineated from a total of 3031 possible genes present in any the 10 clusters. Notably, unbiased hierarchical clustering of these 1426 genes resulted in correlation of expression between the TIL related genes (purple) and both CD4\(^+\) and CD8\(^+\) KIT differentially expressed genes (Figure 5C). GSEA further supported the finding that the CD8\(^+\) KITs are similar to PD-1\(^+\) Tim3\(^+\) TILs with significant positive correlation for gene clusters C1 and C2, and negative correlation for the naïve cell clusters C4 and C5 (Supplemental Figure 7).

CD8\(^+\) KITs exhibited increased transcription of IRs, in agreement with the flow cytometry data (Figures 3 and 5D) and decreased transcription of metabolism related genes defined in LCMV exhaustion (37). Interestingly, CD8\(^+\) exhausted cells express increased levels of inflammatory cytokine transcripts, while expression of these proteins was actually reduced (Figures 2 and 5D). The finding of increased transcripts by RNA-seq is in line with microdissection studies of KITs in LN that revealed increased cytokine transcripts (14, 18). The phenomenon of discordant mRNA and protein levels is well documented (45), particularly for cytokines that undergo significant post-transcriptional regulation (46, 47). Importantly, this regulation may play an integral role in T cell exhaustion in KITs, as a similar discordant protein/transcript phenomenon was previously observed for LCMV-specific CD8 T cells (37).

Exhausted CD8 T cells express increased Eomes (48) and decreased Tcf1 (49). Thus, we next examined whether the expression pattern of the transcription factors Eomes and Tcf1 in KITs mirrored that of T cell exhaustion in the LCMV model of chronic viral infection. KITs were compared to PD-1\(^{high}\), Tim3\(^{high}\) (exhausted) T cells from LCMV infected mice and control non-exhausted cells. Notably, KITs expressed a similar Tcf1
and Eomes profile to that of exhausted T cells after LCMV infection (Figure 5E) with a significant decreased Tcf1 expression compared to control (non-exhausted T cells), and splenic derived T cells from matched MRL/lpr mice, with a concomitant increase in Eomes compared to non-exhausted T cells (Figure 5E). This suggests that the transcriptional phenotype observed in KITs may be mediated by mechanisms similar to those regulating T cell exhaustion in chronic viral infections.

**PD-L1 expression is increased in lupus nephritis kidney compared to non-inflamed kidney.**

Several studies suggest that the PD-L1/PD-1 signaling axis is protective in murine lupus (50-52). These studies relied on global gene deletion, which would have affected activation of cells in secondary lymphoid tissues as well as target organs. Thus, to begin to determine how T cell exhaustion is mediated in nephritic kidneys, we investigated in situ PD-L1 expression in kidneys of diseased MRL/lpr mice. T cell infiltrates were observed both in the peri-glomerular space as well as the interstitium of nephritic mice (Figure 6, top panel, and Supplemental Figure 8) but not in younger, pre-diseased mice (Figure 6 bottom panel). PD-L1 was highly expressed in 4 of 6 nephritic mice on both parenchymal and infiltrating cells, and in 2 of 6 mice on predominantly infiltrating cells, while none of the control, pre-diseased MRL/lpr mice had any PD-L1 staining (Figure 6, Supplemental Figure 8). Flow cytometric analysis showed that the majority of infiltrating cells expressing elevated PD-L1 levels were CD11b⁺ (Supplemental Figure 9). The finding of PD-L1 expression in murine nephritic kidneys parallels two studies of human LN kidneys, which showed increased PD-L1 in biopsy samples (53, 54). Hence, in the context of autoimmune nephritis, PD-L1 is induced in both mice, based on our data, and SLE patients, based on published studies, providing a potential mechanism for induction of an exhausted-like T cell phenotype.
Discussion:

A paradigm in the field of autoimmunity is that aggressive, activated self-reactive T cells infiltrate tissue and destroy target organs. Nonetheless, there has been little direct characterization of tissue-infiltrating cells in disease, while cells found in blood and lymphoid organs have been extensively studied. Herein, we present an in-depth characterization of tissue-infiltrating T cells in the kidneys of mice with lupus-like nephritis. Our central and most unexpected finding is that the great majority of KITs are not activated effector cells, as had been thought; rather, they demonstrate suppressed cytokine production and proliferative capacity, with enhanced expression of IRs. In a potential mechanistic link, these cells have reduced metabolic flux, reduced SRC and reductions in mitochondrial numbers and function. Perhaps most telling, we observed that the transcriptional profile of KITs mimicked the T cell exhaustion profile observed in other contexts (36, 37). Taken together, the data indicate that KITS closely resemble T cells found in chronic infection and infiltrating tumors (35-37, 44).

A central concept in tumor immunology is that the tumor microenvironment actively suppresses the function of invading T cells through specific adaptive mutations and increased expression of ligands for IRs (55). It may have been assumed, therefore, that non-malignant parenchymal tissues generally lacked the capacity to suppress infiltrating cells or at the least, that such capacity was compromised to permit autoimmunity. The conceptual advance driven by our current study is that normal parenchymal tissue has the ability to suppress T cells in a similar fashion to tumors, even in the context of genetic predisposition to- and clinical existence of- autoimmunity. We suggest that target tissues may not be as susceptible to autoimmune infiltrates as previously thought and may be endowed with multiple mechanisms to naturally suppress locally destructive adaptive immunity, with these mechanisms still operating or even enhanced in the face
of ongoing autoimmune attack. Given that the KIT exhausted phenotype, as we described herein, correlates well with that of tumor infiltrating lymphocytes, we hypothesize that it may reflect the existence of a general phenotype of T cells infiltrating parenchymal tissues during inflammation. In this context, it is interesting to consider autoimmunity, which is observed in numerous organ systems, that emerges upon therapeutic inhibition of PD-1/PD-L1 in cancer immunotherapy (56). This complication may result from increased activation of autoreactive T cells in secondary lymphoid tissue; however, our data highlight another potential mechanism—that checkpoint inhibition could uncover latent autoimmunity by de-repression of previously exhausted infiltrating cells that were previously causing no clinically significant harm. This mechanism would also be consistent with the relatively rapid onset and aggressive nature of checkpoint blockade-induced autoimmunity (57).

Interestingly, transcriptional profiling revealed that TNFα and IFN-γ mRNAs were more highly expressed in the CD8⁺ KITs compared to the effector splenic CD8⁺ T cell population; whereas protein expression was just the opposite, with KITs expressing much less than peripheral effectors. Though seemingly paradoxical, it is actually not surprising, as a similar phenomenon was described for LCMV-driven T cell exhaustion (37). Our data and these prior data on LCMV-driven exhausted CD8⁺ T cells together imply that a major mechanistic checkpoint for enforcement of reduced effector function by exhausted cells is via post-transcriptional regulation of cytokine, and perhaps other, genes. How this takes place has yet to be determined. In any case, these data suggest that T cell exhaustion and suppression of cytokine signaling is an active process and not just another form of anergy.
How parenchymal tissues under autoimmune attack respond to inhibit the functional status of infiltrating T cells is not yet clear, and is likely to be at least as complex as mechanisms at play in tumors, an area of long-term and extensive research that still is not fully resolved. Nonetheless, from the tumor and chronic viral infection fields, signaling via PD-1 has emerged as a major, but non-exclusive, axis that enhances the exhausted phenotype. Based on this we looked for, and found, local expression of PD-L1 on infiltrated but not non-infiltrated kidneys in MRL/pr mice, in agreement with published studies of LN in humans (53, 54). Interestingly, PD-L1 expression occurs in close proximity to infiltrating T cells, which suggests that parenchymal tissues sense inflammatory signals from infiltrating T cells and respond by upregulating PD-L1 (and potentially other inhibitory ligands). The importance of PD-L1 upregulation is further supported by prior studies that show that PD-L1 expression on kidney derived tubular epithelial cells lines can suppress T cell function (58, 59). A similar relationship between infiltrating cells and PD-L1 expression has been described in tumors (60). If this were true, it would represent a tissue adaptation to inflammation manifesting even in an autoimmune-prone mouse.

Given the profound phenotype of KITs observed in mice with active lupus-like disease, we examined the potential of tissue parenchyma to induce a T cell exhaustion phenotype in the absence of overt autoimmunity. KITs from B6 mice indeed have suppressed cytokine production and an altered metabolic profile similar to that observed in lupus prone mice. Additionally, like MRL/pr KITs, these “non-lupus prone” derived Kidney T cells differed significantly from splenic comparators with an increased frequency of “effector cells” (CD44+, CD62L-) as well as increased frequencies of CD69 and PD-1 expressing cells. Yet they did not fully resemble KITs from MRL/pr mice in several respects. Compared to MRL/pr KITs, B6 KITs had lower frequencies of IR
expression and expressed fewer overall IRs; contained a larger naïve cell population, and had a metabolically intact subset of CD4 KITs. Given the 100-fold lower number of KITs infiltrating the kidney in B6 compared to MRL/lpr mice the impact of these cells on tissue function is unknown. One hypothesis to explain these KITs and their partially exhausted phenotype is that they represent an age-dependent naturally occurring autoreactive population. In normal mice we propose that the parenchyma and other factors successfully suppress these cells, resulting in no clinically apparent disease phenotype. Since the exhausted KIT phenotype is observed in B6 mice it remains possible that this phenotype is not entirely dependent on the autoimmune/inflammatory microenvironment, Studies in which particular antigen-specific T cells are observed in either inflammatory or quiescent contexts would be needed to distinguish these possibilities, which are not mutually exclusive. Either scenario is consistent with the notion that the kidney possesses suppressive qualities, which if confirmed would have implications for hypertension, ischemia reperfusion injury, and renal transplantation.

A major question raised by these results is: since T cells become exhausted in the target tissue, then how is damage mediated? In this context, it is important to note that exhausted T cells, while dysfunctional, are not inert. Exhausted T cells in infection play a non-redundant role in viral control despite their apparent exhaustion, as demonstrated by severe exacerbation of disease and death when these exhausted cells are experimentally depleted(61, 62). Hence, we suggest that though most infiltrating cells are exhausted, they nevertheless retain some effector function and in fact cause the slow rather than acute destruction and decline that characterizes chronic autoimmunity in these lupus nephritis models. Further, in these models there are still some cells that do not display IRs and thus could conceivably not yet be exhausted. Thus, an additional or alternative model to explain disease pathogenesis in the face of exhaustion is that
these IR-negative T cells, which could be newly arrived, initially cause damage and subsequently undergo exhaustion.

Given our knowledge about CD8\(^+\) T cell exhaustion in other settings, it is likely that there are, in addition to PD-1, other T cell-intrinsic IRs which play a role in regulating lupus pathogenesis. We identified increased expression on KITs of Lag3, Tim3, and 2B4; whether these (or even PD-1) are functionally important, and where the relevant ligands are expressed, will need to be addressed in future work that ideally would involve tissue-specific genetic manipulation. It is important to distinguish tissue-localized exhaustion and PD-L1 expression from the regulation of autoimmune responses in general, which could emanate from initial or chronic activation in lymphoid tissues. Indeed, several reports demonstrate roles for PD-L1 in regulating autoimmune disease (50-52). These studies, which depended on global inactivation, hypothesized a role for PD-L1/PD-1 in peripheral T cell activation. Critically, prior genetic or inhibitor studies neither targeted nor characterized T cells in tissues.

Recent work has suggested that altered metabolism is a driving factor in T cell exhaustion (39, 40). As with TILs, KITs exhibited a markedly altered metabolic profile. Mitochondrial mass, flux capacity, and membrane potential are all suppressed in KITs. Furthermore, KITs demonstrate reduced glucose uptake, which may in part be responsible for suppression of cytokine production, since it is reported that suppression of aerobic glycolysis regulates translation of inflammatory cytokines (41). The kidney itself has a high oxygen and glucose demand (63), and this may play a role in altering the metabolic profile of infiltrating T cells. Such a mechanism would be in keeping with our observation of reduced cytokine protein synthesis despite similar or increased gene transcription in KITs.
Another potential factor that could mediate exhaustion and inactivation of KITs is co-infiltrating CD11b+ cells, which was the second most numerous cell type in lupus kidneys and most numerous cell type in non-lupus kidneys. These myeloid cells are diverse, based on initial flow cytometric analysis (not shown). Nonetheless, since tumor infiltrating myeloid cells are thought to be suppressive in the setting of the tumor microenvironment (64), it will be worthwhile to further explore the potential suppressive properties of kidney-infiltrating myeloid cells in lupus and how this may in turn impact on KIT phenotypes.

The metabolic profile of KITs may have implications for understanding and designing therapy for LN. Inhibition of mitochondrial and glucose signaling was recently shown to suppress disease in murine models of SLE (22). These studies hypothesized a role of metabolic pathways in attenuating peripheral CD4+ T cell activation. Based on the observations presented here, we suggest that, additionally, KITs—which are already metabolically suppressed—may be uniquely susceptible to further metabolic inhibition, which could cause further suppression or even apoptosis.

While to our knowledge exhaustion of T cells within target organs has not been linked to autoimmunity, a recent landmark study correlated the presence of exhausted CD8+ T cells in the peripheral blood with prolonged flare-free survival in lupus and ANCA vasculitis (65). Their presence in blood of some patients who will progress more slowly may reflect a genetic proclivity in these patients for inducing exhaustion, which in turn could result in target organ-resistance to T cell-mediated damage. The origin of these prognostic cells, however, remains unknown. It could be assumed that circulating exhausted CD8+ T cells become exhausted in secondary lymphoid organs. Our data
suggest an alternate scenario that connects exhaustion to lack of target-organ damage
in non-progressors: circulating exhausted CD8\(^+\) T cells could derive from cells that
infiltrated organs, became exhausted at that site, and then recirculate. Regardless of
which explanation applies, considering the two studies together raises the intriguing
possibility that flares may be related to events that break homeostatic tolerance or
exhaustion, rather than de novo activation of new clones.

Our observations could also have implications for disease monitoring and novel therapy
design as well as for therapies already in the clinic. With respect to disease stratification
and tracking, an exhaustion phenotype in kidney biopsies might be predictive of disease
outcomes or subtypes, analogous to blood signatures seen by McKinney et. al (65).
Effective therapy could promote more rapid exhaustion in nephritis, for example via PD-1
agonists. Furthermore, since exhausted T cells differ from both naïve and activated T
cells, it may be possible to design drugs that selectively eliminate such cells, without
unduly suppressing anti-foreign activated T cells.
Methods:

Animals

All mice were housed in specific pathogen free conditions prior to use. MRL.Fas<sup>lpr</sup> (MRL/lpr) (ages 18-24 weeks) Fcgr2b<sup>-/-</sup>. Yaa (age 26-32 weeks) and MRL.Tlr9<sup>-/-</sup> (age 9-10 months) mice bred in our lab (Fcgr2b<sup>-/-</sup>. Yaa mice were a kind gift from Silvia Bolland), Tlr9<sup>-/-</sup> mice (66) of a mixed genetic background (B6 and 129Sv) were bred to MRL/lpr, in our colony and backcrossed for at least 10 generations. MRL/lpr, BALB/c, and B6 mice were purchased from the Jackson Laboratory. Lupus prone mice were evaluated at the range of ages defined above, when they met the additional criteria of being nephritic as determined by a proteinuria level of 3 or 4 using colorimetric dipstick assay (Albustix; Bayer). In the experiments outlined in Supplemental Figures 1, 3, and 4, MRL/lpr mice were used at 11-12 weeks of age and B6 mice were used at 5 and 21 weeks of age, as defined in the figure legend.

Isolation of KITs

After sacrifice, spleens were removed and animals were perfused with 40 ml of HBSS, until complete blanching of liver and kidney occurred. Kidneys were removed and the capsular layer was dissected away. KITs were isolated using the Octodissociator (Miltenyi Biotech) in the presence of 1600 Kunitz Units/ml collagenase D (Roche Diagnostics) and 0.2mg/ml DNAse IV (Sigma-Aldrich) for 30 min at 37°C. Digestion was quenched using metabolism buffer (2.5% HEPES buffer (Gibco), 0.5% bovine serum albumin, 2.5 mM EDTA pH 8.0 in PBS. Homogenates were allowed to settle for 15 minutes and supernatant, which contained the majority of immune cells, was collected. RBC lysis was performed and cells were filtered through a 70µM mesh. Splenocytes were isolated using mechanical dissociation between two frosted glass slides and
filtered through a 70µM mesh filter after RBC lysis. Cells were then used in various assays of T cell function as denoted in each experiment.

**Flow Cytometry**

Single cell suspensions were stained with antibodies against surface molecules in ice-cold PBS with 3% calf serum and 5 mM EDTA in the presence of FcR blocking Ab 2.4G2. Antibodies to the following targets were used: CD4 (GK1.5, Biologend and in-house), CD8 (TIB-105, in-house), CD44 (Pgp-1, in-house), CD62L (Mel-14, Biolegend), TCR (H57-597, Biolegend), CD11b (M1/70, Biolegend), PD-1 (G4, in-house), and Tim3 (215008, R&D), Lag3 (4-10-C9, gift from Vignali laboratory), 2B4(REA-524, Miltenyi), PD-L1 (10F.9G2, Biolegend), CD90.1 (1A14, in house). Ghost BV510 (Tonbo) was used to exclude dead cells. For all "in-house" antibodies, hybridoma clones are commercially available. Antibodies were purified from HB101 (Irvine Scientific) or Hybridoma Serum Free Media (Gibco, Thermo Fisher) supernatants using protein G column (GE healthcare) purification. Purity was monitored by acrylamide gel electrophoresis. Prior to conjugation, antibodies were concentrated and dialyzed into a 0.1M NaHCO3 pH 8.4 using Amicon 4 centrifugal devices 30K MWCO Membrane (Millipore). For Alexa NHS esters (Thermo Fisher) and Pacific Blue (Thermo Fisher), antibodies 10 uL of a 10 mg/mL solution of dye in DMSO was added per mg of antibody at a 2 mg/mL concentration. The dye-antibody mixture was rotated in the dark for 1 hour at room temperature. After an hour, the buffer was exchanged using Amicon 4 filter tubes to 1x PBS w/ 0.05% NaN3 and resuspended in final concentration of 0.5mg/mL in 1x PBS/ NaN3. For biotin conjugations, 75 µl of 1.5 mg/ml Biotin XX, SSE (Invitrogen Catalog # B1606) dissolved in DMSO was added per mg of antibody, which was at a 1 mg/mL concentration. The biotin-antibody mixture was rotated in the dark for 4 hours at room temperature, after which buffer was exchanged similarly to the Alexa
dyes. Appropriate dilutions and specificity were determined by titration on spleen cells. Cells were fixed in 1% paraformaldehyde in PBS. Samples analyzed for transcription factors were fixed/permeabilized with eBioscience FoxP3 staining kit. Antibodies to the following targets were used: Tcf1 (C63D9, cell signaling), and Eomes (Dan11mag, ebioscience). All data were collected on an LSRII or Fortessa Analyzer (BD Bioscience) and analyzed with FlowJo software (Tree Star, Inc.). An example of our generalized gating strategy can be seen in Supplemental Figure 10.

**Intracellular Cytokine Assay**

For intracytoplasmic cytokine staining, single cell suspensions of cells (5 × 10^6/well in 200µl) were stimulated in vitro with 50 ng/ml phorbol myristate acetate (PMA) (EMD) and 1 µg/ml ionomycin (EMD) for 4 h in the presence of 1:1000 Brefeldin A (Biolegend). Cells were then harvested and stained with antibodies to CD8, CD4, TCR and PD-1 before fixation. Intracellular staining was performed using Cytofix/Cytoperm and Perm/Wash buffers (BD). Permeabilized cells were then stained for IL-2 (JES6-5H4, Biolegend), TNF-a (MP6-XT22, eBioscience), and IFN-γ (XMG-1.2, Biolegend), IL-10 (JES5-16E3, BD Pharmingen) and analyzed by flow cytometry as described above.

**Cell Proliferation Assay**

Single cell suspensions made from spleen or kidney were loaded with 10µM Cell Proliferation Dye eFluor450 (eBioscience) according to manufacturer instructions (20’ at RT in PBS). They were then quenched on ice with RPMI-1640 supplemented with 5% FCS (Gemini Benchmark), Penicillin and Streptomycin (Gibco), 10 mM HEPES (Gibco), 2 mM L-glutamine (Gibco), for five minutes. Cells were stimulated in complete media with 1ug/ml of plate-bound anti-CD3e (Tonbo) and 1ug/ml soluble anti-CD28
(Tonbo) in the presence of IL-2 (100IU/ml) for indicated times. They were then collected and processed for flow cytometry analysis as described above.

**Staining for Flow Cytometric Analysis of Metabolic Status**

Single cell suspensions made from spleen or kidney were isolated in metabolism buffer. Samples were then incubated with 60 uM 2-NBDG (Cayman Chemical) in RPMI-1640 supplemented with 2% FCS (Gemini Benchmark), Penicillin and Streptomycin (Gibco), 10 mM HEPES (Gibco), 2 mM L-glutamine (Gibco), for 30 min at 37˚C. Cells were surface-stained and loaded with 20nM MitoTracker FM (ThermoFisher) or 10nM Mitostatus (BD Pharmingen) dyes to measure mitochondrial mass and function, and counterstained with antibodies, as indicated.

**Seahorse Analysis**

Single cell suspensions were made and stained as noted above in metabolism assays. Unfixed cells were sorted using a FACSAria (BD Bioscience) for CD4^{+} CD90.1^{+}, and CD8^{+} CD90.1^{+} populations. Cells were maintained on ice until they were plated on Cell-Tak coated Seahorse culture plates (200,000 T cells/well), and analyzed using a Seahorse XFe96 (Agilent) as described (39).

**RNA-seq analysis**

T cells isolated from kidney and spleen were sorted using a FACSAria (BD Bioscience). 1 x 10^{6} CD4^{+} and CD8^{+} T cells were isolated from matched kidneys and spleens from 3 individual MRL/lpr mice (ages 23 weeks). RNA was isolated using the RNeasy Plus Micro Kit (Qiagen). Samples were sequenced using Illumina NextSeq 500 with 75bp paired-end reads and aligned to the mm10 genome using the STAR aligner (67). The
number of uniquely aligned reads ranged from 10 to 12 million. Gene-level counts were
determined using featureCounts (42) and raw counts were analyzed for differential
expression using the “voom” method (68) in the “limma” R package (43). All gene-set
enrichments were preformed using the “rankSumTestWithCorrelation” function in
“limma”, which explicitly corrects for correlation among genes in the gene set being
interrogated. Specific gene sets used for GSEA correlation were derived as follows:
In Figure 5A, B our gene set was correlated with genes defined in Supplementary Table
1 from ref (44). In Figure 5C and Supplemental Figure 6 our gene set was correlated
with genes defined in Supplementary Table 1 from ref (36). In Supplemental Figure 7
our gene set was correlated with genes defined in Table 1 from ref (37).

Presence of certain kidney specific transcripts indicated unavoidable kidney cell
contamination in the kidney sample; to prevent this from confounding our analysis,
differential expression was performed only on T cell-expressed genes, which were
defined as having at least an average of 20 counts in the splenic sample. The RNA-seq
data generated in this paper has been deposited in the Gene Expression Omnibus
(GEO) under accession numbers: GSM3324012- GSM3324023.

**Immunofluorescence**

Tissue sections were isolated from nephritic (age >18 weeks) MRL/lpr mice. Samples
were fixed in 2% PFA for 4 hours. Each sample was incubated in 30% sucrose
phosphate buffer overnight at 4 degrees, and then frozen in OCT compound (Tissue-
TEk). 7μm sections were cut on a cryostat. Blocking was performed with 3% BSA and
10% rat serum in PBS for 30 mins. Primary antibody staining was performed using anti-
CD4-Al488, anti-CD8-Al647, and anti-PD-L1-PE (10F.9G2, Biolegend) antibodies. To
improve photostability of PD-L1 staining, a secondary stain with anti-PE Al555
(Rockland) was performed. Sections were mounted using ProLong Gold Antifade Mountant with DAPI (Life Technologies). Once the mounting media cured, slides were sealed with nail polish. Imaging was performed on an Olympus IX83. Image display settings and exposure times were kept constant for all samples using CellSens software (Olympus).

**LCMV infection**

LCMV Clone 13 are from Dr. Rafi Ahmed (Emory) and propagated and titered as described previously (69). For chronic LCMVcl13 infections, mice were infected with 2x10^5 PFU intravenously (i.v.). Necropsy was on day ≥30 post infection.

**Statistics**

Statistical analysis was performed using Prism 6.0 (Graphpad). Paired two-tailed Student’s t test or ANOVA analysis were performed where indicated. Each experiment shown is representative of at least two independent experiments unless otherwise indicated. A p-value of <0.05 was considered statistically significant, although lower p-values are indicated in individual figures.

**Study Approval**

Animal work was done in accordance with the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh.

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Author Contributions

J.S.T. and M.J.S. conceived the project and designed experiments. J.S.T. performed most of the experiments and analyzed the data. L.A. performed proliferation assays and helped with flow staining. A.M. performed Seahorse metabolism experiments. G.M.D assisted with design of metabolism and Seahorse experiments and data interpretation. R.A.G. and L.P.K. assisted with data interpretation and experimental design and paper edits. J.S.T. and M.J.S. wrote the manuscript.

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Figure 1. Characterization of T cell populations infiltrating the kidneys of nephritic MRL/lpr mice.

(A) The frequency of CD45+ CD11b+, T cells (CD45+ TCR+), and B cells (intracellular Igκ+) in kidney infiltrates was determined using flow cytometry (n=8 per group). Each dot denotes an individual mouse, horizontal lines represent the mean and error bars show 1 standard deviation.

(B) The frequency of different T cell subsets was determined in the kidneys and spleens of matched MRL/lpr mice with nephritis (n=8 per group).

(C) Representative flow plots of CD44 and CD62L expression on CD4+ and CD8+ T cells from indicated organs is shown with percentages ± standard deviation of each gated subpopulation CD62L+CD44-, CD62L+CD44+, and CD62L-CD44+ (n=4 per group).

(D; left panel) Representative histogram of CD69 expression on CD4+ and CD8+ T cells from indicated organs (blue = spleen, red = kidney, grey = Balb/c). (D; right panel) Summary data from spleens and kidneys of lupus prone mice (n=10 mice per group). For tabulated data each dot denotes an individual mouse and horizontal lines represent the mean. A paired Student's t test was used to determine statistical significance between spleen and kidney samples (* p<0.05, **** p<.0001)
Figure 2. Kidney-infiltrating T cells have suppressed functional capacity. T cells were isolated from the kidney (red) and spleen (blue) of nephritic MRL/lpr and Fcgr2b⁻/⁻.Yaa. mice. (A-D) Cells were stimulated in bulk culture with PMA and ionomycin in the presence of brefeldin A for 4 hours and cytokine expression was assessed by flow cytometry. (A, B) Representative contour plots showing cytokine production by CD4⁺ and CD8⁺ T cells from MRL/lpr (upper panel) and Fcgr2b⁻/⁻.Yaa (lower panel) mice. (C, D) Cytokine production by T cells of MRL/lpr (C) Fcgr2b⁻/⁻.Yaa (D) represented as the percent of positive cells (upper panel) and MFI of producers (lower panel). (E) Proliferation of CD8⁺ T cells in bulk culture after 3 days of anti-CD3/anti-CD28 stimulation (upper panels), and 5 days for CD4⁺ T cells (lower panel) from indicated organs. Cells were labeled with Cell Proliferation Dye prior to culture and its staining is shown on the x-axis. Proliferation index and division index were calculated for CD8⁺ (n=5), and CD4⁺ (n = 7) T cells. For tabulated data (C-E), each dot denotes an individual mouse, horizontal lines represent the mean with error bars representing one standard deviation. A paired Student’s t test was used to determine statistical significance between spleen and kidney samples (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001)
Figure 3. Kidney infiltrating T cells increase expression of inhibitory receptors.

Representative histograms of IR expression on CD8⁺ (A) and CD4⁺ (B) T cells from kidney (red) and spleen (blue) of MRL/lpr mice and B6 (grey), with gating parameters (left column). Right columns show summary data from MRL/lpr (solid, n=5/group), Fcgr2b⁻⁻ Yaa. (open, n=4/group) and B6, non-lupus controls (black, n=4/group). For tabulated data, each dot denotes an individual mouse, horizontal lines represent the mean with error bars representing one standard deviation. One-way ANOVA with Tukey’s multiple comparison was performed with * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. These findings are representative of 3 replicates of MRL/lpr mice and 2 replicates of Fcgr2b⁻⁻ Yaa. mice.
Figure 4. Kidney-infiltrating T cells are metabolically suppressed.

(A) Representative oxygen consumption rate (OCR) trace (left) from sorted CD4+ and CD8+ T cells isolated from kidney (red) and spleen (blue) of MRL/lpr mice. A metabolic stress test was performed by injection of Oligomycin (Oligo), mitochondrial decoupler (FCCP), and glucose uptake inhibitor (2-DG) and Antimycin A/Rotenone (Ant/Rot). Spare respiratory capacity (SRC) was calculated as the difference between basal OCR values and maximal OCR values achieved after FCCP uncoupling. Summary data of SRC Ratio, defined as SRC divided by basal OCR, is shown on the right (n=7 per group). Error bars at each time point in the trace represent means +/- 1 SEM of triplicate wells, with tabulated data in dot plots (right panels).

(B) Mitochondrial membrane potential was assessed by flow cytometry using the MitoStatus dye. Representative histograms (red: kidney and blue: spleen) of MitoStatus from T cell lineages as indicated from both MRL/lpr (upper left panel) and Fcgr2b-/-.Yaa (lower left panel), with tabulated data in dot plots (right panels) (n=5 per group.)

(C) Mitochondrial mass was assessed by flow cytometry using MitoTracker DR. Representative histograms (red: kidney and blue: spleen) of MitoTracker DR from T cell lineages as indicated from MRL/lpr mice with summary data in dot plots (right) (n=5 per group).

(D) Representative contour plots of Balb/c splenic, or MRL/lpr splenic- or kidney-derived CD4+ (upper panel) and CD8+ (lower panel) T cells showing 2-NBDG (glucose uptake) and MitoStatus (mitochondrial membrane potential) staining, with summary data in dot plots (right, Balb/c n=2, MRL/lpr n=5 mice).

For tabulated data (b-d), each dot denotes an individual mouse, horizontal lines represent the mean with error bars representing one SD. A paired Student’s t test was used to determine statistical significance between spleen and kidney samples (* p<0.05, **p<0.01, ***p<0.001, **** p<0.0001)
Figure 5. The transcriptional profile of KITs is consistent with T cell exhaustion. (A,B) RNA-seq data were used to construct gene set enrichment plots illustrating genes differentially regulated in kidney-compared to splenic-derived T cells (n=3 per group) with respect to a known set of 194 CD4+ expressed and 200 CD8+ expressed genes specific for LCMV induced T cell exhaustion (44), in both the CD8+ (A) and CD4+ (B) compartments. P-values calculated using the conservative “rankSumTestWithCorrelation” function in the limma package. (C) Unbiased hierarchical clustering was performed using 1426 genes previously identified from 10 clusters comparing tumor-infiltrating T cells (TILs) to activated and naïve T cell populations (36) and represented as a heatmap. Row annotation on the heatmap shows association of clustered genes with PD-1+ Tim3+ TILs (purple), naïve, effector, and PD-1+ Tim3+ TILs (grey), or those with enhanced gene expression in both subgroups (orange) (36). GSEA was also performed on individual clusters (Supplemental Figure 6). (D) Differential expression between kidney and splenic CD8 T cells for selected inhibitory receptors, T cell products, and metabolic genes as determined by RNA-seq. (E) Representative contour plots for Tcf-1 and Eomes expression in non-exhausted (black), exhausted (PD-1+, Tim3+ T cells from LCMV infected mice) (green), or MRL/lpr kidney (red) or splenic derived (blue) CD8+ T cells. For tabulated data each dot denotes an individual mouse and bars represent the mean with error bars indicating standard deviation. One way ANOVA was used to determine statistical significance using Tukey’s test for multiple comparisons with (** p<0.01, **** p<.0001).
Figure 6. Nephritic kidneys from MRL/Pr mice locally express PD-L1 near sites of T cell infiltration. PD-L1 expression on kidneys of nephritic (>20 weeks) and pre-nephritic (4 weeks) MRL/Pr mice was assessed by immunofluorescence microscopy: CD4+ (green), CD8+ (white), and PD-L1 (red) expression. Merged and single color images are shown for each stain.