Leukocyte–Renal Epithelial Cell Interactions Regulate Lupus Nephritis

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Summary: Renal disease is the major cause of morbidity in patients with lupus. MRL-*Fas*^{lpr} mice share features with human lupus. The tempo, predictability, and homogeneous expression of disease in MRL-*Fas*^{lpr} mice make them an excellent tool to probe the pathogenesis of lupus nephritis and to identify therapeutic targets. This article focuses on the concepts that renal parenchymal cells are active participants that regulate immune responses in the kidney, and that the interaction between parenchymal cells and leukocytes (macrophages, T cells) determine whether the kidney is protected or destroyed during lupus nephritis. In particular we review the role of macrophages, fueled by the principal macrophage developmental molecule, colony stimulating factor-1, in lupus nephritis, and we review T cells and costimulatory pathways and the interaction of these leukocytes with renal parenchymal cells that regulate lupus nephritis.

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Lupus is one of the most mysterious diseases.[1] Although tissue injury can affect nearly every organ, damage to the kidney is largely responsible for morbidity and mortality. When I first began exploring the mechanisms of renal disease the dogma was that the kidney was an innocent bystander, and that circulating antigen–antibody complexes lodge in the renal vascular walls, attract polymorphonuclear leukocytes, and these leukocytes release mediators that destroy the kidney. These concepts have been challenged. We now appreciate the following: (1) the kidney is not an innocent bystander but rather parenchymal cells are active participants that regulate immune responses in the kidney, (2) macrophages initiate renal parenchymal cell destruction (apoptosis), and (3) the interaction between parenchymal cells and leukocytes (macrophages, T cells) can determine whether the kidney is protected or destroyed. This article reviews the central elements that support the concept that the interaction of macrophages, fueled by its principal growth factor, colony stimulating factor-1 (CSF-1), T cells and their costimulatory pathways, and renal parenchymal cells regulate lupus nephritis.

MRL-*Fas*^{lpr} MICE: A MODEL OF LUPUS NEPHRITIS

Fortunately, there are excellent mouse models that mimic aspects of human lupus. In particular, the MRL-*Fas*^{lpr} model provides a valuable tool for researchers to dissect the pathogenesis of disease and to explore therapeutic strategies. In the MRL-*Fas*^{lpr} mice there are spontaneous characteristic features that are pronounced including a plethora of autoantibodies, lymphadenopathy, splenomegaly, and multiple tissues that are damaged including the kidney, skin, lungs, liver, salivary and lacrimal glands, joints, and so forth.[2,3] Similar to human autoimmune diseases, female MRL-*Fas*^{lpr} mice have a more rapidly progressive illness. Although the MRL-*Fas*^{lpr} strain has a mutation in *Fas*^{lpr}, the deletion in Fas is not sufficient to cause lupus, but rather the interaction of the MRL background

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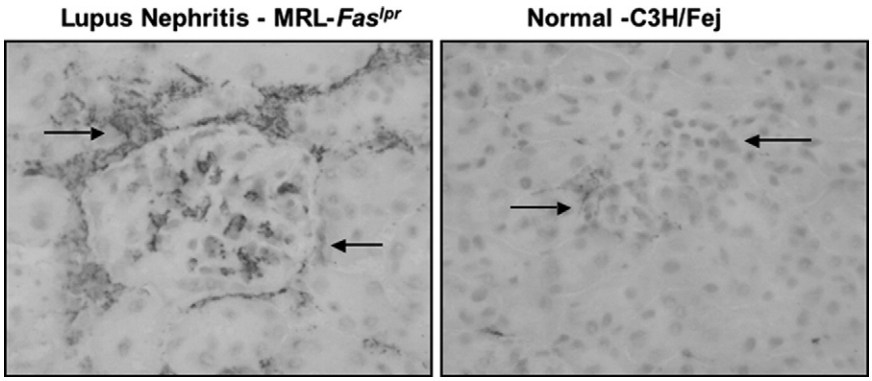

Figure 1. Macrophages are abundant in the MRL-*Fas*^{lpr} kidney with lupus nephritis. Macrophages, identified by the expression of CD68, are located within glomeruli, surrounding glomeruli, within the interstitium, and surrounding vessels (not shown) in MRL-*Fas*^{lpr} mice with lupus nephritis. Intrarenal macrophages are increased dramatically in MRL-*Fas*^{lpr} mice as compared with the C3H/Fej mouse with normal kidneys. Reprinted with permission from Lenda et al.[15] Copyright 2004 The American Association of Immunologist, Inc.

genes and the *Fas*^{lpr} mutation accelerates the tempo of nephritis. These mice are particularly valuable for understanding lupus nephritis because the kidney disease has a rapid onset (age, 3 mo), increases until death (50% mortality at age 5 mo), is relatively homogenous, and is ubiquitous. Thus, disease in this strain is predictable, consistent, and the tempo is slow enough to tease apart the mechanisms, yet sufficiently rapid to be efficient and economic. It would be naive to equate disease in the MRL-*Fas*^{lpr} mice with all of the diverse forms of lupus nephritis, nevertheless, understanding the pathogenesis central to tissue injury in this model provides candidate therapeutic targets for at least some forms of human lupus and other autoimmune and kidney diseases.

MACROPHAGES MEDIATE TUBULAR APOPTOSIS DURING NEPHRITIS

The macrophage is central to the pathogenesis of kidney diseases. These leukocytes are abundant in human and mouse lupus nephritis. Thus, it is reasonable to suspect that macrophages are active participants in renal injury. In fact, we uncovered a central mechanism responsible for macrophage-mediated kidney damage during nephritis. Activated macrophages, either by releasing molecules or via cell-to-cell contact, induce apoptosis in tubular epithelial cells (TECs).[4] Macrophage-mediated apoptosis is not limited to epithelial cells; macrophages initiate apoptosis of renal mesangial cells, however, cell-to-cell contact is required.[5] The molecules responsible for inducing apoptosis in renal parenchymal cell types may differ. For example, although some suggest that tumor necrosis factor-α mediates mesangial cell apoptosis, others suggest that this cytokine does not mediate TEC apoptosis.[6] Regardless of the mediators responsible for inducing apoptosis, it is clear that activated macrophages within the kidney during the initiation phase of the lesion are harmful.

CSF-1, THE PRINCIPAL MACROPHAGE GROWTH FACTOR, IS LINKED TO LUPUS NEPHRITIS

Macrophages are especially abundant in the kidney of MRL-*Fas*^{lpr} mice, rendering this strain particularly valuable for identifying molecules responsible for macrophage recruitment into the kidney, and for determining the mechanisms that arm these cells to destroy renal parenchymal cells (Fig. 1). Thus, it would be reasonable to suggest that CSF-1, the principal macrophage developmental molecule, regulates lupus nephritis. In fact, substantial evidence indicates that CSF-1 is linked to lupus nephritis in MRL-*Fas*^{lpr} mice. CSF-1 is a harbinger of autoimmune-mediated lupus nephritis.[7] CSF-1 is detected in neonatal MRL-*Fas*^{lpr} mouse serum. This increase in circulating CSF-1 is evident well in advance of clinically detectable

kidney disease. CSF-1 increases even further in the serum in proportion to the advancing renal injury. Similarly, CSF-1 has been identified in the MRL-*Fas*^{lpr} kidney in advance of overt pathology using in situ hybridization.[8] Intrarenal CSF-1 expression continues to increase as kidney damage advances. By comparison, serum and intrarenal CSF-1 levels are not detectable in normal mouse strains that are not destined to develop nephritis. In addition, the kidney is a major source of circulating CSF-1 in these MRL-*Fas*^{lpr} mice. Transplanting a single MRL-*Fas*^{lpr} kidney expressing CSF-1 and rich in macrophages into a congenic MRL-++ mouse after bilateral nephrectomy of the normal kidneys results in an increase of CSF-1 in the serum to approximately one half of the amount detected in wild-type MRL-*Fas*^{lpr} mice.[9] Within the kidney, the parenchymal cells, mainly renal TECs, and to a lesser extent the mesangial cells, and cells in the vascular wall generate CSF-1 during nephritis.[8] Recently, we have more specifically localized the major source of intrarenal CSF-1 to the proximal tubules by using β-gal expression driven by the CSF-1 promoter and lectins that identify select tubules during renal inflammation (unilateral urethral ligation) (unpublished data). Notably, the macrophage-richest areas within the kidney are adjacent to the cells generating the largest amount of CSF-1. The link between CSF-1 and macrophages was supported further by showing that macrophages propagated from glomeruli during the early, but not the later, phase of nephritis in MRL-*Fas*^{lpr} mice were dividing in culture, but required CSF-1 to proliferate and survive.[8] These experiments established that at least some of the macrophages within the kidney at the onset of lupus nephritis are undifferentiated and are dependent on CSF-1. Taken together, macrophage-rich lupus nephritis in MRL-*Fas*^{lpr} mice is linked to CSF-1 generated by renal parenchymal cells.

CONTINUOUS INDUCTION BY A CIRCULATING STIMULANT IN MRL-*Fas*^{lpr} MICE IS REQUIRED TO INDUCE CSF-1

What turns on renal parenchymal cells to generate CSF-1 during lupus nephritis? To determine whether a circulating stimulant in the autoimmune milieu of MRL-*Fas*^{lpr} induces renal parenchymal cells to produce CSF-1, a single kidney from an MRL-++ (age, 4 mo) that did not develop nephritis during the first year of life was transplanted into a MRL-*Fas*^{lpr} recipient with nephritis (age, 4 mo) after bilateral nephrectomy. CSF-1 and macrophage-rich nephritis were induced rapidly (2 wks) in the donor kidney.[10] Thus, a circulating stimulant in the autoimmune milieu induces CSF-1. Conversely, transplanting a single MRL-*Fas*^{lpr} kidney into MRL-++ recipients after bilateral nephrectomy (normal kidneys) leads to a rapid (2 wk) disappearance of CSF-1 and macrophage-rich nephritis in the donor kidneys.[9] Taken together, this suggests that CSF-1 is a proximal stimulus in triggering cytopathic kidney autoimmune injury, and intrarenal expression of CSF-1 requires continual stimulation from a circulating factor(s).

What is the circulating factor in the autoimmune milieu that is responsible for inducing CSF-1 in the renal parenchymal cells? This is a difficult question to answer. We noted that CSF-1 and macrophage-rich nephritis are eliminated in T-cell deficient (CD4, and class I selected CD8), and the unique double negative (DN) T cells (T-cell receptor [TCR]α/β^+, CD4$^-$, CD8$^-$, B220 epitope of CD45$^+$) characteristic of MRL-*Fas*^{lpr} mice, and interferon-γ receptor–deficient MRL-*Fas*^{lpr} mice.[11,12] However, this indicates that these cells/cytokines are necessary, but they may not be sufficient, to stimulate the expression of CSF-1 by renal parenchymal cells. Thus, it is difficult to pinpoint the exact molecules in the circulation that induce CSF-1 in the MRL-*Fas*^{lpr} kidneys. Nevertheless, these studies do indicate that factors in the circulation initiate a dynamic interaction between macrophages, T cells, and renal parenchymal cells that culminate in tissue injury during lupus nephritis.

CSF-1 REGULATES LUPUS NEPHRITIS

To test the concept that overexpression of CSF-1 in the adult kidney recruits macrophages and incites lupus nephritis, a gene transfer strategy was constructed to deliver CSF-1 locally into the mature kidney. TECs were modified

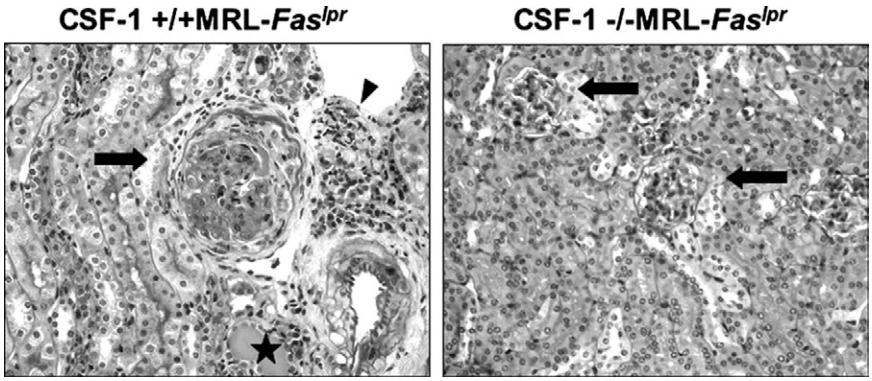

Figure 2. CSF-1 null MRL-*Fas*^{lpr} are protected from lupus nephritis. CSF-1 +/+ MRL-*Fas*^{lpr} mice have glomulonephritis (arrow), casts within tubules (star), and a profound perivascular leukocytic (arrowhead) infiltrate rich in macrophages and T cells (age, 6 mo). By comparison, age- and sex-matched CSF-1 null (−/−) MRL-*Fas*^{lpr} mice are protected from renal disease.

genetically by using a recombinant retrovirus vector to express CSF-1.[13] These TECs are efficiently converted into carrier cells, which secrete stable, sustained levels of CSF-1. Implanting these carrier cells under the renal capsule persistently delivers CSF-1 into the kidney and circulation. By using this gene transfer strategy, intrarenal CSF-1 elicited an influx of macrophages, followed by T cells, resulting in interstitial nephritis in strains with *Fas*^{lpr}, but not in normal strains. Because *Fas* is instrumental in deleting autoreactive T cells, we suspect that CSF-1 draws macrophages into the kidney, but requires autoreactive T cells to incite local inflammation.

Because intrarenal expression of CSF-1 incites lupus nephritis, this suggests that eliminating CSF-1 from the MRL-*Fas*^{lpr} mice will suppress disease. In fact, CSF-1 null MRL-*Fas*^{lpr} mice are protected from nephritis and injury to other tissues as compared with the wild-type strain (Fig. 2).[14] Kidney protection in CSF-1 null MRL-*Fas*^{lpr} mice is attributed, at least in part, to a decrease in intrarenal macrophages and T cells and a reduction in activated macrophages in the kidney. We have determined that macrophage accumulation and activation in the kidney is dependent on CSF-1, and that CSF-1 mediates macrophage-induced TEC apoptosis.[15] On the other hand, the decrease in nephritis may be related to a reduction in autoantibodies. Autoantibodies and immunoglobulin (Ig) isotypes in the circulation, and IgG and complement within glomeruli are reduced in CSF-1 null MRL-*Fas*^{lpr} mice. This reduction of autoantibodies and Igs may be related to an enhanced B-cell apoptosis in the bone marrow and spleens of these CSF-1 null MRL-*Fas*^{lpr} mice.[15] However, our laboratory[4,16] and others[17] have uncoupled circulating and glomerular deposits of autoantibodies, and the development of lupus nephritis. Thus, it is unclear whether the reduction in autoantibodies in the CSF-1 null MRL-*Fas*^{lpr} suppresses lupus nephritis. Taken together, because CSF-1 regulates several leukocytic cell types (macrophages, B cells, and T cells), CSF-1 may be instrumental in multiple mechanisms that result in lupus nephritis.

DISTINCT MACROPHAGES LINKED TO THE MRL BACKGROUND INCREASE NEPHRITIS

Although CSF-1 attracts macrophages into the kidney, the fate of these cells is regulated by the CSF-1 receptor, termed *c-fms*. Macrophages within the MRL background accumulate more rapidly in the kidney than other normal strains. This is in part related to a heightened proliferative response to CSF-1.[18] We have implanted bone-marrow macrophages along with a source of CSF-1, TECs genetically modified to constitutively express CSF-1, under the kidney capsule in mice with the MRL and the C3H background that do not develop lupus nephritis. The expansion of intrarenal macrophages is increased dramatically in these MRL-*Fas*^{lpr} mice as compared with the C3H-*Fas*^{lpr} strain.[18] The factors responsible for this enhanced proliferative response in

the MRL-*Fas*^{lpr} strain are not entirely clear. It is possible that a defect in the downregulation of *c-fms* in the macrophages of the MRL strains leads to the increase in intrarenal macrophage proliferation in MRL-*Fas*^{lpr} mice.[18] Because the failure to downregulate the CSF-1 receptor may be linked to a modifying gene in the MRL background it is intriguing to speculate that identification of these genes in mice may provide clues to identifying the counterpart in human lupus patients. Taken together, determining the expression and regulation of receptors for molecules that are integral in the pathogenesis of lupus nephritis, such as CSF-1, may be prognostic indicators of the disease tempo.

Macrophages in the MRL-*Fas*^{lpr} strain have other abnormal functions. These include a decrease in cytokine production,[19-21] increased antibody-dependent cellular cytotoxicity and hydrogen peroxide production,[22] and decreased Fc-mediated binding and impaired phagocytosis.[23] In many of these studies it is not clear whether the impaired macrophage function is a consequence of the disease, or whether it is linked genetically to either the *Fas*^{lpr} mutation or MRL background. As in the mouse, defective macrophage functions are a feature of human systemic lupus erythematosus.[24] It is critical to determine whether these defects are identifiable in healthy individuals before the loss of renal function, or are a consequence of the disease. Nevertheless, a systematic exploration of the regulation of intrarenal macrophage functions and their impact on kidney disease undoubtedly will lead to the design of novel therapeutics for lupus nephritis.

CSF-1 IN HUMAN LUPUS NEPHRITIS

Macrophage growth factors are increased in glomeruli of patients with lupus. Upregulation of CSF-1 and another macrophage growth factor (granulocyte-macrophage growth factor), have been identified in glomeruli in patients with lupus nephritis.[25] However, a more comprehensive evaluation of human renal injury is required to determine the importance of macrophage growth factors in the expansion of macrophages within the kidney and the extent of kidney disease. Nevertheless, we would predict strategies that prevent the intrarenal expression of CSF-1, and perhaps granulocyte-macrophage CSF, will protect the kidney from injury in individuals genetically susceptible to autoimmune illnesses.

CSF-1: A THERAPEUTIC TARGET FOR LUPUS NEPHRITIS?

It is clear that CSF-1 is required for lupus nephritis in the MRL-*Fas*^{lpr} mouse. However, CSF-1 null mice have numerous problems including osteopetrosis, male and female infertility, deafness, blindness, and emphysema.[26-28] The issue remains, will blocking CSF-1 in adult human kidney with lupus nephritis halt the progression of disease without compromising the individual? To help address this concern we are currently determining the role of the 3 distinct isoforms of CSF-1, a membrane-spanning cell surface CSF-1, a secreted glycoprotein CSF-1, and a secreted proteoglycan CSF-1 during lupus nephritis. In addition, it will be important to eliminate CSF- 1, CSF-1 receptor, and individual CSF-1 isoforms (antibody, fusion protein, conditional knockout, and so forth) during stages of lupus nephritis to determine whether targeting this interaction has a beneficial impact in lupus nephritis without the deleterious consequences.

T-CELL EFFECTORS IN LUPUS NEPHRITIS

T cells regulate a wide range of diverse functions that are pivotal in tissue destruction and protection. T cells are notable within the kidneys in human and mouse forms of lupus. Therefore, it is important to elucidate the pathways that regulate T-cell activation, the T-cell interaction with macrophages and renal parenchymal cells, and its importance in lupus nephritis. The T cells in *Fas*-deficient *Fas*^{lpr} strains that escape apoptosis are distinctive. The massive enlargement of lymph nodes and spleens in this strain is a result of infiltration by unique DN T cells, the origin, proliferation, and migration of which remain controversial.[29-34] The abundant expression of 2 activation determinants, B220 and FasL, and the spontaneous cytotoxic activity suggests that DN T cells were activated previously.[35] Notably, DN T-cell clones propagated from the kidney of MRL-*Fas*^{lpr} mice ex-

clusively proliferate to renal parenchymal TECs and mesangial cells, and not to other tissues.[36] In addition, infiltrating DN cells are evident in the kidney, lymph nodes, and spleens simultaneously. Based on these findings, we suggest that DN T cells expand in the kidney. DN T cells are class I major histocompatibility complex selected, and are reduced in the MRL-*Fas*^{lpr} strain deficient in β-2-microglobulin (ie, class I).[37] Class I–deficient MRL-*Fas*^{lpr} mice are spared from an intrarenal infiltration of DN and CD8 T cells and have drastically reduced CD4 T cells. These mice are protected form renal injury.[11,38] Thus, class I–selected T cells are necessary for autoimmune kidney injury in the MRL-*Fas*^{lpr} strain.

The CD4 T cells in the MRL-*Fas*^{lpr} strain are distinctive.[39-41] CD4+ and DN T cells account for the majority of T cells in nephritic MRL-*Fas*^{lpr} kidneys. Genetic deletion of CD4 T cells in MRL-*Fas*^{lpr} mice protects the kidney from glomerular, tubular, and vascular pathology, an outcome similar to that achieved by deleting T-cell receptor α/β cells. Thus, autoimmune kidney disease in this strain requires both multiple T-cell populations.[11]

REGULATING T CELLS: COSTIMULATORY PATHWAYS

Immune cell interactions and T-cell costimulatory pathways are instrumental in autoimmune disease induction and provide key targets for therapeutic intervention. Antigen recognition alone is not sufficient for full T-cell activation. T cells require 2 distinct signals to become fully activated.[42,43] The first signal is provided by the engagement of the TCR with the major histocompatibility complex and peptide complex on antigen-presenting cells (APCs). The second costimulatory signal is provided by engagement of one or more TCRs with their specific ligands on APCs.[44-46] Signaling through the TCR alone in the absence of a costimulatory signal leads to prolonged T-cell unresponsiveness, anergy.[43]

The engagement of CD28 expressed on T cells with APCs bearing B-7 is a potent costimulatory pathway.[47] The CD28/CTLA4-B7-1/B7-2 T-cell costimulatory pathway is a unique and complex pathway that regulates T-cell activation.[48] Interaction of CD28, constitutively expressed on T cells, with B7-1 and B7-2, expressed on APCs, provides a second positive signal that results in T-cell activation. During T-cell activation cytokines are generated, the clones expand, and T-cell anergy is prevented. Furthermore, T-cell survival, clonal expansion, and differentiation are defective in CD28 null mice.[49] Thus, the CD28-B7 interaction is critical for T-cell survival.

The CD28 family molecules are promising therapeutic targets for lupus nephritis. Eliminating the B7 pathway (genetic deletion of B7-1 and B7-2) in MRL-*Fas*^{lpr} mice protects this strain from renal and other tissue injury.[50] Furthermore, prophylactic treatment of MRL-*Fas*^{lpr} mice with CTLA4Ig (a fusion protein that binds to B7 and blocks this pathway) diminishes kidney disease, but not lung disease, in this strain.[51] This emphasizes the differential impact of the CD28/CTLA4 (CD152) pathway on target tissues in the MRL-*Fas*^{lpr} strain. Similarly, CD28 null MRL-*Fas*^{lpr} mice develop less severe glomerulonephritis, but greater splenomegaly than wild-type mice.[52] Treating mild renal disease in another form of lupus nephritis, the New Zealand Black (NZB) × New Zealand White (NZW) F1 hybrid females (BW), delays the onset of proteinuria. However, delaying treatment until the BW mice have advanced nephritis requires CTLA4Ig in combination with cyclophosphamide (double therapy) to retard the onset of proteinuria.[53] Nevertheless, BW mice survive longer whether treatment with CTLA4Ig is initiated in mice with mild or advanced nephritis as compared with controls. Moreover, short-term administration of this double therapy, or triple therapy (CTLA-4Ig, cyclophosphamide, and anti-CD154 antibody) in BW mice with established nephritis leads to a disappearance of proteinuria in the majority of mice and prolonged survival.[54] These experimental studies have prompted clinical trials using CTLA4Ig in patients with lupus nephritis.

THE PROGRAMMED CELL DEATH PATHWAY: A NEGATIVE REGULATOR OF IMMUNE RESPONSES

A new pathway in the CD28 superfamily is the programmed cell death (PD-1) pathway.[55] PD-1, similar to CD28 and CTLA4, is a transmembrane

protein and belongs to the Ig superfamily. PD-1 has an immunoreceptor tyrosine-based inhibitory motifs (ITIM) within its cytoplasmic tail. PD-1 is expressed on activated T, B, and myeloid cells including macrophages.[56,57] PD-1 binds 2 known ligands, PD-L1 and PD-L2. PD-L1 is expressed broadly. PD-L1 is expressed constitutively on bone marrow–derived cells including T cells, B cells, macrophages, and dendritic cells, and is up-regulated further after activation of these cells.[56,58] In addition, PD-L1 is expressed on parenchymal cells, including vascular endothelial cells, β cells in the pancreas, glial cells in the brain, cells in the lung,[59-61] and epithelial cells in kidney.[62] In contrast, PD-L2 expression is limited to activated macrophages and dendritic cells.[56,58] The expression patterns of PD-1 and PD-Ls provide opportunity for bidirectional signaling.[63] The concept that the PD-1 pathway is a negative regulatory checkpoint in immune reactions initially was based on PD-1 null mice spontaneously developing autoimmune diseases. These autoimmune diseases were dictated by the strain's background genes and include splenomegaly, B-cell expansion with increased serum immunoglobulins, arthritis, and cardiomyopathy.[64] Importantly, PD-1 null C57Bl/6 (B6) mice resulted in a low incidence of mild glomerulonephritis, while the incidence and severity increased in the B6-*Fas*^{lpr} strain. However, the interstitial and adjacent tubular pathology, the area richest in invading macrophage and T cells in renal inflammation, was not explored.[64] Because macrophage and T cells do not infiltrate the kidney in the B6-*Fas*^{lpr} strain, it is plausible that deletion of PD-1 expression renders the kidney susceptible to invading macrophage and T cells because the PD-1–dependent protective barrier is lost. This may be related to T-cell interactions with APCs (macrophage, dendritic cells, and so forth) outside or within the kidney.

PD-L1 EXPRESSING PARENCHYMAL CELLS: A BARRIER TO INVADING T CELLS

PD-L1 expression on parenchymal cells in peripheral tissues fueled the concept that the PD-1 pathway is central to preserving tolerance within target tissues. Most recent studies in nonobese diabetic[60,65] and autoimmune encephalomyelitis[66] indicate that the PD-1 pathway is a negative checkpoint that suppresses tissue injury. Within this framework, increasing evidence suggests that expression of PD-L1 on parenchymal cells shields parenchymal cells from invading leukocytes. For example, in nonobese diabetic mice, blocking PD-L1 on pancreatic islet β cells or eliminating PD-1 results in enhanced T-cell invasion of islets, increased islet destruction, and more severe diabetes.[60,65] In addition, fetal-maternal tolerance expression of PD-L1 on the decidual cells may provide a barrier to protect against invading PD-1–bearing T cells in mice.[67] Thus, PD-L1–expressing parenchymal cells, at least in the pancreas and placenta, appear to serve as a checkpoint to protect tissues from immunoinflammatory processes.

RENAL PARENCHYMAL CELLS: POSITIONED TO REGULATE IMMUNE REACTIONS

Kidneys in the vast majority of people remain normal. To protect the kidney after random T-cell contact with parenchymal cells, T cells must be more readily inactivated than activated. Bone marrow–derived macrophages, dendritic cells, and B cells are professional APCs. Because TECs, nonprofessional APCs, comprise 80% of the renal cortex, TECs are well positioned to turn on or turn off T-cell activation. Are T-cell activation and anergy regulated by TEC surface determinants? More than a decade ago, we determined that TECs in MRL-*Fas*^{lpr} mice expressed the major histocompatibility complex determinant, Ia. This prompted studies determining that Ia^{+} TECs, process and present antigen to T cells.[68,69] We determined that parenchymal cells and professional APCs show unique functional attributes. TEC are poised to turn off T-cell activation. TECs do not express B7 molecules, the potent costimulatory signals that promote T-cell activation. Moreover, PD-L1 expression on a TEC line after stimulation with interferon-γ inhibits T-cell activation.[62] In addition, TECs stimulated by interferon-γ down-regulate the proliferation of autoreactive T-cell clones derived from MRL-*Fas*^{lpr} kidneys.[70] This suggests that TECs deliver signals that limit the expansion of autoreactive T cells in lupus ne-

phritis. Moreover, intrarenal PD-1, PD-L1, and PD-L2 are upregulated in the MRL-*Fas*^{lpr} mice during lupus nephritis, and PD-L1 is expressed on TECs (unpublished data). Thus, PD-L1 on TECs may pose a negative regulatory checkpoint for lupus nephritis. If this is the case, it is clear that this barrier alone is not sufficient to protect the kidney. It is conceivable that an imbalance in activating and anergic signals delivered to T cells results in lupus nephritis. The challenge remains to identify the negative regulatory checkpoints and signals that drive tissue damage so that we tip the balance toward protection and spare the kidney.

CONCLUSIONS

Taken together, dissecting the cellular and molecular interactions between macrophages, T cells, and renal parenchymal cells in experimental systems that mimic lupus nephritis should provide candidate therapeutic targets for the human illness.

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