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Toll-like receptor signaling in transplantation

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Abstract

Purpose of the review—This review summarizes recent advances on the role of endogenous and exogenous Toll-like receptor (TLR) ligands in the activation and inhibition of immune responses in transplantation.

Recent findings—During an alloresponse, TLRs can be engaged by both damage-induced endogenous ligands or microbial-associated molecular patterns. The damage-induced molecule high mobility group box 1 protein (HGMB1) and its binding to TLR4 have been identified as major initiators of anti-tumor and anti-transplant immune responses. Type I interferon (IFN) signaling plays an important role in the pro-rejection effect mediated by TLR agonists and some bacteria. However, similar pathways in neonates can result in inhibition rather than activation of alloimmune responses.

Summary—The consequences of TLR engagement by endogenous and exogenous ligands in transplantation may depend on the relative induction of inflammatory and regulatory pathways and the stage of development of the immune system.

Keywords

TLR; microorganisms; acute rejection; tolerance

Introduction

TLR-mediated signals play a role in acute allograft rejection and antagonize the induction of transplantation tolerance. However, TLR engagement can also promote tolerance in several experimental models. Recent developments in the biology of TLR signaling in transplantation include a better understanding of endogenous and exogenous ligands that can generate TLR signals in allograft recipients, as well as increased attention to possible negative regulatory loops induced by TLR ligation.

1. TLR signals promote acute allograft rejection

TLRs are pattern-recognition receptors expressed on both hematopoietic and non-hematopoietic cells such as endothelial and epithelial cells. At least 13 TLRs have been identified to date. All of them require adaptor MyD88 for signal transduction except for TLR3, which utilizes the adaptor Trif, and TLR4, which can signal via both MyD88 and Trif pathways. Using mice deficient in MyD88 or MyD88 and Trif, it has been shown that TLR signals by donor and recipient cells are absolutely necessary for the rejection of allografts expressing

minor histocompatibility antigen mismatches [1,2], while they promote, but are not required for, acute rejection of allografts with major mismatches [3]. Furthermore, genetic ablation of MyD88 facilitates the induction of transplantation tolerance by costimulation-targeting therapies [4,5] and, conversely, administration of synthetic TLR agonists prevents transplantation tolerance by the same regimens [5,6]. The current model states that antigen-presenting cells stimulated by TLRs at the time of transplantation will incur maturation and enhanced presentation of alloantigen, resulting in augmented responses of alloreactive T cells that may then become resistant to tolerance induction. However, the ligands that trigger these TLR signals after transplantation remain to be ascertained. It is clear that molecular patterns expressed by microorganisms can engage TLRs and we have proposed that translocation of commensal bacteria (or bacterial products) during the surgical procedure, or concurrent infections in donors or recipients, may result in TLR signaling and its consequences on alloresponses [5]. However, several reports have also argued for the existence of endogenous non-microbial ligands generated as a consequence of cellular damage. This concept had initially been met with skepticism given the difficulty to definitively exclude the contribution of potential microbial contaminants [7]. However, recent results lend credence to a role of damage-associated molecular patterns (DAMPs) *in vivo* and a role for selected endogenous ligands of TLRs in transplant responses is emerging.

2. Non-microbial endogenous ligands of TLRs

Several endogenous ligands have been described that are released during inflammation, cellular stress and damage and can engage TLRs. These include heat shock proteins (HSPs), extracellular matrix-derived products, HMGB1, β -defensin, surfactant protein A, and minimally modified LDL [8]. To date, only HMGB1, HSPs and hyaluronan have been linked to transplant responses and will be discussed herein.

2.1 HMGB1

HMGB1 was described as a nuclear protein with fast migration properties during electrophoresis and was named accordingly [9]. Its sequence has been highly conserved during evolution and HMGB1 proteins from all mammals are virtually identical. Its expression is essential as mice deficient in HMGB1 die shortly after birth from hypoglycemia [10]. HMGB1 mostly resides in the nucleus where it is known to bind without sequence specificity to minor DNA grooves, resulting in DNA bending and promotion of protein assembly [11]. HMGB1 can both be passively released by necrotic cells [12], or be actively secreted by upon cell exposure to tumor necrosis factor (TNF), interleukin-1 β (IL-1 β), lipopolysaccharide (LPS) or other TLR agonists [13] [14,15]. Thus, it can be present in soluble form during both sterile and non-sterile inflammation.

Soluble HMGB1 has been shown to bind the receptor for advanced glycation end products (RAGE), expressed at low levels on many cells types [11], but also to selected TLRs, namely TLR 2 and TLR4. Ligation of HMGB1 to TLR2 was initially controversial as macrophage cell lines transfected with dominant negative constructs of these TLRs showed reduced HMGB1 signaling [16], but not macrophages from TLR2-deficient mice [17]. These discrepancies may be due to redundancy between RAGE and TLR signaling in response to HMGB1. More recently, HMGB1 has also been shown to associate with TLR9 [14,18]. HMGB1 interacted with CpG-A oligonucleotides resulting in enhanced activation of plasmacytoid dendritic cells (pDCs), B cells and macrophages in a RAGE- and TLR9-dependent manner [14,18]. Thus the association of HMGB1 with TLR9, though indirect, may facilitate TLR9 signaling by DNA-containing immune complexes and perhaps contribute to autoimmunity.

In support of an important role for the HMGB1/TLR pathway in immune responses *in vivo*, recent evidence shows that the anti-tumor immune response elicited by radiation therapy or

chemotherapy is due to the release by dying tumor cells of HMGB1, which acts on TLR4/MyD88 on dendritic cells. This anti-tumor effect is abolished in either anti-HMGB1-treated mice or mice deficient in TLR4 but not in other TLRs [19]. These results strongly support an important role for endogenous HMGB1 via TLR4 engagement in the absence of bacterial contamination. At least part of the inflammatory effect of HMGB1 may be due to its ability to induce IL-1 production by monocytes in a CD14 and TLR4-dependent manner, resulting in production of IFN- γ and IL-17 by T cells [20]. Furthermore, HMGB1 signaling via TLRs activates the transcription factor NF- κ B, which itself induces further upregulation of HMGB1 and its receptors thereby amplifying inflammation in a positive feedback loop [15].

The role of HMGB1 in transplantation may be many fold. Ischemia/reperfusion injury (IRI) induces tissue damage and liver IRI has been shown to result in rapid release of HMGB1 [21]. Its production by ischemic hepatocytes was demonstrated to be TLR4-dependent and due to induction of reactive oxygen species and subsequent calcium/calmodulin-dependent kinases signaling [22]. Extracellular HMGB1 in turn serves as a chemoattractant for inflammatory cells and leads to hepatic tissue injury, as damage can be reduced using blocking antibodies to HMGB1. These harmful effects of HMGB1 depend on TLR4 expression on hematopoietic cells [23,24].

Following IRI, damage of a transplanted organ can occur as a consequence of alloresponses or autoreactivity. It was recently shown that cellular targets of cytotoxic T cells can release HMGB1 [25]. In addition, activated immune cells, as found infiltrating transplanted organs undergoing acute rejection, can actively secrete HMGB1. Blockade of HMGB1 using a recombinant A-box that acts as a selective antagonist by reducing activity of full-length HMGB1, resulted in prolonged survival of fully mismatched cardiac allografts in mice, that correlated with reduced intra-graft expression of TNF, IFN- γ and impaired Th1 immune responses [26]. Similar results in another murine cardiac allograft model were obtained upon blocking HMGB1 with soluble RAGE, which may prevent engagement of HMGB1 to all its ligands [27]. Whether these pro-rejection effects of HMGB1 are mediated via TLRs 2, 4 or 9, RAGE or a combination of these receptors remains to be established. Interestingly, endogenous production of anti-HMGB1 antibodies was identified during a tolerance phase after liver transplantation in rats, as well as in a patient following withdrawal of immunosuppressive therapy [28]. Although this study did not demonstrate that these endogenous antibodies could block HMGB1 ligation to its receptors, it further suggests that blockade of HMGB1 (either therapeutic or spontaneous) may facilitate graft acceptance.

2.2 Heat shock proteins

HSPs are important chaperones for protein folding and cytoprotection and are mostly found in the cytoplasm of cells, although some are also encountered in mitochondria or nuclei [29]. HSPs are classified into families according to their approximate molecular weight. Although they are expressed at low levels in all cells, their expression is markedly induced under conditions of cellular damage that can also result in their extracellular release by a mechanism that is not yet fully understood [30]. HSPs have been reported to use several surface receptors, including CD14, CD40, CCR5 and TLRs [29]. Both HSP60 and HSP70 are thought to signal via TLR2 and TLR4 [30].

The role of endogenous HSPs in allograft rejection remains to be firmly established. Expression of HSPs, including HSP60 and HSP70, has been detected in transplanted organs [31] and transgenic expression of HSP60 on donor skin has been shown to accelerate allograft rejection [32]. However, the combined deficiency in HSP70.1 and 70.3, the two forms of HSP70 induced by stress in the mouse, in both donor and recipient animals did not affect allograft rejection, dendritic cell maturation or Th1 alloresponses in a minor mismatched murine skin graft model whose rejection is known to depend on MyD88-mediated signals [33]. This result is in contrast

to earlier work in which single deletion of HSP70.1 in only donor skin resulted in a statistically significant prolongation of allograft survival in a fully mismatched mouse model, albeit only by a couple of days [34]. It is possible that various HSPs have redundant roles and would all need to be targeted to observe more biologically significant impact on allograft survival.

Hyaluronan—Hyaluronan is a component of the extracellular matrix that accumulates during tissue injury. Clearance of hyaluronan products is mediated by the main hyaluronan receptor CD44. However, hyaluronan fragments can also bind TLR2 and TLR4 resulting in activation of macrophages [35]. Levels of hyaluronan have been shown to be elevated during acute rejection in rat serum and cardiac allografts [36] and in mouse skin allografts [37] and to be associated with bronchiolitis obliterans syndrome in human lung allograft recipients [37]. Treatment of dendritic cells with hyaluronan fragments induced their maturation and enhanced their capacity to initiate alloimmunity *in vitro* in a manner dependent on signaling via TIR-associated protein (TIRAP), an adaptor for TLR2 and TLR4, but independent from MyD88 and Trif [37]. Thus, components of the extracellular matrix can act as immune adjuvants and potentiate alloreactivity.

2. Microbial ligands of TLRs

Transplantation, like other major surgical procedures can result in translocation of commensal bacteria from skin incisions and intestinal stress. In particular, bacterial and endotoxin translocation have been identified following clinical liver and intestinal transplantation [38, 39]. Because transplanted patients are heavily immunosuppressed to prevent allograft rejection, they are more susceptible to pathogenic viral, bacterial and fungal infections. It is conceivable that exposure to both commensal and pathogenic microorganisms promotes allograft responses in transplanted patients.

2.1 Common pathogens in transplanted patients

The incidence and clinical characteristics of infections after solid organ transplantation was described in a retrospective study of 2702 solid organ transplant recipients from the database of the Spanish Network of Infection in Transplantation (RESITRA) [40]. The incidence of infections in the first month after transplantation was observed to be significantly higher (3.5 episodes per 1000 transplant-days) compared to that in the late period (0.4 episodes per 1000 transplantation days), with higher rates of infections following kidney-pancreas, lung and liver allografts (11.52–14.47 episodes per 1000 transplantation days) and lower rates for kidney and heart allografts (4.91–8.78 episodes per 1000 transplantation days). In the late period of >6 months post-transplantation, infection rates were highest for lung allografts (1.4 episodes per 1000 transplantation days), intermediate (0.76 episodes per 1000 transplantation days) for liver, and low for kidney and heart allografts (0.28–0.34 episodes per 1000 transplantation days). Bacterial infections were the most common, and represented 80% of infections in the first month post-transplantation and 50% of all infections after six months post-transplantation. Viral infections, especially CMV, were the second most frequent, contributing 13% and 46% of all infections in the first and >6 months post-transplantation. The rate of fungal infections was 2.5–5% for the study duration, which had median follow-up of 13 months.

The incidence of blood stream infections after solid organ and hematopoietic stem cell transplantation was examined from the same RISTRA database [41]. The incidence of blood stream infections was 7.3–10% for kidney, heart, lung and liver recipients and 20% for pancreas recipients, and significantly higher for hematopoietic stem cell transplant recipients (42.3% for allogeneic recipients and 26% for autologous recipients). Blood stream infections were largely the result of Gram-negative and Gram-positive bacterial infections, with coagulase-negative staphylococci (37%) being the most frequent, followed by *E. coli* (17%).

Fungal, bacterial and viral infections can profoundly impact the morbidity and mortality of transplant patients [42–44]. In addition, there is evidence that viral infections, especially CMV, contribute to the development of acute and chronic rejection [45]. The impact of bacterial infections on graft rejection is less appreciated. Nonetheless, there is a significant literature correlating bacterial infections and graft rejection (reviewed in [46]). The evidence for fungal infections precipitating rejection is least well documented. Because of adjustments to immunosuppression made in response to infections, the relationships between immunosuppression, infections and allograft rejection are difficult to definitively appreciate solely from clinical observations.

2.2 Pathogen recognition by TLRs

Pathogens, including bacteria, viruses and fungi, can be recognized by host innate immune cells through an array of pattern recognition receptors (PRRs), of which, the best characterized are the TLRs. TLRs that are located on the cell surface recognize cell wall components of bacteria and fungi or envelop proteins of viruses, while endosomally located TLRs recognize viral dsRNA or ssRNA, or unmethylated CpG motifs in DNA. Table I lists potential ligands of pathogens commonly found in clinical transplantation and the TLRs they can engage. Non-TLR PRRs include retinoic-acid-inducible gene I-like helicases (RLH), nucleotide-oligomerization domain leucine-rich repeat (NOD-LRR), C-type lectin and cytosolic DNA-dependent activator of IFN-regulatory factors (DAI) proteins (reviewed in [47–50]). All PRRs can trigger distinct intracellular signals that induce the production of cytokines and inflammatory mediators, providing the cues for the development of adaptive immune responses that, in most instances, control the infection.

The relative frequency of bacterial, viral and fungal infections post-transplantation, the ability of the innate immune system to sense these infections, and the cross-talk between innate and adaptive immunity predicts an impact of infections on alloreactivity and graft rejections. We and others had previously reported that TLR-ligation, with CpG (TLR9), LPS (TLR4), Pam₃CysK₄ (TLR1/2) and polyinosinic:polycytidylic acid (poly I:C, TLR3), at the time of allograft transplantation, prevents the ability of anti-CD154-based therapy to induce long-term graft survival [5,6]. More recently, Garantziotis and colleagues reported that inhaled LPS promoted lymphocytic bronchiolitis, epithelial injury and obliterative bronchiolar lesions in allogeneic bone marrow transplant recipients [51]. The inflammation induced by the inhaled LPS was shown to be dependent on TLR4-expression on donor-derived hematopoietic cells and not on recipient structural lung tissue, and resulted in sustained elevations of the inflammatory chemokine, CCL5, but not the cytokine, TNF. In a model of established bone marrow mixed chimeras, Chakraverty and colleagues reported that the transfer of donor T cells does not trigger GVHD. In contrast, the induction of inflammation by administration a TLR7 agonist, imiquimod to the skin, at the time of T cell transfer, triggers massive infiltration of T cells to the skin and localized GVHD [52]. These observations demonstrate that tissue inflammation, triggered by TLRs, can induce the trafficking and activation of alloreactive T cells, and allude to the existence of a tissue-localized checkpoint for GVHD. More recently, Durakovic and colleagues reported that the co-administration of CpG or imiquimod, intraperitoneally augmented the effector function of adoptively transferred donor lymphocyte infusions into stable mixed bone marrow chimeras and promoted graft-versus-host and graft-versus-leukemia activities [53]. These experimental data complement previous clinical reports of possible associations between TLR4 mutations and the risk for acute GVHD, although potentially increased risk for bacterial infections may complicate the interpretation of data in the clinical studies [54,55].

2.3 Effector pathways: Type I IFNs

Both viral and bacterial infections can elicit the production of type I IFNs, which is controlled by two major classes of PPRs: endosomal or plasma membrane-associated TLRs and cytosolic receptors, such as RLHs and DAI [55,56]. The membrane-bound TLR4, and the endosomally-localized TLRs 3, 7, 8 and 9 can signal the production of type I IFNs. pDCs express high levels of TLR7 and TLR9, which stimulate the production of type I IFNs in a MyD88-dependent manner. Conventional DCs (cDCs) preferentially express TLR3, TLR8 and low levels of TLR4. TLR3 stimulate type I IFN production in a MyD88-independent and Trif-dependent pathway, while TLR4 does so in a TRAM/Trif and TIRAP/MyD88-dependent manner. TLR8 bears significant homology to TLR7, which stimulates type I IFN production in a MyD88-dependent manner. Kumaigai *et al.* reported that systemic infection with Newcastle disease virus resulted in the production of IFN- α by plasmacytoid DCs in a TLR-dependent manner, and by conventional DCs and macrophages in a RIG-I-like helicase-dependent manner [57]. In contrast, lung infections induced IFN- α production by alveolar macrophages and conventional DCs in a RIG-I-like helicase-dependent manner, but no production of IFN- α by plasmacytoid DCs [57]. Thus the cells responsible for, and the signaling pathways controlling, type I IFN production are defined by the infectious agent as well as the location of the infection [48,57].

Mechanistic studies by Thornley and colleagues revealed that both LPS and poly I:C were able to prevent the induction of skin tolerance by anti-CD154 plus donor specific transfusion [6]. LPS was shown to utilize a TLR4- and MyD88-dependent pathway, while the effects of poly I:C were independent of TLR-3 [58]. Nonetheless, the ability of both LPS and poly I:C to antagonize the effects of anti-CD154-based tolerance induction was dependent on IFN- α/β receptor I (IFN- α/β RI) signaling [58]. The administration of IFN- β recapitulated the effects of LPS and poly I:C, demonstrating the sufficiency of type I IFNs to prevent the induction of allograft tolerance.

While synthetic ligands have proven invaluable for dissecting the impact of TLRs on alloreactivity, an important extension of the observations by Thornley *et al.* [58] is to investigate if real infections also alter allogeneic immune responses. To this end, we have tested the impact of a *Listeria monocytogenes* infection in a mouse model of heart allograft tolerance [59]. *Listeria* is a Gram-positive intracellular bacterium that, upon invasion into the cytosol of resting macrophages, induces the production of IFN- β [60]. We observed that *Listeria* infection at the time of transplantation antagonizes the ability of anti-CD154 and donor specific transfusion to induce long-term heart or skin allograft survival [59]. The effect of *Listeria* infection was lost in IFN- α/β RI-deficient mice, and recapitulated in wildtype mice injected with IFN- β . The pro-rejection effect of *Listeria* and its ability to induce IFN- β production were both largely independent of MyD88, consistent with previous reports that type I IFN production is dependent on the sensing of *Listeria* by cytosolic receptors in infected macrophages [60, 61].

These observations demonstrate that viral and bacterial infections that elicit a strong production of type I IFNs, either through TLRs or non-TLR signaling pathways, may antagonize the induction of tolerance. It is clear that some infections do not elicit type I IFN production, and it would be of interest to determine whether such infections can also prevent the induction of tolerance and if so, define their effector mechanisms.

3. Inhibitory effects of TLR signaling

TLR activation leads to immunity, however excessive TLR signaling would induce inflammation and subsequent disease. TLR induced inflammation is controlled by negative feedback pathways via several inhibitory adaptor proteins. These include IRAK-M, A20,

SOCS1 [62–64]. Previous studies have demonstrated that mice deficient in these genes manifest exaggerated responses to TLR activation and subsequent inflammation [62,63]. Additionally, repeated TLR activation *in vitro* can lead to reduced inflammatory responses, for example the phenomenon of endotoxin tolerance [65]. It was recently shown that altered chromatin modifications lead to gene silencing in endotoxin tolerance [66], although the *in vivo* significance of this finding is not clear. Furthermore, TLR activation directly on regulatory T cells can diminish immune responses [67–69]. In sum, although TLR activation leads to the initiation of potent inflammatory responses, immune regulatory pathways co-evolved to dampen immune responses and prevent undesirable immune pathology.

3.1 Immunoregulatory pathways in neonates

Prior studies have demonstrated that neonates are more prone to infectious disease and to immune tolerance [70–73]. Indeed, in human cardiac transplantation, neonatal recipients can receive ABO incompatible allografts under the cover of generalized immunosuppression [74]. One possible explanation for these findings is that neonates manifest exaggerated immune regulatory pathways and recent studies support this postulate. The work from the Lo-Man laboratory has elegantly demonstrated that neonatal B cells possess unique immunoregulatory properties, specifically upon TLR9 activation [75]. Their initial work demonstrated that neonatal DCs were capable of promoting Th1 immune responses [75]. However, neonatal CD5⁺ B cells reduced DC inflammatory responses upon CpG activation via an IL-10 dependent process. Work published this year by the same group demonstrated that neonatal CD5⁺ B cells are critical for protecting neonates from lethal CpG challenge [76]. In this study, neonatal B cells produced type I IFNs, and these cytokines down graded inflammatory responses upon TLR activation both *in vitro* and *in vivo*. In contrast, type I IFNs in adult mice were inflammatory. Thus, these data demonstrate that neonatal B cells possess specific immunoregulatory properties that impair potent pro-inflammatory signals. These pathways may have evolved to limit inflammation in the neonatal period during the initial exposure to the environment.

In agreement with the Lo-Man laboratory, we have found that TLR-activated neonatal B cells (in response to activation from either TLR 2/6 via peptidoglycan, TLR4 via LPS or TLR9 via CpG) alter DC maturation responses [77]. Specifically, we determined that TLR activated neonatal B cells impaired the ability of adult myeloid DCs to upregulate costimulatory molecules. Furthermore, we found that TLR activated neonatal B cells impaired the ability of DCs to produce IL-12 upon TLR ligation. The presence of TLR activated neonatal B cells skewed T cells to a Th2 phenotype during *in vitro* mixed lymphocyte reactions. *In vivo*, we found that TLR activated neonatal B cells, reduced Th1 immune priming in response to immunization with allogeneic spleen cells or in response to skin allografts. Similar to findings from the Lo-Man laboratory [76], we found that IL-10 produced by TLR-activated neonatal B cells was both necessary and sufficient for the ability of neonatal B cells to alter *in vitro* alloimmune responses [77]. Thus, TLR activated neonatal B cells, in contrast to adult counterparts, modulate both *in vitro* and *in vivo* alloimmune responses. Clearly, it will be important in future studies to determine if such pathways contribute to the immune tolerance-prone state of neonates.

3.2 Immunoregulatory pathways of endogenous TLR ligands

Similarly to well known reports of regulatory pathways triggered by exogenous ligands of TLRs, such as endotoxin tolerance [78], emerging evidence suggests that endogenous ligands of TLRs can also inhibit immune responses under certain circumstances. In contrast to the pro-inflammatory effects of HMGB1 described earlier, pre-conditioning with HMGB1 has been shown to protect against hepatic damage induced by liver IRI [79]. This anti-inflammatory

effect was dependent on intact TLR4 signaling and was associated with upregulation of IRAK-M, one of the inhibitory proteins in the TLR pathways.

Growing evidence suggests that HSPs can also carry anti-inflammatory properties [29]. HSP gp96 is an endoplasmic reticulum chaperone for TLRs. Its predominant role appears to be pro-inflammatory as macrophages from mice with a macrophage-specific targeted deficiency in gp96 failed to respond to engagement of TLRs 2, 4, 5, 7 and 9, resulting in mice that are resistant to endotoxin shock *in vivo* [80]. However, HSP gp96 can also inhibit immune responses, as treatment with HSP gp96 resulted in improved survival of mouse skin allografts bearing either minor or major mismatches [81]. In a model of rat cardiac allograft, administration of high doses of HSP gp96 purified from donor but not recipient strain livers also resulted in prolongation of graft survival that was associated with T cell hyporesponsiveness to polyclonal stimuli [82]. Finally, although transgenic expression of membrane-bound HSP gp96 resulted in hyper-responsiveness to LPS and a TLR4-dependent lupus-like syndrome, it was also shown to increase the suppressive function of Tregs in a TLR4-dependent manner, providing a putative mechanism for the regulatory properties of damage-associated TLR ligands [83]. Collectively, these results point to potential therapeutic uses of stress-induced molecules.

Conclusion

In summary, growing evidence points to engagement of TLRs by damage-induced endogenous ligands after transplantation resulting in enhanced alloimmune responses. In addition, ligation of TLRs by exogenous ligands can prevent transplantation tolerance in a mechanism dependent on the production of type I IFNs, which may be shared by pathogenic microorganisms. Finally, despite the well known pro-inflammatory role of TLR signals, it is becoming clear that TLR pathways can also lead to immunosuppression and such mechanisms may be harnessed in the future for novel therapeutic manipulations in transplanted patients.

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References

1. Goldstein DR, Tesar BM, Akira S, Lakkis FG. Critical role of the Toll-like receptor signal adaptor protein MyD88 in acute allograft rejection. *J Clin Invest* 2003;111:1571–1578. [PubMed: 12750407]
2. McKay D, Shigeoka A, Rubinstein M, Surh C, Sprent J. Simultaneous deletion of MyD88 and Trif delays major histocompatibility and minor antigen mismatch allograft rejection. *Eur J Immunol* 2006;36:1994–2002. [PubMed: 16874736]
3. Tesar BM, Zhang J, Li Q, Goldstein DR. TH1 immune responses to fully MHC mismatched allografts are diminished in the absence of MyD88, a toll-like receptor signal adaptor protein. *Am J Transplant* 2004;4:1429–1439. [PubMed: 15307830]
4. Walker WE, Nasr IW, Camirand G, Tesar BM, Booth CJ, Goldstein DR. Absence of innate MyD88 signaling promotes inducible allograft acceptance. *J Immunol* 2006;177:5307–5316. [PubMed: 17015716]
5. Chen L, Wang T, Zhou P, Ma L, Yin D, Shen J, Molinero L, Nozaki T, Phillips T, Uematsu S, et al. TLR engagement prevents transplantation tolerance. *Am J Transplant* 2006;6:2282–2291. [PubMed: 16970798]
6. Thornley TB, Brehm MA, Markees TG, Shultz LD, Mordes JP, Welsh RM, Rossini AA, Greiner DL. TLR Agonists Abrogate Costimulation Blockade-Induced Prolongation of Skin Allografts. *J Immunol* 2006;176:1561–1570. [PubMed: 16424185]

7. Tsan MF, Baochong G. Pathogen-associated molecular pattern contamination as putative endogenous ligands of Toll-like receptors. *J Endotoxin Res* 2007;13:6–14. [PubMed: 17621541]
8. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. *Semin Immunol* 2007;19:3–10. [PubMed: 17275324]Epub 2007 Feb 2001
9. Goodwin GH, Sanders C, Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. *Eur J Biochem* 1973;38:14–19. [PubMed: 4774120]
10. Calogero S, Grassi F, Aguzzi A, Voigtlander T, Ferrier P, Ferrari S, Bianchi ME. The lack of chromosomal protein Hmg1 does not disrupt cell growth but causes lethal hypoglycaemia in newborn mice. *Nat Genet* 1999;22:276–280. [PubMed: 10391216]
11. Bianchi ME, Manfredi AA. High-mobility group box 1 (HMGB1) protein at the crossroads between innate and adaptive immunity. *Immunol Rev* 2007;220:35–46. [PubMed: 17979838]
12. Steer SA, Scarim AL, Chambers KT, Corbett JA. Interleukin-1 stimulates beta-cell necrosis and release of the immunological adjuvant HMGB1. *PLoS Med* 2006;3:e17. [PubMed: 16354107]Epub 2005 Dec 2020
13. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248–251. [PubMed: 10398600]
- 14**. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, Parroche P, Drabic S, Golenbock D, Sirois C, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* 2007;8:487–496. [PubMed: 17417641]Epub 2007 Apr 2008. One of two papers describing TLR9 as an additional ligand of HMGB1
15. van Beijnum JR, Buurman WA, Griffioen AW. Convergence and amplification of toll-like receptor (TLR) and receptor for advanced glycation end products (RAGE) signaling pathways via high mobility group B1 (HMGB1). *Angiogenesis* 2008;9:9.
16. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem* 2004;279:7370–7377. [PubMed: 14660645]
17. Kokkola R, Andersson A, Mullins G, Ostberg T, Treutiger CJ, Arnold B, Nawroth P, Andersson U, Harris RA, Harris HE. RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. *Scand J Immunol* 2005;61:1–9. [PubMed: 15644117]
- 18**. Ivanov S, Dragoi AM, Wang X, Dallacosta C, Louten J, Musco G, Sitia G, Yap GS, Wan Y, Biron CA, et al. A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA. *Blood* 2007;110:1970–1981. [PubMed: 17548579]Epub 2007 Jun 1974. One of two papers describing TLR9 as an additional ligand of HMGB1
- 19**. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med* 2007;13:1050–1059. [PubMed: 17704786]Epub 2007 Aug 2019. This paper demonstrates that the anti-tumor immune response elicited by radiation therapy and chemotherapy depends on HMGB1 binding to TLR4 on DCs
- 20*. Rao DA, Tracey KJ, Pober JS. IL-1alpha and IL-1beta are endogenous mediators linking cell injury to the adaptive alloimmune response. *J Immunol* 2007;179:6536–6546. [PubMed: 17982042]A manuscript demonstrating among other things a possible role for IL-1 as an effector mechanism for HMGB1
21. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, et al. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005;201:1135–1143. [PubMed: 15795240]
- 22*. Tsung A, Klune JR, Zhang X, Jeyabalan G, Cao Z, Peng X, Stolz DB, Geller DA, Rosengart MR, Billiar TR. HMGB1 release induced by liver ischemia involves Toll-like receptor 4 dependent reactive oxygen species production and calcium-mediated signaling. *J Exp Med* 2007;204:2913–2923. [PubMed: 17984303]Epub 2007 Nov 2915. A paper demonstrating that induction of HMGB1 following hepatic ischemia depends on TLR4 activation and specific downstream pathways
23. Tsung A, Hoffman RA, Izuishi K, Critchlow ND, Nakao A, Chan MH, Lotze MT, Geller DA, Billiar TR. Hepatic ischemia/reperfusion injury involves functional TLR4 signaling in nonparenchymal cells. *J Immunol* 2005;175:7661–7668. [PubMed: 16301676]

- 24*. Tsung A, Zheng N, Jeyabalan G, Izuishi K, Klune JR, Geller DA, Lotze MT, Lu L, Billiar TR. Increasing numbers of hepatic dendritic cells promote HMGB1-mediated ischemia-reperfusion injury. *J Leukoc Biol* 2007;81:119–128. [PubMed: 17062605]Epub 2006 Oct 2024. A manuscript suggesting that functional TLR4 on DCs is necessary for HMGB1-mediated induction of liver IRI
- 25*. Ito N, DeMarco RA, Mailliard RB, Han J, Rabinowich H, Kalinski P, Stolz DB, Zeh HJ 3rd, Lotze MT. Cytolytic cells induce HMGB1 release from melanoma cell lines. *J Leukoc Biol* 2007;81:75–83. [PubMed: 16968820]Epub 2006 Sep 2012. A paper showing that HMGB1 can be released following cytolysis of T cell target cells
- 26*. Huang Y, Yin H, Han J, Huang B, Xu J, Zheng F, Tan Z, Fang M, Rui L, Chen D, et al. Extracellular hmgbl functions as an innate immune-mediator implicated in murine cardiac allograft acute rejection. *Am J Transplant* 2007;7:799–808. [PubMed: 17331117]Epub 2007 Feb 2028. A manuscript showing expression of HMGB1 in allografts after transplantation and demonstrating that blockade of HMGB1 with a soluble truncated form of HMGB1 prolongs allograft survival
- 27*. Moser B, Szabolcs MJ, Ankersmit HJ, Lu Y, Qu W, Weinberg A, Herold KC, Schmidt AM. Blockade of RAGE suppresses alloimmune reactions in vitro and delays allograft rejection in murine heart transplantation. *Am J Transplant* 2007;7:293–302. [PubMed: 17241110]Another manuscript demonstrating that blockade of HMGB1 favorably affects allograft survival, though blockade here is achieved via soluble RAGE
- 28*. Nakano T, Goto S, Lai CY, Hsu LW, Kao YH, Lin YC, Kawamoto S, Chiang KC, Ohmori N, Goto T, et al. Experimental and clinical significance of antinuclear antibodies in liver transplantation. *Transplantation* 2007;83:1122–1125. [PubMed: 17452904]A paper showing that rats and patients can become spontaneously immunized to HMGB1 following transplantation and develop anti-HMGB1 antibodies that appear to correlate with tolerance
29. Pockley AG, Muthana M, Calderwood SK. The dual immunoregulatory roles of stress proteins. *Trends Biochem Sci* 2008;33:71–79. [PubMed: 18182297]Epub 2008 Jan 2007
30. Asea A. Heat shock proteins and toll-like receptors. *Handb Exp Pharmacol* 2008;111–127. [PubMed: 18071657]
31. Pockley AG, Muthana M. Heat shock proteins and allograft rejection. *Contrib Nephrol* 2005;148:122–134. [PubMed: 15912031]
32. Birk OS, Gur SL, Elias D, Margalit R, Mor F, Carmi P, Bockova J, Altmann DM, Cohen IR. The 60-kDa heat shock protein modulates allograft rejection. *Proc Natl Acad Sci U S A* 1999;96:5159–5163. [PubMed: 10220435]
- 33*. Tesar BM, Goldstein DR. Acute allograft rejection occurs independently of inducible heat shock protein-70. *Transplantation* 2007;83:1513–1517. [PubMed: 17565327]A manuscript demonstrating normal rejection kinetics of skin grafts despite deficiency in HSP70.1 and HSP70.3 in both donor and recipient mice
34. Oh KH, Kim JY, Kim D, Lee EM, Oh HY, Seo JS, Han JS, Kim S, Lee JS, Ahn C. Targeted gene disruption of the heat shock protein 72 gene (hsp70.1) in the donor tissue is associated with a prolonged rejection-free survival in the murine skin allograft model. *Transpl Immunol* 2004;13:273–281. [PubMed: 15589740]
35. Jiang D, Liang J, Noble PW. Hyaluronan in tissue injury and repair. *Annu Rev Cell Dev Biol* 2007;23:435–461. [PubMed: 17506690]
36. Johnsson C, Tufveson G. Serum hyaluronan--a potential marker of cardiac allograft rejection? *J Heart Lung Transplant* 2006;25:544–549. [PubMed: 16678033]Epub 2006 Apr 2011
37. Tesar BM, Jiang D, Liang J, Palmer SM, Noble PW, Goldstein DR. The role of hyaluronan degradation products as innate alloimmune agonists. *Am J Transplant* 2006;6:2622–2635. [PubMed: 17049055]
- 38*. Abdala E, Baia CE, Mies S, Massarollo PC, de Paula Cavaleiro N, Baia VR, Inacio CA, Sef HC, Barone AA. Bacterial translocation during liver transplantation: a randomized trial comparing conventional with venovenous bypass vs. piggyback methods. *Liver Transpl* 2007;13:488–496. [PubMed: 17436334]A manuscript that demonstrates endotoxin translocation after liver transplantation that can be detected in hepatic artery and portal vein
39. Cicalese L, Sileri P, Green M, Abu-Elmagd K, Kocoshis S, Reyes J. Bacterial translocation in clinical intestinal transplantation. *Transplantation* 2001;71:1414–1417. [PubMed: 11391228]

- 40*. San Juan R, Aguado JM, Lumberras C, Diaz-Pedroche C, Lopez-Medrano F, Lizasoain M, Gavalda J, Montejo M, Moreno A, Gurgui M, et al. Incidence, clinical characteristics and risk factors of late infection in solid organ transplant recipients: data from the RESITRA study group. *Am J Transplant* 2007;7:964–971. [PubMed: 17391136] This study defines the incidence, clinical characteristics and risk factors for infection in the first 13 months post-solid organ transplantation from the database of the Spanish Network of Infection in Transplantation
- 41*. Moreno A, Cervera C, Gavalda J, Rovira M, de la Camara R, Jarque I, Montejo M, de la Torre-Cisneros J, Miguel Cisneros J, Fortun J, et al. Bloodstream infections among transplant recipients: results of a nationwide surveillance in Spain. *Am J Transplant* 2007;7:2579–2586. [PubMed: 17868067] Epub 2007 Sep 25. This work defines the incidence, microbiology and outcomes of bloodstream infections in the first 13 months post-solid organ or hematopoietic stem cell transplantation from the Spanish Network of Infection in Transplantation database
- 42*. Shepherd RW, Turmelle Y, Nadler M, Lowell JA, Narkewicz MR, McDiarmid SV, Anand R, Song C. Risk factors for rejection and infection in pediatric liver transplantation. *Am J Transplant* 2008;8:396–403. [PubMed: 18162090] Epub 2007 Dec 20. Analysis of the rates of rejection (46%) and infection (52%), as important adverse events after 2291 pediatric liver transplantation from the US and Canada Pediatric Liver Transplantation (SPLIT) database
- 43*. Silveira FP, Husain S. Fungal infections in solid organ transplantation. *Med Mycol* 2007;45:305–320. [PubMed: 17510855] Review of fungal infections in solid organ transplant recipients, which are a significant cause of morbidity and mortality. *Candida*. and *Aspergillus* represent the most invasive fungal infections
44. Husain S, Chan KM, Palmer SM, Hadjiladis D, Humar A, McCurry KR, Wagener MM, Singh N. Bacteremia in lung transplant recipients in the current era. *Am J Transplant* 2006;6:3000–3007. [PubMed: 17294526]
- 45*. Legendre C, Pascual M. Improving outcomes for solid-organ transplant recipients at risk from cytomegalovirus infection: late-onset disease and indirect consequences. *Clin Infect Dis* 2008;46:732–740. [PubMed: 18220478] Prophylaxis is more effective than preemptive therapy at preventing persistent low-level CMV infection, which has been linked with transplant-associated vasculopathy, and an increased risk of opportunistic infection and graft rejection
- 46*. Ahmed EM, Alegre ML, Chong A. The role of bacterial infections in allograft rejection. *Exp Rev Clin Immunol*. 2008 in press. Review examining the correlation between bacterial infections and rejection, and discussing the molecular interface between bacterial infections and alloreactivity
47. Beutler B, Eidenschenk C, Crozat K, Imler JL, Takeuchi O, Hoffmann JA, Akira S. Genetic analysis of resistance to viral infection. *Nat Rev Immunol* 2007;7:753–766. [PubMed: 17893693]
48. Takeuchi O, Akira S. Recognition of viruses by innate immunity. *Immunol Rev* 2007;220:214–224. [PubMed: 17979849]
49. Takeuchi O, Akira S. Signaling pathways activated by microorganisms. *Curr Opin Cell Biol* 2007;19:185–191. [PubMed: 17303405] Epub 2007 Feb 20
50. Willment JA, Brown GD. C-type lectin receptors in antifungal immunity. *Trends Microbiol* 2008;16:27–32. [PubMed: 18160296] Epub 2007 Dec 20
- 51*. Garantzotis S, Palmer SM, Snyder LD, Ganous T, Chen BJ, Wang T, Cook DN, Schwartz DA. Alloimmune lung injury induced by local innate immune activation through inhaled lipopolysaccharide. *Transplantation* 2007;84:1012–1019. [PubMed: 17989607] This paper demonstrates a critical role for localized innate immune activation triggered by lipopolysaccharide inhalation in stimulating alloimmune lung injury in a murine bone marrow transplant model
52. Chakraverty R, Cote D, Buchli J, Cotter P, Hsu R, Zhao G, Sachs T, Pitsillides CM, Bronson R, Means T, et al. An inflammatory checkpoint regulates recruitment of graft-versus-host reactive T cells to peripheral tissues. *J Exp Med* 2006;203:2021–2031. [PubMed: 16880259] Epub 2006 Jul 20
- 53*. Durakovic N, Radojic V, Skarica M, Bezak KB, Powell JD, Fuchs EJ, Luznik L. Factors governing the activation of adoptively transferred donor T cells infused after allogeneic bone marrow transplantation in the mouse. *Blood* 2007;109:4564–4574. [PubMed: 17227829] Epub 2007 Jan 4. A manuscript showing in murine models of mixed bone marrow chimeras that the induction of optimal alloreactivity for graft versus host and graft versus leukemia reactivities requires donor T cells, host APCs and TLR ligands

54. Lorenz E, Schwartz DA, Martin PJ, Gooley T, Lin MT, Chien JW, Hansen JA, Clark JG. Association of TLR4 mutations and the risk for acute GVHD after HLA-matched-sibling hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001;7:384–387. [PubMed: 11529488]
55. Elmaagacli AH, Koldehoff M, Hindahl H, Steckel NK, Trenchel R, Peceny R, Ottinger H, Rath PM, Ross RS, Roggendorf M, et al. Mutations in innate immune system NOD2/CARD 15 and TLR-4 (Thr399Ile) genes influence the risk for severe acute graft-versus-host disease in patients who underwent an allogeneic transplantation. *Transplantation* 2006;81:247–254. [PubMed: 16436969]
56. Uematsu S, Akira S. Toll-like receptors and Type I interferons. *J Biol Chem* 2007;282:15319–15323. [PubMed: 17395581]Epub 12007 Mar 15329
- 57*. Kumagai Y, Takeuchi O, Kato H, Kumar H, Matsui K, Morii E, Aozasa K, Kawai T, Akira S. Alveolar macrophages are the primary interferon-alpha producer in pulmonary infection with RNA viruses. *Immunity* 2007;27:240–252. [PubMed: 17723216]Using a knockin mouse in which green fluorescence protein (GFP) was expressed under the control of the *Ifnα 6* promoter, systemic infection with the RNA virus, Newcastle disease virus (NDV), induced the production of IFNα 6 in plasmacytoid DCs that was dependent on the MyD88/TLR system
- 58*. Thornley TB, Phillips NE, Beaudette-Zlatanova BC, Markees TG, Bahl K, Brehm MA, Shultz LD, Kurt-Jones EA, Mordes JP, Welsh RM, et al. Type 1 IFN mediates cross-talk between innate and adaptive immunity that abrogates transplantation tolerance. *J Immunol* 2007;179:6620–6629. [PubMed: 17982052]Type 1 IFN generated as part of an innate immune response to TLR3 or TLR4 ligation can in turn activate adaptive immune responses that abrogate transplantation tolerance
- 59*. Wang T, Chen L, Ahmed EM, Ma L, Yin D, Zhou P, Shen J, Xu H, Wang CR, Alegre ML, et al. Prevention of allograft tolerance by bacterial infection with *Listeria monocytogenes*. *J Immunol*. 2008in press. Type 1 IFN generated in a MyD88-independent manner as part of an innate immune response to *Listeria* infection can activate alloreactive immune responses and abrogate the induction of transplantation tolerance
60. O’Riordan M, Yi CH, Gonzales R, Lee KD, Portnoy DA. Innate recognition of bacteria by a macrophage cytosolic surveillance pathway. *Proc Natl Acad Sci U S A* 2002;99:13861–13866. [PubMed: 12359878]
- 61*. Herskovits AA, Auerbuch V, Portnoy DA. Bacterial ligands generated in a phagosome are targets of the cytosolic innate immune system. *PLoS Pathog* 2007;3:e51. [PubMed: 17397264]Activation of macrophages with IFN-γ leads to rapid killing and degradation of *Listeria monocytogenes* in the phagosome and the generation of bacterial ligands that activate TLRs and cytosolic microbial sensors, and the production of IFN-β
62. Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, Hurley P, Chien M, Chai S, Hitotsumatsu O, et al. The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. *Nat Immunol* 2004;5:1052–1060. [PubMed: 15334086]Epub 2004 Aug 1029
63. Kobayashi K, Hernandez LD, Galan JE, Janeway CA Jr, Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell* 2002;110:191–202. [PubMed: 12150927]
64. Liew FY, Xu D, Brint EK, O’Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5:446–458. [PubMed: 15928677]
65. Beeson PB. With the Technical Assistance of Elizabeth R. TOLERANCE TO BACTERIAL PYROGENS: I. FACTORS INFLUENCING ITS DEVELOPMENT. *J Exp Med* 1947;86:29–38.10.1084/jem.86.1.29
- 66*. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* 2007;447:972–978. [PubMed: 17538624]Epub 2007 May 2030. This study examines the genetic control of endotoxin tolerance
67. Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF. Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. *Science* 2005;309:1380–1384. [PubMed: 16123302]
68. Netea MG, Suttmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH, Hartung T, Adema G, Kullberg BJ. Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. *J Immunol* 2004;172:3712–3718. [PubMed: 15004175]
69. Suttmuller RP, den Brok MH, Kramer M, Bennis EJ, Toonen LW, Kullberg BJ, Joosten LA, Akira S, Netea MG, Adema GJ. Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Invest* 2006;116:19.

70. McKenney WM. Understanding the neonatal immune system: high risk for infection. *Crit Care Nurse* 2001;21:35–47. [PubMed: 11767763]
71. Wilson CB. Immunologic basis for increased susceptibility of the neonate to infection. *J Pediatr* 1986;108:1–12. [PubMed: 3511202]
72. Wilson CB, Lewis DB, English BK. T cell development in the fetus and neonate. *Adv Exp Med Biol* 1991;310:17–27. [PubMed: 1808993]
73. Streilein JW. Neonatal tolerance of H-2 alloantigens. Procuring graft acceptance the “old-fashioned” way. *Transplantation* 1991;52:1–10. [PubMed: 1858135]
74. Fan X, Ang A, Pollock-Barziv SM, Dipchand AI, Ruiz P, Wilson G, Platt JL, West LJ. Donor-specific B-cell tolerance after ABO-incompatible infant heart transplantation. *Nat Med* 2004;10:1227–1233. [PubMed: 15502841][see comment]
75. Sun CM, Deriaud E, Leclerc C, Lo-Man R. Upon TLR9 signaling, CD5+ B cells control the IL-12-dependent Th1-priming capacity of neonatal DCs. *Immunity* 2005;22:467–477. [PubMed: 15845451]
- 76**. Zhang X, Deriaud E, Jiao X, Braun D, Leclerc C, Lo-Man R. Type I interferons protect neonates from acute inflammation through interleukin 10-producing B cells. *J Exp Med* 2007;204:1107–1118. 10.1084/jem.20062013 This study determined that neonatal B cells produce type I interferons upon CpG activation and protect neonates from lethal inflammation, whereas CpG induced type I interferon production in adults induces lethality
- 77**. Walker WE, Goldstein DR. Neonatal B cells suppress innate toll-like receptor immune responses and modulate alloimmunity. *J Immunol* 2007;179:1700–1710. [PubMed: 17641036] This study determined that TLR activated neonatal B cells, unlike adult counterparts, possess immunoregulatory properties that alter DC responses and alloimmunity. This effect occurred via an IL-10 dependent process
78. Fan H, Cook JA. Molecular mechanisms of endotoxin tolerance. *J Endotoxin Res* 2004;10:71–84. [PubMed: 15119998]
79. Izuishi K, Tsung A, Jeyabalan G, Critchlow ND, Li J, Tracey KJ, Demarco RA, Lotze MT, Fink MP, Geller DA, et al. Cutting edge: high-mobility group box 1 preconditioning protects against liver ischemia-reperfusion injury. *J Immunol* 2006;176:7154–7158. [PubMed: 16751357]
- 80**. Yang Y, Liu B, Dai J, Srivastava PK, Zammit DJ, Lefrancois L, Li Z. Heat shock protein gp96 is a master chaperone for toll-like receptors and is important in the innate function of macrophages. *Immunity* 2007;26:215–226. [PubMed: 17275357] Epub 2007 Feb 2001. A study showing that macrophage-specific deletion of HSP gp96 results in impaired responses to ligation of TLRs 2, 4, 5, 7 and 9 by macrophages and in mice that are resistant to endotoxic shock
81. Kovalchin JT, Mendonca C, Wagh MS, Wang R, Chandawarkar RY. In vivo treatment of mice with heat shock protein, gp 96, improves survival of skin grafts with minor and major antigenic disparity. *Transpl Immunol* 2006;15:179–185. [PubMed: 16431284] Epub 2005 Aug 2009
- 82*. Slack LK, Muthana M, Hopkinson K, Suvarna SK, Espigares E, Mirza S, Fairburn B, Pockley AG. Administration of the stress protein gp96 prolongs rat cardiac allograft survival, modifies rejection-associated inflammatory events, and induces a state of peripheral T-cell hyporesponsiveness. *Cell Stress Chaperones* 2007;12:71–82. [PubMed: 17441509] A paper showing that administration of HSP gp96 can have immunosuppressive effects in a rat cardiac allograft model
- 83*. Dai J, Liu B, Ngoi SM, Sun S, Vella AT, Li Z. TLR4 hyperresponsiveness via cell surface expression of heat shock protein gp96 potentiates suppressive function of regulatory T cells. *J Immunol* 2007;178:3219–3225. [PubMed: 17312170] A study showing that transgenic expression of HSP gp96 resulted in enhanced Treg suppressive function in a TLR4-dependent manner

Table I**Common infections in transplant recipients and their potential ligands for TLRs**

CMV (cytomegalovirus) HSV-I (herpes virus simplex type I)

Infections	Microbial Agent or Component	TLR
Bacterial	LPS	TLR4
	Diacyl lipopeptides	TLR2/TLR6
	Triacyl lipopeptides	TLR2/TLR1
	Lipoteichoic acid	TLR2/TLR6
	Phenol-soluble modulin	TLR2
	Atypical LPS	TLR2
	Flagellin	TLR5
	CpG DNA	TLR9
Viral	Uropathogenic bacteria	TLR11
	Envelope Proteins of CMV and HSV-1	TLR2
	F protein of respiratory syncytial virus (RSV)	TLR4
	Double strand RNA virus	TLR3
	Single strand RNA virus	TLR7/TLR8
Fungal	DNA virus/CpG DNA	TLR9
	Apergillus	TLR2/TLR4
	Saccharomyces – Zymozan	TLR2/TLR6
	Saccharomyces – Mannan	TLR4
	Candida – phospholipomannan	TLR2
	Cryptococcus - Glucuronoxymannan	TLR4