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Innate immunity for better or worse govern the allograft response

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Abstract

Purpose of review—To update knowledge concerning the cause and consequences of the detrimental forms of innate immunity that inevitably occurs in peri-transplant period tissue and cellular transplants. In addition we review information a newly discovered engraftment promoting and tolerance inducing macrophage population is identified and characterized.

Recent Findings—The allograft response mounted by adaptive immune cells is shaped by innate immunity. The early allograft response is uniquely intense as a result of activation of the innate immune response created by ischemia reperfusion injury in organ transplants, delayed revascularization of cell transplants and hypoxia. Inflammation is created by both cellular “debris” and cytokines. On the other hand a newly discovered prominent, albeit fragile, tissue resident, non-invasive and immunoregulatory macrophage promotes engraftment and tolerance. The role of intracellular “debris” as well as inflammation in evoking detrimental rejection provoking peri-transplant inflammation is emphasized as well as characterization of a prominent and highly immunoregulatory albeit fragile macrophage population that is tissue resident and does not circulate is characterized.

Summary—Opportunity lies in the ability to rein in detrimental peri-transplant inflammation and in the ability to promote the longevity of a subpopulation of highly potent tissue resident immunoregulatory macrophages.

Keywords

Tissue resident macrophages; Damage Associated Molecular Pattern molecules (DAMPs)

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Conflicts of interest

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Introduction

While there are center-to-center, organ specific, recipient and donor age related differences with respect to the preferred immunosuppressive protocol utilized, the immunosuppressive protocols utilized in the early transplant period are very intense and include high dose anti-inflammatory corticosteroids than maintenance regimens. Why? The circumstances that attend harvest, preservation and transplantation of organ and cellular transplants inevitably create an intense inflammatory state in the early post-transplant period. This circumstance has impact upon the resilience of the transplant and upon the quantitative and qualitative aspects of the host anti-donor adaptive immune response.

In renal transplantation, delayed graft function (DGF) and acute rejection (AR) are widely but not universally regarded as inter-related post-transplant complications that can contribute to impaired intermediate- and long-term graft function and survival. We have tested the hypothesis that molecular evidence of intra-graft expression of pro-inflammatory cytokines especially when linked with suboptimal expression of anti-apoptotic genes and evidence of active T cell immunity present intra-operatively 10–15 minutes after revascularization and detected via PCR-based transcriptional profiling are linked to adverse post-transplant clinical outcomes such as DGF, AR within 3 months following transplantation, and the quality of graft function 6 months and even 2 years post-transplantation[1, 2]. As a sidelight to this investigation we determined that as compared to normal kidneys every deceased donor renal transplant, even those without evidence of DGF, exhibit robust expression of pro-inflammatory cytokines. Taken together these date suggest that detrimental intra-graft inflammation is universal in the early post-transplant period and may point to the reason that potent anti-inflammatory agents such as high dose corticosteroids are required in the peri-transplant period.

The fine molecular texture of inflammation within the microenvironment in which T cells recognize antigen directs the commitment of T cells into either tissue protective (regulatory T cells; Tregs) or destructive helper T cell phenotypes. The presence of pro-inflammatory cytokines within this microenvironment polarizes antigen activated CD4+ T cells into tissue destructive helper T cell (e.g., Th1, Th2, and Th17) phenotypes. An environment dominated by transforming growth factor beta leads to commitment to the CD4+ regulatory T cells (TRegs) phenotype. In sharp contrast, the prominent appearance of certain pro-inflammatory cytokines such as IL-6 and IL-21, a member of the T cell growth factor family, favors differentiation into the Th17 phenotype and totally obviates the potential to direct naïve T cells into TRegs, thereby leading to weak if not unopposed dominance of effector type Th1, Th2 and Th17 alloreactive T cells in the allograft response[3]. As noted above IL-6 and other pro-inflammatory cytokines are abundantly expressed as a consequence of ischemia reperfusion injury in organ transplants[2] and in the disaster that leads to massive death of cell transplants before neo- angiogenesis of the transplant occurs.

Danger Molecules and Ischemia Reperfusion Injury

Intracellular components of all normal cells are privileged in that they escape host recognition. Following ischemia reperfusion injury, prolonged hypoxia and particularly with delayed reperfusion, these intracellular molecules, no longer contained within a membrane

barrier, function as distress signals rapidly eliciting a potent innate immune response in a manner similar to pathogens. All tissue injury releases Damage Associated Molecular Pattern molecules (DAMPs), a series of intracellular proteins released as a result of tissue injury. The most well described DAMPs include those from i) subcellular organelles and include HMGB1 and calreticulin, and ii) the cytoplasm including heat shock proteins, galectins and S100A proteins. More recent data identifies mitochondria, nucleotides including ATP as well as heme as powerful endogenous signaling molecules that emerge from the damaged cell and activate innate immunity. Each DAMP bears cognate receptor and these receptor molecules include RAGE, mincle, purinergic, and toll like receptors (TLRs). Mitochondrial DAMPs (mtDAMPs) are important contributors to inflammation[4, 5]. Mitochondria are evolutionary endo-symbionts derived from proteobacteria; thus, it is logical that mitochondria would bear molecular motifs that function as DAMPs. Indeed mitochondrial debris-containing DAMPs enter the circulation after injury and contribute to systemic inflammation. In fact, mitochondrial DNA (mtDNA) circulates in critical illness in concentrations that predict systemic inflammation, sepsis and clinical outcome[6]. In addition to mtDNA, mitochondria debris includes formyl peptides, mitochondrial DNA (mtDNA) and heme among other motifs. MT formyl peptides activate formyl peptide receptors (FPR) and are potent chemo-attractants; mtDNA activates immune cells via the direct and especially indirect effects of TLRs. Heme release up-regulates heme oxygenases as a stress response gene to metabolize the pro-oxidant, heme. Adenosine triphosphate and adenosine are powerful signaling molecules that act on a battery of purinergic receptors that also contribute to modulation of an inflammatory response and alert the immune system of necrosis[7]. There is great direct tissue insult occurring during the harvest and transplantation of a solid organ. The requisite trauma followed by ischemia reperfusion injury without question results in the release of a tremendous payload of DAMPs that wreak havoc both locally in the transplanted organ, but in all likelihood contribute to systemic inflammatory responses in the recipient. Exposure of dendritic cells to DAMPs rapidly enhances their maturation and amplifies the T cell responses[8]. HMGB1 is required for the accumulation of T cells into tissues directed by macrophages and dendritic cells[9]. Mitochondrial debris released after tissue injury leads to activation of γδ T cells therein initiating a sterile inflammatory response that ultimately impacts the healing process[10].

In short, the early post-transplant period is attended by a potent innate immune response created by the release of immune stimulating and tissue damaging intracellular DAMP molecules that amplify immune cell activation and expression of cytokines that seriously limit commitment of naïve T cells to the TRegs phenotype and reciprocally foster potent tissue destructive effector immunity. DAMPs also increase endothelial cell permeability and parenchymal cell expression of pro-fibrotic tissue remodeling proteins that further contribute to chronic disease including transplant vascular stenosis[6].

Interfering with DAMP-mediated immune events that otherwise lead to pathology will prove challenging. There are a number of active efforts underway led by biotech and pharmaceutical companies. The characterization of antibodies against formyl peptides and mitochondria as well as medicinal chemistry efforts towards design of novel small molecule formyl peptide receptor inhibitors are in early preclinical development. Other approaches include inhibition of HMGB1 with ethyl pyruvate or sRAGE, antagonists of TLR4 and

RAGE, and even the use of rasburicase to remove uric acid as a pro-inflammatory molecule. One of the more notable, yet underappreciated DAMPs is heme, which is present in large amounts in hemoglobin-packed erythrocytes, yet a large pool exists in all cells where heme serves as a critical moiety in the function of numerous enzyme complexes. Free heme is recognized by TLR4 and rapidly degraded by heme oxygenase into three bioactive products including the bile pigments and carbon monoxide that have been shown to modulate the inflammatory responses. Given the potentially large number of DAMPs contained within cells, many of which have yet to be identified, a successful approach to interfering with their activity will prove challenging and not without potential collateral damage. In short, the early post-transplant period is attended by a potent innate immune response created the release of immune stimulating and tissue damaging intracellular DAMP proteins and amplified expression of cytokines that seriously limit commitment of naïve T cells to the TRegs phenotype and reciprocally foster potent tissue destructive effector immunity. That intracellular molecules activate the immune response has led to an explosion of research and the likely emergence of an entire new field of study with direct applicability to transplantation.

Peritransplant Inflammation is Dangerous

As noted above, in renal transplantation DGF and AR are linked, suggesting that the heightened inflammatory response that is causal for DGF is also linked to rejection. The issues arising from inflammation in the peri-transplant period in the setting of islet transplant are even more detrimental as most islets die from a macrophage rich inflammatory state within the peri-transplant period. The losses are so profound that clinical application of islet transplantation has been slowed by the inability to routinely utilize islets from a single donor to restore euglycemia, even short-term, and by the unacceptably high 5 year failure rate even for islet transplants that function at 1 year post-transplantation[11]. As a result of non-immunologic processes, many islets perish during harvest, purification and shortly following transplantation[12, 13]. Moreover, the marked non-immunologic loss of islets may render the recipient prone to beta cell exhaustion long-term[14].

Alpha 1-antitrypsin (AAT), an acute phase reactant serine protease inhibitor with anti-inflammatory, anti-leukocyte migratory, anti-thrombotic, complement inhibition, and anti-apoptotic effects but without direct effects on isolated T cells[15–18], exerts cytoprotective effects upon islets in vitro[17, 19]. Short-term AAT treatment restores euglycemia and self-tolerance to islets in overtly type 1 diabetic, non-obese diabetic (NOD) mice. We studied the impact of short term AAT treatment upon the fate of marginal mass syngeneic islet transplants in highly reproducible syngeneic mouse and autologous non-human primate models. In the mouse model, primary non-function was noted in all recipients of untreated mice but syngeneic islets functioned 90% of treated mice[20]. In the autologous monkey islet transplant model, frank diabetes was noted in each control by 6 months post-transplantation but short term AAT treatment insured long-term, apparently permanent graft survival in each recipient[3]. Using genome wide arrays and systems biology analysis, AAT treatment produced potent inhibition of a powerful intra-graft inflammatory process. AAT treatment also grossly inhibited graft infiltration by circulating macrophages. Studies are now in progress to determine whether AAT treatment can be used safely and effectively as an

adjunct to several other regimens that tilt the allograft response toward tolerance in cynomolgus monkey allograft models.

Tissue Resident anti-inflammatory macrophages—It is now evident that Tregs are not the only immunoregulatory cells that can inhibit rejection and promote tolerance. It is beyond the scope of this review to comment upon the role of regulatory B cells, mesenchymal derived stem cells, and immunoregulatory dendritic cells and previously described conventional circulating immunoregulatory macrophages. Instead we will briefly describe a subpopulation of tissue resident macrophages, that exert powerful immunosuppressive effects and promote long-term engraftment and tolerance of allogeneic mouse cardiac transplants[21]. These tissue resident macrophages unlike monocyte derived circulating macrophages are Ly6C null and express CD169 and other cell surface siglec molecules. This Ly6C null, CD160+ population is heterogeneous and not all tissue resident macrophages are immunoregulatory. To our surprise we found that the primary macrophage population present in hearts is a population that is Ly6c-, CD169+, F4/80+ and expresses other macrophage markers. This population also robustly expresses T cell Immunoglobulin and Mucin (TIM-4). TIM-4 and other TIM proteins possess a phospatidylserine binding pocket that commit these cells to bind to dead and dying cells as phospatidylserine is present on the surface of dead and dying cells.

The previously uncharacterized (TIM-4^{hi}) subset of $CD169^{+}$ M2-like tissue-resident macrophages is a major subset of tissue-resident macrophages and is present in normal as well as transplanted hearts[21]. While tissue resident macrophages have been regarded as sessile these cell home to draining lymph nodes following oxidative stress and are therefore capable of delivering alloantigen to transplant draining lymph nodes. These cells exhibit an immunoregulatory and hypostimulatory phenotype as they express many of the immunoregulary molcules produced by Tregs. This phenotype is maintained following migration to secondary lymphoid organs. As compared to conventional allogeneic $Ly6C^{+}$ macrophages, the TIM-4hi subset of CD169+ M2-like tissue-resident macrophages the favors (5X fold) preferential induction of antigen-stimulated Tregs in the mixed lymphocyte response[21]. Yet these immunoregulatory macrophages are fragile and disappear from the transplant just before rejection occurs.

We learned that this subpopulation of tissue resident macrophages is also very fragile in vitro and highly susceptible to apoptosis in an oxidation stress model. In contrast, these Ly6C null, CD169+ tissue-resident macrophages are resistant to oxidation stress-induced apoptosis in TIM-$4^{-/-}$ mice. Compared to wild type heart allografts, TIM-$4^{-/-}$ heart allografts placed into untreated recipients survived over a month longer and were easily tolerized with suboptimal doses of sirolimus[21]. Thus M2-like TIM-4^{hi}, $CD169^{+}$ tissue-resident macrophages are immunoregulatory and promote engraftment of cardiac allografts but their influence is diminished by TIM-4-dependent programmed cell death.

We conclude that a TIM-4^{hi} subset of $Ly6C^{-}CD169^{+}$ tissue-resident macrophages: 1) constitutes a major subset of $Ly6C^{-}$ tissue-resident macrophages, 2) homes to draining lymph nodes following oxidative stress, a major site for T cell activation, 3) exhibits an immunoregulatory and hypostimulatory phenotype that is maintained following migration to

dLNs, 4) favors preferential induction of antigen-stimulated Tregs, 5) is highly susceptible to apoptosis, and 6) is rendered resistant to apoptosis via genetic ablation of TIM-4 expression. As a consequence, TIM-$4^{-/-}$ heart allografts placed into untreated recipients survived much longer than wild type hearts and survived indefinitely with the addition of a short, suboptimal rapamycin treatment regimen. Inflammation creates a milieu in which there is oxidative stress. Thus TIM-$4^{hi}CD169^{+}$ tissue-resident macrophages are immunoregulatory and hypostimulatory but their long-term influence is diminished by their susceptibility to programmed cell death in response to oxidative stress. We are now testing the hypothesis that anti-TIM-4 monoclonal antibodies able to protect the TIM-4^{hi} subset of $CD169^{+}$ M2-like tissue-resident macrophages from apoptosis will promote long-term engraftment and tolerance.

Conclusion

In this review, we have described the detrimental influence of pro-inflammatory tissue injury and cell death that occurs in the peri-transplant period. This situations leads to dysfunction of organ allografts, tremendous loss of islet cell transplants and leads directly to heightened anti-donor immunity. A major effort to modify and diminish this detrimental form of immunity seems justified. A new population of engraftment and tolerance promoting tissue resident macrophages has been identified. This population is fragile and efforts to promote their longevity lead to prolonged engraftment and high susceptibility to tolerance. A strategy to identify therapies that promote their longevity and translation to the clinic is outlined.

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Key points

- Ischemia reperfusion injury, graft hypoxia, and delayed neo-angiogenesis create a virulent form of inflammation in the immediate post-transplant period.
- This detriment form of inflammation is likely the reason we are dependent upon corticosteroid treatment, albeit only modestly successful in curbing inflammation, in this period.
- This detrimental inflammation is generated by liberation of immune activating intracellular protein; pro-inflammatory cytokines heightens graft destroying anti-donor immunity and is a huge hurdle to tolerance induction.
- A newly discovered of potent tolerance promoting immunoregulatory tissue resident macrophages has been discovered.
- Efforts to promote the longevity of this inherently fragile population promote transplant tolerance.