Innate Immunity-Mediated Allograft Rejection and Strategies to Prevent It

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ABSTRACT

Experimental and clinical evidence has accumulated in support of the notion that oxidative injuries to allografts induce an adaptive alloimmune response which leads to acute rejection. The link between the initial injury and subsequent rejection is the innate immune system represented by injury-activated donor-derived and recipient-derived dendritic cells which interact with naive T cells of the recipient to induce an alloimmune T-cell response. Therefore, time is mature to consider potential therapeutic strategies that are able to suppress events of innate immunity. Such strategies refer to a “time-restricted therapeutic window” that includes treatment of the donor during organ removal and the recipient during allograft reperfusion. Major targets of such treatment include (1) mitigation of the oxidative allograft injury; (2) inhibition of injury-induced activation of complement; (3) inhibition of Toll-like receptor (TLR)-mediated and innate lymphocyte-triggered maturation of dendritic cells; and (4) blockade of innate effector functions. A considerable variety of promising experimental studies about the prevention/inhibition of innate immune events has already been performed, including the successful experimental use of gene silencing methods, eg, using RNA interference technology with the application of small interfering RNA (siRNA). In addition, a few clinical trials with antioxidants (edaravone, SOD-mimetics), complement inhibitors (pexelizumab, TP-10) in patients with acute myocardial infarction, and TLR4 antagonists (TAK-242, E-5564) in patients with sepsis have been performed or are underway. Performance of similar clinical trials in transplant patients with antioxidative drugs, complement inhibitors, and/or TLR4 antagonists is urgently warranted; siRNAs appear to be extremely attractive for investigation in experimental allogeneic transplant models.

Unlike MEDICATION is an evolutionarily highly conserved rapid first line of host defense in mammals, plants, and insects. The term does not appear appropriate and I suggest to call it “innate defensity”—regarded as a subunit of “supervivere” (a term created from the Latin word supervivere, meaning “to survive”)—in terms of a superimposed concept of evolutionarily conserved biological systems that have phylogenetically developed to provide biodefenses and survival strategies (“struggle for life”). In mammals, “innate defensity” subsequently initiates immunity, the classical term for adaptive immunity (immunization). Blood coagulation, simultaneously evolved with “innate defensity” and evolutionarily linked to inflammation, represents another biological subunit of “supervivere.” Shape modifications (eg, mimicry, white skin color of polar animals) may also be regarded as such a biological subunit.

As reviewed,1,2 mounting evidence suggests that the innate immune system reacts in every situation of a dangerous tissue injury induced not only by pathogens but any injurious event, eg, as occurs during allograft reperfusion. In mammals, a whole family of cells (eg, dendritic cells [DCs], macrophages, innate lymphocytes, vascular and epithelial cells) is involved which are equipped with pattern recognition receptors such as Toll-like receptors (TLRs). These receptors recognize specific structures of microorganisms, the pathogen-associated molecular patterns (PAMPs) in terms of exogenous ligands, as well as injury-
induced altered host-derived (“self”) structures, the damage-associated molecular patterns (DAMPs) in terms of endogenous ligands. After recognition, TLRs initiate intracellular signal transduction pathways that result, via activation of transcription factors such as NFκB, AP-1, and IRF3, in the expression of genes involved in inflammation, antiviral responses, and maturation of immature dendritic cells (iDCs). Matured DCs interact with naïve T lymphocytes, initiate a T-cell response, and thus represent the link to adaptive immunity. As illustrated in Fig 1, the innate immune response appears to be shaped like an hourglass. However, as suggested in 2002, and recently confirmed, the hourglass may not be so narrow at its center since many of the active downstream kinases and transcription factors may be regulated through oxidant-dependent posttranslational modifications, resulting in an additional (ie, higher) level of TLR signaling (Fig 2). Importantly, the TLR-mediated DC maturation as a prerequisite for the initiation of an adaptive immune response is not the only cell-maturing mechanism. Recent studies have shown that innate lymphocytes, NK-, NKT-, and γδ T cells, trigger DC maturation following detection of pathogen-derived antigens or injury-induced neo-antigens.

Besides cells, humoral factors such as complement and natural monoclonal IgM antibodies represent classical instruments of the innate immune system as well. New evidence suggests that “innate” IgM antibodies initially bind to a reperfusion injury-induced, host-derived neo-antigen providing a binding site for mannan binding lectin (MBL), a circulating recognition molecule. The bound MBL, once involved in this “innate” antigen-antibody reaction, activates the complement cascade via the lectin pathway. These events may subsequently contribute to additional tissue injury.

![Fig 1. Oversimplified schematic illustration of intracellular TLR-triggered/mediated signaling pathways resulting, via activation of transcription factors NFκB, AP-1, and IRF3, in the expression of “innate” genes involved in inflammation, antiviral responses, and maturation of DCs. The innate immune response appears to be shaped like an hourglass. The top of the hourglass is wide, indicating that 10 human TLRs recognize a large variety of PAMPs and DAMPs, then the hourglass narrows to represent a smaller number of 4 adaptor proteins (MyD88, TIRAP/Mal, TRIF, and TRAM) and only 2 initial kinases. Then it widens again to reflect involvement of more than 10 activated distal kinases followed by transcriptional activation of more than 500 genes. AP-1 = activator protein 1; DAMPs = damage-associated molecular patterns; HMGB-1 = high mobility group box 1; HSPs = heat shock proteins; IRF-3 = interferon regulatory factor 3; IKKε = IkB kinase epsilon; IKKs = IkB kinases; IRAK = interleukin 1-receptor-associated kinase; LPS = lipopolysaccharide; MAPks = mitogen-activated protein kinases; MyD88 = myeloid differentiation marker 88; NF-κB = nuclear factor-kappa B; PAMPs = pathogen-associated molecular patterns; ROS = reactive oxygen species; TBK1 = TANK-binding kinase 1; TIRAP = TIR-associated protein; TLR = Toll-like receptor; TRAF6 = TNF receptor-associated factor 6; TRIF = TIR-domain containing adaptor inducing INF-β; TRAM = TRIF-related adaptor molecule.]
INNATE IMMUNITY AND ORGAN TRANSPLANTATION
As reviewed, innate immune events play a critical role in organ transplantation.1,2 Accumulating evidence suggests that reactive oxygen species (ROS)-mediated allograft injury, occurring during donor brain death condition and graft reperfusion during implantation in the recipient, canonically leads to TLR-mediated maturation of donor-derived DCs (residing already in the graft) and recipient-derived DCs (entering the graft during reperfusion). Efficient uptake and subsequent presentation of alloantigens on major histocompatibility complex (MHC) molecules by DCs as well as TLR-triggered up-regulation of costimulatory molecules on matured DCs, in concert with secretion of proinflammatory mediator substances, orchestrate the development of antigen-specific adaptive alloimmunity. Innate lymphocyte-triggered DC maturation may potentiate and expand the alloimmune response. In addition, growing evidence from experimental studies on reperfusion injury models suggests that TLRs, in particular TLR4 and TLR2, following interaction with DAMPs such as HSP70 and high mobility group box 1 (HMGB-1), appear to trigger the same intracellular signaling pathways as initiated by TLRs following recognition of PAMPs. Also, the possibility can be discussed that the high intragraft concentrations of ROS, acting as so-called second messenger molecules, up-regulate the transcriptional gene activation via posttranslational modifications of the TLR-signaling cascade. The result is a dramatically up-regulated innate immune response (Figs 1 and 2).

Fig 2. Oversimplified schematic illustration of intracellular TLR-triggered/mediated signaling pathways resulting, via activation of transcription factors NFκB, AP-1, and IRF3, in the expression of “innate” genes involved in inflammation, antiviral responses, and maturation of DCs. Widening of the “hourglass”: The high intracellular concentrations of reactive oxygen species, generated during reperfusion injury and acting as second messenger molecules, facilitate the signaling pathways via oxidant-dependent posttranslational modifications. The result is an up-regulation of transcriptional gene activation associated with an increase of the innate immune response. For abbreviations, see legend to Fig 1.

SUPPRESSION OF INNATE IMMUNE EVENTS
"Time-Restricted Therapeutic Window" and Potential Targets for Suppression of the Donor’s and Recipient’s Innate Immune Systems
As mentioned, donor brain death condition as well as donor organ reperfusion injury in the recipient represent a significant oxidative allograft injury that leads to activation of the innate immune system of both the donor and the recipient. Logically, prevention of innate immunity-mediated acute allograft rejection must take into account therapeutic strategies applied to the donor during organ removal and the
recipient during/immediately after allograft reperfusion. Therefore, this strategic concept reflects an a priori time-restricted therapeutic window! The main goal to inhibit innate immune events in these situations refers to the application of antioxidative agents, namely for 2 reasons: first, to avoid the oxidative allograft injury associated with development of an intragraft inflammatory milieu which sustains and expands innate immune processes, in particular DC maturation; and second, to keep the hourglass of the innate signaling cascades as narrow as possible by avoiding oxidant-dependent posttranslational modifications, ie, to diminish “innate” transcriptional gene activation. Another major goal of preventive treatment modalities is the inhibition of DC maturation, a fulminating innate process which is induced by injury, mediated by TLRs, triggered by innate lymphocytes, and primarily controlled by the transcription factor NFκB. This therapeutic approach is based on the paradigm of tolerogenic/immature versus inflammatory/mature DCs. In fact, iDCs are prone to induce regulatory T cells and hence promote tolerance, probably by secreting suppressive cytokines.9,10 Finally, inhibition of innate effector functions appears worthwhile to be considered. As reviewed,11 a large list of agents exists which may principally and potentially inhibit events of innate immunity, starting from inhibition of the oxidative injury (ie, the initial event), and proceeding to inhibition of downstream events: inhibition of innate lymphocyte-triggered/TLR-mediated DC maturation, and inhibition of innate effector functions. As partially outlined in the text, some of the agents are already registered for treatment of certain diseases such as autoimmune diseases, some agents are currently in clinical phase II/phase III trials, eg, in patients with acute myocardial infarction and in patients with sepsis, some compounds have been tested successfully in experimental models of innate immunity and some substances are of theoretical interest.

Fig 3. A list of agents which may principally and potentially inhibit events of innate immunity, starting from inhibition of the oxidative injury (ie, the initial event), and proceeding to inhibition of downstream events: inhibition of innate lymphocyte-triggered/TLR-mediated DC maturation, and inhibition of innate effector functions. As partially outlined in the text, some of the agents are already registered for treatment of certain diseases such as autoimmune diseases, some agents are currently in clinical phase II/phase III trials, eg, in patients with acute myocardial infarction and in patients with sepsis, some compounds have been tested successfully in experimental models of innate immunity and some substances are of theoretical interest.

Mitigation of Oxidative Allograft Injury

As shown (Fig 1), innate immune pathways are characterized by great complexity and probable redundancy. Therefore, with the aim of suppressing them, attempts should focus mainly on bypassing this complexity and redundancy by trying to address the early “upstream” initiating event rather than the subsequent “downstream” cascading events. In fact, during the past years, a large list of powerful antioxidative agents has been published. Some agents have gained increasing attention, among them edaravone and SOD-mimetics.12,13 Edaravone is approved in Japan for treatment of patients with acute cerebral infarction. Another promising antioxidant is the SOD-mimetic M-40403, which has been investigated clinically in patients after tooth extraction. Recent reports of experimental data on the prevention of the reperfusion injury with the use of small interfering RNA (siRNA), in order to silence the caspase 3 and caspase 8 genes, are promising and may announce the start of future clinical trials harnessing the phenomenon of RNA interference.14 In view of the fact that the Nobel Prize for Medicine and Physiology in 2006 was awarded to A.Z. Fire and C.C. Mello for their discovery of the RNA interference in 1998,15 increasing popularity of this therapeutic modality to inhibit events of innate immunity can be expected in the near future.
Inhibition of the MBL-Dependent Complement Cascade

Potential strategies for suppressing innate immune events include attempts to interfere with the MBL-mediated complement activation pathway. Recently, pexelizumab, which prevents complement-mediated myocardial damage from myocardial ischemia and reperfusion, has been developed. Two large clinical trials with pexelizumab, a novel anti-C5 complement monoclonal antibody fragment, are currently underway in patients with acute myocardial infarction or undergoing coronary artery bypass surgery. In addition, TP-10, a soluble complement-receptor-1 inhibitor which inhibits the activation of the complement system by inactivating C3a and C5a convertases, has been investigated in reducing ischemia-reperfusion injury in lung transplantation. Recent experimental data on the prevention of the reperfusion injury-induced complement activation using siRNA for silencing the C3 gene or the complement 5a receptor gene, are encouraging and may also call for the design of clinical trials.

Prevention of DC Maturation by Blockade of TLR4 or by Silencing the NFκB Gene

A novel small molecule TLR4 antagonist, TAK-242, has been shown in in vitro experiments to suppress production of multiple cytokines by selectively inhibiting TLR4 intracellular signaling. Interestingly, this TLR4 antagonist is currently undergoing clinical trials for the treatment of sepsis. A similar compound, Eritoran (E-5564), a synthetic antagonist of bacterial endotoxin, already investigated in a phase 1 trial, is also being developed to treat and/or prevent clinical sepsis. Recent experimental data from in vitro studies to generate tolerogenic DCs using decoy ODNs or siRNA for silencing the NFκB gene are extremely promising. In particular, in another in vitro study, treatment of iDCs with the antioxidant and NFκB inhibitor pyrroliidine dithiocarbamate (PDTC) led to an arrest in their maturation.

Prevention of DC Maturation by Deletion of Innate Lymphocytes

As known, polyclonal and monoclonal anti-T-cell preparations are routinely used to suppress alloreactive lymphocyte proliferation in transplant patients. For example, polyclonal Fresenius ATG “S” or the monoclonal antibody alemtuzumab (Campath-1H) can presumably also be used for the deletion of innate lymphocytes when administered to the donor during organ removal and to the recipient during allograft reperfusion. The proven beneficial effect on graft survival of only one shot of ATG during allograft implantation may be a mirror of its effect on events of innate immunity.

Blockade of Innate Effector Functions

Strategies to suppress events at the efferent arm of innate immunity include the use of monoclonal antibodies or fusion proteins against cytokines, chemokines, or adhesion molecules. Currently, humanized/chimeric monoclonal antibodies and fusion proteins against TNF-α (eg, infliximab [Remicade], adalimumab [Humira], and etanercept [Enbrel]) are registered and approved for the treatment of certain autoimmune diseases. Details may be found in the literature concerned. In addition, polyclonal anti-T-cell preparations such as Fresenius ATG “S” containing specificities against cytokines and chemokines should also be considered for this indication. With the aim of suppressing innate immune events in organ transplantation, these agents could be useful to treat (“off-label”) the donor during organ removal and the recipient during allograft reperfusion. Monoclonal antibodies against the adhesion molecules ICAM-1 (enlimomab) and LFA-1 (efalizumab) have been developed for treating transplant patients, however, data on these antibodies from prospective multicenter trials show no convincing efficacy. Application of polyclonal anti-T-cell antibodies containing specificities against adhesion molecules to the donor during organ removal and to the recipient during allograft reperfusion should also be considered.

The first gene-silencing studies aimed at inhibiting innate effector functions have also been performed successfully using either antisense ODNs or siRNAs. Thus, in a clinical trial, a beneficial effect of ICAM-1 antisense ODN (ISIS 2302) administered to kidney transplant patients could be observed.

REFERENCES

1. Land WG: The role of postischemic reperfusion injury and other nonantigen-dependent inflammatory pathways in transplantation. Transplantation 79:505, 2005


