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REVIEW

Modifying graft immunogenicity and immune response prior to transplantation: potential clinical applications of donor and graft treatment

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Summary

Many studies have shown a strong association between initial graft injury and poor long-term graft outcome. Events initiated by unspecific immune-activating processes including brain death and ischemia/reperfusion injury occurring prior to transplantation reduce graft functionality and amplify the host immune response. These events may be particularly relevant for less than optimal grafts with reduced resistance to unspecific injuries. Several approaches to ameliorate immune activation of the graft by treating the donor or the graft have been studied. While various substances have been shown to have protective effects in experimental transplantation, only a few drugs have been tested clinically and have demonstrated beneficial effects. We review the results of experimental and clinical studies on donor or graft immunomodulation prior to transplantation and analyze the evidence to support clinical application of these strategies.

Introduction

Immunosuppressive protocols have usually focused on the manipulation of the recipient's immune system. However, more recently, it has been shown that the allograft is not only the target of immune activation, but also an active component in initiating an immune response [1].

The quality and immune activation of the graft prior to transplantation is influenced by a variety of factors. Donor age, previous diseases in addition to the individual genetic profiles determine the physiological capacities of the transplant [2,3]. Unspecific immune-activating processes as a consequence of brain death and ischemia/reperfusion (I/R) injury may increase the immunogenicity in synergy with the quality of the graft.

Cadaver donor management, although not the main focus of this review, is of major importance and has been cited as the most neglected area of transplantation medicine [4,5]. In the early days of transplantation, the importance of donor maintenance and organ preservation was underestimated. After the observation that unrelated living donor grafts do better than HLA-matched cadaver grafts [6], antigen-independent risk factors received more attention. The fact that transplants between identical twins develop chronic graft deterioration also highlights the contribution of nonimmunological risk factors on overall graft function [7]. Today, up to 25% of potential donors are deemed to be unsuitable for transplantation prior to organ procurement [8]. Because of the ever-increasing discrepancy between supply and demand, the use of nonoptimal or expanded-criteria grafts has dramatically increased with currently 12% of kidney grafts coming from expanded criteria donors [9,10]. These organs have limited functional reserve and an increased susceptibility to ischemia, which, in turn, increases immunogenicity and predisposes to delayed graft function (DGF) as well as increased rates of

acute and chronic rejection [9,11–13]. For the extended criteria donors, the discard rate is even worse, with 40% of kidney grafts being discarded prior to organ procurement [14]. The increasing utilization of marginal grafts increases the need for more advanced donor management and specifically for measures to enhance the graft's resistance to inflammatory damages [15].

Graft activation and immunogenicity

Many studies have shown a strong association between organ quality, nonspecific damages prior to transplantation and poor long-term graft outcome [16–20]. Poor organ quality and cellular injury prior to transplantation increase the risk for DGF, acute, and chronic rejection while potentially preventing tolerance induction [17,21]. In addition, the initiation of the rejection process may also be activated by the injured graft itself [22]. The most important determinants of graft survival are the age of the donor, brain death, and I/R injury [23].

Reduced capacities to cope with cellular stress and activated antigen-presenting cells (APC) may contribute to an immune activation in organs from elderly donors [11,24,25]. Gene expression profiles demonstrated alterations with increasing age, however exceptions in those patterns also point toward the importance of 'biological' aging [3].

Kidney grafts from living donors have a significantly prolonged long-term graft survival compared with cadaveric organs from brain dead donors [6]. Clinical studies showed that the gene expression profile in kidneys from brain-dead donors is different compared with grafts obtained from living donors [29–32]. While most of the upregulated genes in cadaveric kidneys were related to inflammation, redox state, metabolism, cell-cycle regulation, and protein modification cytokine profiles of liver transplant recipients were different in cadaveric grafts compared with grafts from living donors [31–33].

After brain death, a series of neural, hormonal, and molecular changes occur, resulting in cellular stress and inflammatory response [26,27]. These events lead to reduced cell defense mechanisms and increased graft immunogenicity inducing a host alloimmune response even in the absence of nonself antigens. It is hypothesized that the initial injury initiates allograft rejection by activating complement and coagulation pathways, recruiting inflammatory cells, promoting trafficking of dendritic cells (DCs) into the allograft, inducing the expression of major histocompatibility complex (MHC) molecules and costimulatory signals, as well as regulating T-cell differentiation [28]. The initial graft injury associated with brain death, the harvesting procedure, and consequences of I/R limit the function of 'marginal grafts' even more [9,13,34].

Relationship between innate and adaptive graft recognition

Traditionally, recognition of alloantigens and triggering of the immune response to the allograft have been associated with the adaptive immune system and mediated by antigen-specific T- and B cells. The innate immune system, composed of monocytes, macrophages, neutrophils, eosinophils, and natural killer (NK) cells, was believed to be important in protecting the host against infectious agents rather than being involved in specific immune responses. These cells express semi-specific receptors [Toll-like receptors (TLRs)] that recognize ligands (e.g. lipopolysaccharides, teichoic acids, double stranded RNA, etc.) present on a broad range of pathogens, called pathogen-associated molecular patterns [35]. These receptors activate transcription factors such as NF-κB and induce the expression of inflammatory genes [36]. However, recent studies have shown that the innate immune system also recognizes allogenic and xenogenic grafts through TLRs [37].

Interactions between the innate and adaptive immune response may be implicated in the association of ischemia reperfusion injury with acute and chronic rejection [38]. It has been shown that the innate immune response has a major influence on the adaptive immunity by enhancing T-cell priming [35]. The innate immune system produces cytokines and chemokines that are critical for the trafficking of activated T cells. Some cytokines and chemokines produced in response to allografts are not detected in isografts, implying a more specific response [39–41]. Complement receptors have been found on T cells, B cells and APCs, which may represent a link between innate and adaptive systems. Activation of complement, an unavoidable event after transplantation, increases graft immunogenicity possibly by opsonization of graft cells and enhancement of antigen presentation, which increases the incidence of rejection [42,43].

Toll-like receptors and downstream mediators are thought to link innate and adaptive immunity. TLRs expressed on APCs may regulate co-stimulatory signals and cytokine production, which, in turn, modulate the strength of the adaptive immune response [44]. Mice lacking MyD88, a protein that mediates most TLRs signals, demonstrated an impaired Th1 response and were unable to reject minor-mismatched skin allografts [45]. On the other hand, the innate system also depends on the adaptive immune system. It has been shown that T cells are required for macrophage activation [37] while the adaptive immune response can enhance the innate inflammatory response. Recombination activating gene (RAG) knockout mice lacking adaptive immunity still have a strong immune response briefly after cardiac trans-

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plantation. The expression of innate immunity markers was greatly amplified in the allogenic, but not in syngenic group emphasizing that innate immunity is activated or enhanced by alloantigens [46]. Along the same line, it has been shown that NK cells have different responses depending on the microenvironment [47].

However, the host immune response is not only dependent on cells classically thought of as being part of the innate and adaptive immune response. All cells in the graft but especially DCs are able to initiate an immune response. Parenchymal cells are not just targets of the alloresponse, but also play an active role in the rejection process. Stimulated by inflammatory conditions initiated after brain death, the harvesting procedure and amplified by I/R injury, parenchymal cells can overexpress MHC antigens, produce inflammatory cytokines and adhesion molecules, and finally undergo apoptosis. Under those circumstances, these cells can also express MHC class II antigens [27,48].

Activation of DCs in this scenario is of particular relevance for the increase in graft immunogenicity [49,50]. Solid and cellular grafts contain DCs in an immature stage [51]. There is an increasing body of evidence showing that DCs are activated by 'danger signals', substances produced by distressed or injured cells (DNA, heat-shock proteins, inflammatory cytokines, breakdown products of cellular membrane, etc.) [22]. This initial injury provides the maturation signals that DCs need to migrate and induce T-cell activation [52]. When DCs mature in the presence of inflammatory signals, they increase the expression of class I and II MHC antigens and costimulatory molecules, thus increasing the production of cytokines and amplifying the immune response. In addition, when donor DCs die in the recipient's lymph nodes, they can cross-prime antigens through the indirect pathway of allorecognition [53–57].

Rationale of donor treatment

Minimizing initial cellular stress and damage associated with an inflammatory immune response may impact the overall need for post-transplant immunosuppression while increasing the availability of organs for transplantation [5,27,58,59]. The time between the diagnosis of brain death and organ harvesting, as well as the storage period, could be used to prevent or minimize graft immune activation. Donor therapy may be particularly relevant for the transplantation of extended criteria grafts, which are less apt to cope with cellular stresses [13,60,61]. Experimental studies from our group have demonstrated that the treatment of old donors with immunosuppressive agents significantly improved kidney graft function long term [62,63].

Strategies of donor treatment

Various strategies have been used for donor/graft treatment. Those include pharmacotherapy (immunosuppressive, anti-inflammatory and chemotherapy drugs, cytokines, vasoprotective agents, monoclonal antibodies, and antioxidants), irradiation [gamma or ultraviolet (UV) irradiation of the graft], cell transfer experiments (bone marrow cells, blood, splenocytes, DC, and lymphocytes), temporary controlled-warm ischemia (ischemic preconditioning), and gene therapies (liposomes and virus vectors). These approaches were accomplished either by treating the graft itself during perfusion or cold storage or by treating the donor prior to graft procurement.

Treating the donor has the advantage of preserved cellular metabolic pathways, while most pharmacological agents are inactive or insoluble in hypothermic preservation solutions. In addition, poor permeability of membranes and inhibited active transport mechanisms in hypothermic conditions may compromise drug access [64,65]. Similarly, genetic modification of organs is limited as viral vectors have a very limited transfection rate under these conditions [66].

Machine perfusion preservation with hypothermic perfusion provides the best quality and longest preservation of kidney grafts [67]. It has been postulated that it reduces the accumulation of toxic substrates and free radical formation on reperfusion, thus minimizing the consequences of I/R injury. Continuous perfusion permits, in theory, also the use of normothermic solution, which is more appropriate to promote active graft modulation. When cell metabolism is maintained, both pharmacological agents and viral vectors are more efficient in promoting protection. Normothermic organ preservation may be particularly advantageous when utilizing marginal grafts [68,69]; however, there are many obstacles to be overcome before normothermic preservation will be applied routinely in the clinical settings.

Gene transfer strategies have the advantage of selective delivery of molecules with immunomodulating activity to the graft itself. Experiments on gene therapy using antiapoptotic genes [Bcl-2, Bcl-xL, A20, and HO-1 (heme oxygenase-1)], Th-2 cytokines (IL-10, TGFβ-1), antioxidants (superoxide dismutase, iNOS), donor MHC class I and II antigens, genes blocking costimulatory signals (e.g. CTLA4Ig), and recombinant ligands genes have prolonged graft survival in rodent models [70].

After brain death, there is a progressive deterioration of the graft. The extent of the initial graft injury is associated with the strength of the immune response and further graft damage [21,71]. Therefore, preservation therapies should be initiated immediately after the confirmation of brain death and prior to occurrence of further

immune activation, in order to block the vicious cycle of injury and increased immunogenicity. In most experimental and clinical studies, the time frame between treatment and organ retrieval has ranged from 2 to 18 h. A maximum survival-prolonging effect was obtained with an interval of 6 h between treatment and organ retrieval [72].

There are several nonexclusive theories to explain the beneficial effects of donor treatment. Those include the elimination of highly antigenic cells, the reduction of the overall antigenicity of all cells in the graft by interfering with cell markers and expression, modifications of the spatial conformation of antigens or their release into the recipient circulation, a drug 'carry-over' effect and an optimization of microcirculation [72]. Most approaches to treat donors have focused on immunomodulation of the graft by depletion or modulation of APCs, and/or cytoprotection to increase the resistance to unspecific injuries.

Donor immunomodulation

Modulation of DCs

The most frequent approaches to deplete grafts from DCs include gamma irradiation, cytotoxic drugs, photosensitizer + UV radiation, and antilymphocyte antibodies. Donor or graft treatment of endocrine allografts for a selective elimination of DCs can result in indefinite allograft survival in immune-competent recipients [73]. However, results on solid organ transplantation show less impressive results following DC depletion. There is some evidence that DC function may be necessary for the development of tolerance induction as it can induce regulatory T cells. It has been shown that low-dose UV B pretreatment of human islets may reduce immunogenicity by reducing the expression of costimulatory molecules (e.g. ICAM-1 and B7) on DCs [74]. Immunosuppressive drugs have different effects on DCs [54,75–78]. Calcineurin inhibitors seem to have little effect on DC maturation, although inhibiting allostimulatory capacity and cytokine production. Steroids and vitamin D analogs affect all stages of DC maturation and their function. In our own experimental studies, we were able to demonstrate that donor treatment for the induction of HO-1 was associated with reduced donor-specific DCs in all recipient compartments [79].

An increasing number of experiments using genetically engineered DCs that constitutively express IL-10, TGF-β, FasL, or CTLA-4Ig have been reported. One study demonstrated a single adenovirus administration carrying the gene of CTLA4-Ig to the donor 24 h prior to transplantation associated with an indefinite graft acceptance [80].

Donor cytoprotection

Graft cytoprotection maintains cellular functionality and reduces graft immunogenicity at the same time. Potential candidates include antioxidants, membrane stabilizers, and antiapoptotic molecules. The most investigated approach is the modulation of the HO-1 system.

Many reports have shown beneficial effects of HO-1 in transplantation. HO-1 induction may potentially reduce I/R injury, inflammation, apoptosis, allo-mediated cell toxicity, and graft-versus-host disease (for a comprehensive review, see ref. 81). HO-1 overexpression has been shown to increase the viability of grafts after a prolonged cold storage in a series of transplant models [82–85]. HO-1 overexpression also enhances intracardiac expression of antiapoptotic proteins Bcl2 and bag1 [83] and prevents expression of adhesion molecules on endothelium [86]. In addition, accelerated transplant arteriosclerosis, the main manifestation of chronic rejection, has been inhibited by the induction of HO-1 expression [86]. Most experimental models inducing HO-1 have used metalloproteins (FePP and CoPP) or gene therapy. CO, a by-product of HO-1 metabolism, has been shown to reproduce the protective effects of HO-1 in many transplantation models [87–90]. It has the advantages of being active and penetrating the cell membrane in cold temperatures. Bilirubin and biliverdin, down-stream products of the HO-1 metabolism, have also been also associated with protection against I/R injury [91].

Limitations of donor pretreatment strategies

Gene therapy, although very selective, is frequently limited by low transfection rates, transient gene expression, and a potential immune activation because of viral-vectors [66,70]. The 'one-for-all' principle does not apply for donor pretreatment. Some drugs use organ-specific pathways and toxicity levels may show organ specificities. While dopamine treatment was associated with improved results following kidney transplantation, similar effects could not be observed following liver and heart transplantation. Side effects may have included mesenteric spasms and reduced mitochondrial redox states [92,93].

Clinical studies

Most clinical studies exploring donor therapy were single-center, uncontrolled, and have not investigated the impact on long-term outcome [94–99]. Methylprednisolone has been used in several clinical studies. Guttmann *et al.* [100] showed that kidney recipients of cadaver donors treated with high doses of cyclophosphamide, methylprednisolone, and methotrexate had better renal function

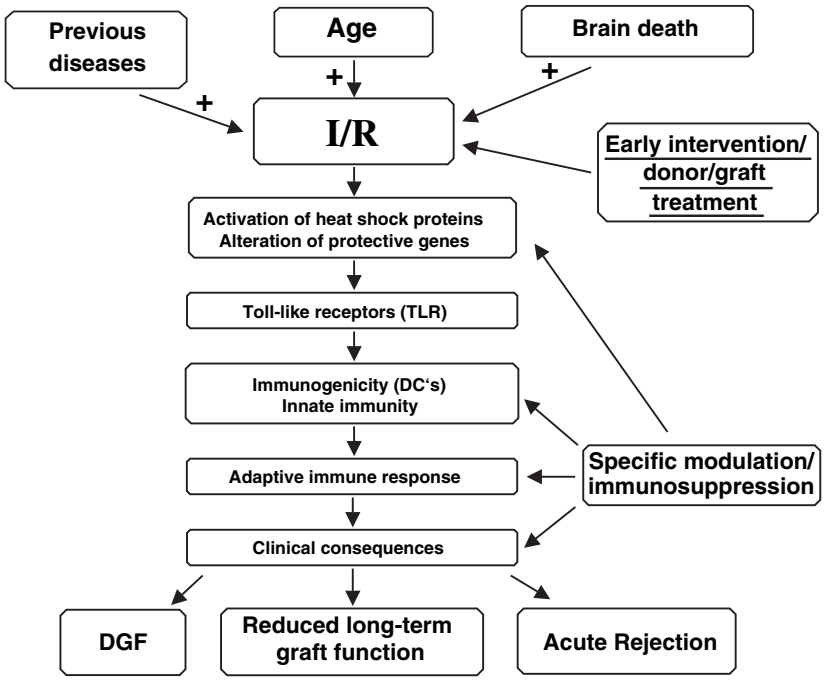

Figure 1 Potential interaction of donor factors with the innate and adaptive immune response to an allograft. Donor treatment may reduce the immunogenicity of an organ transplant, ameliorate the incidence of DGF and acute rejection, and improve long-term graft function.

and showed the reduced frequencies of early and late rejection episodes. In another series, the same author showed that grafts treated with methylprednisolone and cyclophosphamide were superior to those obtained from untreated donors, showing 5-year graft survival rates of 66% vs. 53% [96]. Treatment of brain-dead donors with methylprednisolone 250 mg i.v. bolus followed by 100 mg/h until laparotomy resulted in significant decreased expression of pro-inflammatory cytokines comparable to levels observed in grafts from living donors [33]. Other clinical studies showed that high-dose administration of methylprednisone to brain-dead donors improved oxygenation and significantly increased the recovery of lung grafts [101]. However, not all studies demonstrated beneficial effects following donor steroid therapy [102–104].

In clinical liver transplantation trials, prostaglandin I_2 (Epoprostenol) application has been shown to reduce the levels of transaminases post-transplantation. Proposed mechanisms of protection include splanchnic vasodilatation, decrease of protease and free oxygen radical release, improvement in sinusoidal perfusion, and reduction in leukocyte adherence [105].

Recently, multi-drug treatment strategies of cadaver donors have been proposed. This treatment is based on the administration of the so-called 'Papworth cocktail' consisting of methylprednisolone, insulin, tri-iodothyronine, arginine, and vasopressin in association with intense cardiovascular monitoring and was associated with a significant increase of organs procured and transplanted per donor [106,107]. Two independent clinical studies demonstrated the beneficial effects of catecholamines including dopamine application for the protection of kidney grafts. While kidney graft survival had improved significantly, liver graft survival had not improved and heart transplants showed a trend toward reduced transplant survival [108–110]. In addition to their hemodynamic effects, catecholamines may be effective by modulating cytokine production, adhesion molecules expression, and up-regulation of HO-1 [111–114].

Conclusions

Graft quality and immunogenicity determine, at least in part, the success of organ transplantation. The graft is not only the target, but may also direct the host immune response. Indeed, innate and adaptive immune responses act in concert and can be influenced by donor treatment. Although various experimental studies have shown the benefits of donor treatment, clinical results remain scarce. Clearly, donor treatment offers the opportunity to increase the amount of organs available for transplantation and may ameliorate the immune response (Fig. 1).

Most clinical experiences are based on single-center, noncontrolled, nonrandomized studies and lack long-term results. Prospective randomized multicenter trials are necessary to gain clinical experience. Organ specific differences and early markers of unspecific injuries need to be considered.

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