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INNATE IMMUNITY AND ORGAN TRANSPLANTATION

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INTRODUCTION

The first evidence that initial events of innate immunity may subsequently lead to both acute and chronic rejection of allografts comes from clinical observations on patients at my former Munich institution: In 1994, - without virtually mentioning the term "innate immunity"-, we had described a phenomenon of this host defense system, namely that a non-antigen-dependent, reperfusion-induced inflammatory injury to renal allografts affects their short-term and long-term outcomes. The concept nicely fitted in the "Danger Hypothesis" of POLLY MATZINGER which was published a few months later in 1994. In the following years, 1996-2000, events of innate immunity were discovered by geneticists and immunologists, in particular in studies with Drosophila. Meanwhile, the crucial role of events of innate immunity in organ transplantation has been well recognized. Thus, a couple of months ago, I summarized the current knowledge about this topic in an overview article in "Transplantation" when emphasizing that not only the reperfusion injury to allografts but any non-antigen-dependent inflammatory pathway may impact both acute and chronic rejection after transplantation (1).

Originally it has been thought that the innate immune system represents an evolutionarily conserved, rapid first line of host defense against invading microbial pathogens with the aim to eliminate them. But there is growing experimental and clinical evidence suggesting that it is more than just a defense system against invading microbes.

In analogy to the specificity of the adaptive immune response, innate immunity is extremely selective and has divided the universe into innocuous "self" and potentially noxious and dangerous substances- ranging from "microbial nonself" – the pathogen-associated molecular patterns: the "PAMPs" to "altered, i.e. damaged self" in terms of autologous toxic cell debris, for instance from necrotic cells, which - in analogy to "PAMPs" - I use to call "DAMPs" : damage- associated molecular patterns. Heat shock proteins = stress proteins generated during any stressful tissue injury, for example, represent classical "DAMPs".

The innate system is predominantly represented by cells such as dendritic cells, natural killer cells (NK cells), vascular cells, and epithelial cells, which are able to recognize pathogens via special receptors such as Toll-like receptors (TLRs) and by this, represent first line barriers against microbes that may invade the body via the blood stream and/or, for example, via the skin or the urinary tract. Those cells, once activated, elicit a local response by producing antimicrobial proteins such as proinflammatory cytokines, chemokines as well as defensins and cathelicidins.

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Besides those cells, the complement system is a further classical instrument of innate immunity. Long before the human Toll-like receptors had been discovered, other pathogen sensors have been described such as the mannose-binding lectin (= MBL), a member of the family of the collectins. MBL is a recognition molecule which circulates in the plasma and is able to recognize a variety of pathogens from their sugar structures, followed by activation of the complement cascade - not by utilizing the classical or the alternative pathway - but via the lectin-pathway. Of particular note is that recent studies have shown that the postischemic reperfusion injury is associated with the activation of complement by utilizing this lectin-pathway which additionally contributes to tissue damage.

INNATE IMMUNITY AND HOST DEFENSE

It is now well established that the activation of adaptive immune responses against pathogens requires direction from the innate immune system. In case of pathogen invasion, immature dendritic cells engulf pathogens by phagocytosis, an events that is promoted by a TLR-induced gene programme. In addition, Toll-like receptors recognize pathogen-derived components, the PAMPs, in terms of exogenous ligands of TLRs. These events lead to maturation of dendritic cells, associated with presentation of pathogen-derived peptide antigens, as well as, via gene activation, with TLR-induced up-regulation of costimulatory molecule expression. Phagocytosis-mediated antigen presentation, together with TLR-mediated expression of costimulatory molecules, and secretion of proinflammatory cytokines instruct development of antigen-specific adaptive immunity leading to host defense which really reflects an orchestrated action of innate and acquired immunity.

Stimulation of TLRs by microbial components initiates signal transduction pathways which trigger expression of several genes that are involved in innate immune responses.

One classical example of an innate host defense is the recognition by TLR4 of LPS (endotoxin) from gram-negative bacteria. In brief, the LPS-triggered TLR-signaling pathways, described so far, utilize different adaptor molecules, MyD88 is one important molecule. The MyD88-dependent pathway, specified by another adaptor molecule, TIRAP, leads to the activation of the transcription factors NF-êB as well as - via the MAP-kinase pathway – to another master transcription factor, AP-1. Both factors induce expression of proinflammatory mediator substances such as cytokines and chemokines. In addition, other TIR domain-containing adaptor molecules, TRIF and TRAM, are essential for the MyD88-independent pathway. This pathway mediates activation of transcription factor IRF3 which induces expression of type I interferons, obviously via an autokrine and /or parakrine loop. In addition, this pathway leads to up-regulation of expression of the costimulatory molecules. Interestingly, TLR4-triggered utilization of TRIF/TRAM adaptors leads via TRAF6 to the activation of NF-êB in a later phase.

INNATE IMMUNITY AND ORGAN TRANSPLANTATION

From an evolutionary point of view, the immune defense systems in mammals have been developing over hundreds of millions of years. In view of this immense period of time, the fifty -year -existence of organ transplantation is just an “eye blinking”. Thus, there is no reason to assume that, during this ultra short period of time, nature has created an immune system against allografts that is different from that operating as an efficient host defense against infectious pathogens. My conclusion, therefore, would be that the innate immune response *must* be involved in allograft rejection as well. And what we as transplant surgeons and physicians have to do is to search for the mechanistic links between host defense against pathogens on one side and rejection of allografts on the other side. Indeed, the cardinal link seems to be the initial tissue injury which is not only mediated by a large variety of pathogens associated with PAMPs but also mediated by *any* injury, including allograft injury. All those injuries, when occurring, are associated with the appearance of damage-associated molecular patterns, the DAMPs, in terms of endogenous ligands of TLRs.

EXPERIMENTAL AND CLINICAL DATA IN SUPPORT OF THE CONCEPT:ACUTE ALLOGRAFT REJECTION

xperimental and clinical findings in support of the concept are emerging: First of all, there is a growing list of putative endogenous ligands of TLRs arising during cell/tissue injury including different heat shock proteins which bind to both TLR2 and TLR4, as well as heparan sulfate, hyaluronic acid, and fibronectin. These molecules, in terms of DAMPs, are able to activate cells of the innate immune system via interaction with Toll-like receptors. In fact, expression of those endogenous ligands have already been found in human allografts by the Munich group under the leadership of

HELMUT ARBOGAST: We were able to demonstrate the inducible HSP70 in renal allografts from brain-dead donors before and after reperfusion: HSP 70 - especially located in the tubular cells – is already demonstable after kidney removal and cold storage, but is being dramatically up-regulated 60 min after reperfusion.

As a matter of fact, this endogenous ligand HSP 70 is an important innate ligand that promotes alloimmunity – as reported by the DANIEL GOLDSTEIN´s group at the American Transplantation Congress in Seattle in 2005.

Further, there is accumulating evidence suggesting that the postischemic reperfusion injury activates the innate immune system via TLR4, which triggers the IRF3 – dependent, MyD88-independent innate pathway – as shown in a murine model of liver ischemia/reperfusion injury by JERZY KUPIEC-WEGLINSKI et al. This experiment has been confirmed using the same model by a Chinese group. Very recently, it could also be shown in a rat renal model, that the reperfusion injury activates innate immunity via involvement of TLR2 and TLR 4 and HSP70. In addition, DANIEL GOLDSTEIN et al again reported that minor antigen-mismatched allograft rejection cannot occur in the absence of MyD88 signaling although this finding could not be confirmed by the same group when using a fully MHC- mismatched murine allograft model. Nevertheless, also in this model, the TH1 immune responses are diminished in the absence of the MyD88 - adaptor protein.

Accordingly, the innate pathways leading - in analogy to host defense - to alloimmune – mediated rejection, may be discussed as follows: Immature dendritic cells engulf allogeneic material from damaged donor cells by phagocytosis. In addition, Toll-like receptors recognize DAMPs such as HSP70 in terms of endogenous ligands of TLRs. These events lead to maturation of dendritic cells, associated with presentation of allopeptide/MHC-complexes as well as - via gene activation – to TLR-induced up-regulation of costimulatory molecule expression. Together with secretion of proinflammatory cytokines, these events may lead to the development of alloantigen-specific adaptive alloimmunity resulting in allograft rejection.

Needless to mention, that, in the transplantation setting, we are dealing with donor-derived dendritic cells already residing in the graft (during brain death condition in the donor and reperfusion in the recipient) and leading to direct allorecognition as well as recipient –derived dendritic cells entering the graft during/after reperfusion and leading to indirect allorecognition.

EXPERIMENTAL AND CLINICAL DATA IN SUPPORT OF THE CONCEPT: CHRONIC ALLOGRAFT NEPHROPATHY (CHRONIC REJECTION)

The risk factors contributing to development of chronic allograft nephropathy are well known: besides antigen-dependent factors, there are antigen-independent factors such as reperfusion injury, hypertension, hyperlipidemia, hyperglycemia, HCMV infection, and administration of calcineurin-inhibitors. In fact, there is growing evidence suggesting that antigen-independent risk factors may operate via mechanisms of innate immunity. Notably, these chronic risk factors reflect a “double jeopardy” for successfully transplanted patients: Like a “Damokles” sword, some time after transplantation but any time, they might eventually lead to chronic dysfunction of the donor organ and/or to cardiovascular diseases of the recipient associated with morbidity and mortality. As known, one dominant common feature of these events is the development of atherosclerosis: allo-atherosclerosis of donor vessels and auto-atherosclerosis of recipient vessels. As a matter of fact and according to today´s notion, atherosclerosis does not reflect just a bland lipid storage disease but rather an event of inflammatory innate immune events. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease. As reviewed by QINGBO XU, there is a accumulating data indicating the involvement of heat shock-proteins in the pathogenesis of atherosclerosis which bind to Toll-like receptors and, thus, initiate an innate immune response. In addition, G.WICK, M. KNOFLACH, and Q.XU have reviewed autoimmune and inflammatory mechanisms in atherosclerosis and discussed the possibility that endothelial cells can bind microbial or autologous HSP60 via Toll-like receptors, providing a possibility for targeting adaptive or innate immunological effector mechanisms. The scenario based on data from the literature can nicely be illustrated by using the example of a Chlamydia infection: The induction of a heat shock response by pathogen - induced injury is followed by interaction of HSPs with TLRs -bearing vascular cells such as endothelial cells, smooth muscle cells and macrophages which get activated and, via local secretion of cytokines, chemokines, and growth factors, and expression of adhesion molecules, create an inflammatory milieu in the vessel wall initiating or contributing to atherosclerosis.

And the most interesting question in the context of this issue is: do these risk factors for chronic allograft nephropathy contribute to the development of allo- / auto-atherosclerosis by using the same pattern of innate pathways, especially do they induce endogenous ligands of Toll-like receptors such as heat shock proteins followed by activation of TLR-bearing vascular cells. A look at the current literature reveals the astonishing fact that they may really do so – as recently reviewed elsewhere (2). Thus, the scenario during donor brain death condition and allograft reperfusion injury in the recipient appears to be the clearest one: Oxidative stress induces a heat shock response followed by generation of heat shock = stress proteins, e.g. HSP70 - however, this time produced by damaged host cells! These DAMPs may activate TLR4- or TLR2 - bearing vascular cells resulting – via innate pathways - in alloatherogenesis.

Clearly, hypertension also elicits a heat shock response as demonstrated by many experimental and clinical studies which are not mentioned in this review. Of importance, however, is the underlying mechanism: Shear stress to endothelial cells as well as mechanical stretching of smooth muscle cells lead to non-native damaged proteins which induce an heat shock response associated with the appearance of stress proteins. Subsequent interaction with TLR-bearing vascular cells may lead to their activation resulting in a contribution to the development of inflammatory atherosclerotic lesions.

Hyperlipidemia, another risk factor for chronic allograft nephropathy, is also believed to activate the innate immune system via accumulation of proatherogenic LDL and minimally modified LDL. Indeed, recent in vitro studies have shown that minimally modified LDL operates as an endogenous ligand of TLR4 and induces proinflammatory cytokines which again may contribute – via innate pathways - to development of atherosclerosis. Data from several studies in MyD88-deficient and atherosclerosis-prone, apolipoprotein E-deficient mice as well as in patients with a TLR4 polymorphism support this concept.

Viral infections, such as HCMV infection, activate the innate immune system as well. In fact, there is accumulating evidence for a functional role of the TLRs in mediating antiviral effector mechanism. Further, it was recently shown that TLR2 and its cofactor CD14 recognize human CMV virions and trigger inflammatory cytokine production. Thus, evidence is growing suggesting that HCMV infection contributes to the development of allo-atherosclerosis by utilizing innate immune pathways. One can discuss the possibility that, besides virus-induced appearance of HSPs, HCMV virions interact with and activate TLR2- bearing vascular cells which is followed by the initiation of innate immune pathways.

Of special importance in successfully transplanted patients under immunosuppressive therapy is the atherogenic effect of calcineurin- inhibiting drugs which are known to induce a heat shock response via the induction of oxidative stress. CNI-induced reactive oxygen species lead to appearance of heat shock proteins which may be recognized by TLR – bearing vascular cells and renal epithelial cells, a phenomenon which may lead to the well-known CNI-associated chronic allograft nephropathy. This concept, as proposed by me two years ago, has recently been proven and confirmed by a most remarkable experimental study in rats performed by a South-Korean group.

CONCLUSION

The concept of a role of innate immunity for acute and chronic allograft rejection is based on the assumption that acute and chronic injurious events such as reperfusion injury, hypertension, hyperlipidemia, viral infections, and CNI-mediated injury activate – via induction of damage- associated molecular patterns such as heat shock proteins - cells of innate immunity: dendritic cells which represent the bridge to adaptive alloimmunity, and vascular cells which contribute to the development of allo-atherosclerosis. Indeed, this scenario implies a fascinating field for future research work of dedicated young transplant surgeons and physicians: Many unsolved issue in innate immunity in regard to organ transplantation are waiting for being investigated !

REFERENCES

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