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The Innate Immune System: Its Rediscovery before Toll Was Described

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Abstract

In 1994, in a prospective control trial in cyclosporine-treated, kidney transplant patients, we observed that treatment of a non-specific allograft injury (postischemic reperfusion injury) leads to a significant reduction in the incidence of both specific alloimmune-mediated allograft rejection and chronic allograft failure. From these convincing clinical data, we concluded in terms of an 'argumentum e contrario': it is the tissue injury that induces immunity. As from where we stand today in innate immunity research, these early clinical observations can be regarded as the discovery of the existence of a human innate immune system activated by tissue injury and preceding adaptive immunity.

Current notions in immunology hold that not only pathogen-induced injury but any tissue injury activates the innate immune system leading to infectious/sterile tissue inflammation and preceding adaptive immunity [1–3].

With respect to these modern notions on a fundamental role of the innate immune system in inflammation and adaptive immunity, a peer-reviewed article that dealt with this issue already at an early stage published in *Transplantation* in 1994 appears to be of interest [4]. In this article, we described the existence of a defense system in humans (without calling it 'innate immunity') before first studies on the discovery of the innate immune receptor Toll were published [5, 6].

Interestingly enough, our article appeared just a few months before Matzinger [7] published her famous danger hypothesis which was remarkably in line with our clinical observations.

These clinical observations derived from a prospective, randomized double-blind placebo controlled clinical trial that showed a beneficial effect of human recombinant superoxide-dismutase (SOD) on acute and chronic rejection events in kidney-transplanted patients [4].

The principal design of this clinical study in recipients under cyclosporine-based immunosup-

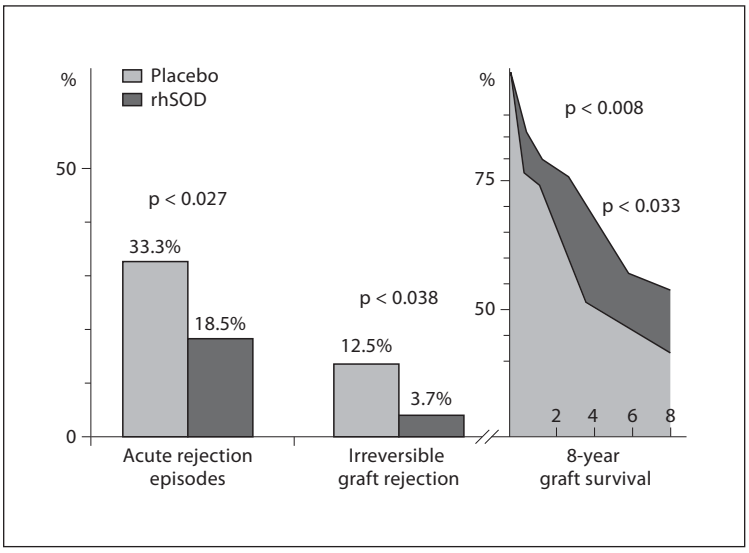

Fig. 1. Clinical data from the Munich SOD trial in kidney-transplanted patients under cyclosporine-based immunosuppression. Left: incidence of acute rejection episodes and irreversible graft rejection during the first year after transplantation was statistically significantly reduced in rhSOD-treated recipients (n = 81) compared to placebo-treated patients (n = 96). Right: long-term results were also significantly improved in rhSOD-treated patients. This difference of survival was most obvious 4 years after transplantation, still statistically significant at 6 years, and still demonstrable at 8 years although having lost its statistical significance.

pression consisted of intravenous administration of 200 mg of the free radical scavenger SOD given just once during surgery, that is, a few minutes before renal allograft reperfusion. During the subsequent 8-year monitoring phase, SOD-treated patients revealed a statistically significant reduction in the incidence of acute rejection episodes to only 18% and irreversible graft loss to 3.7%, respectively. The long-term results were also significantly improved, and most remarkably the beneficial effect was even demonstrable 8 years after a single injection of SOD (fig. 1). The therapeutic effect observed was dramatic: With regard to the incidence of acute rejection episodes under cyclosporine-based immunosuppression, the administration of a single dose of SOD prior to reperfusion is comparable to the application of 2 g mycophenolate mofetil (MMF) daily, that is, an accumulating dose of 730 g MMF within 1 year!

Thus, these clinical observations indicated that treatment of a non-specific allograft injury (=postischemic reperfusion injury) results in a significant reduction in specific adaptive immune events. From these convincing clinical data, we concluded in terms of an 'argumentum e contrario': tissue injury (here: allograft injury) activates a biological immune system that precedes and activates adaptive immunity (here alloimmunity). In the same article [4], this conclusion was extended into a working hypothesis, today known as the Injury Hypothesis. As illustrated in figure 2, a human immune system in its own right was proposed that is activated by non-pathogen-induced tissue injury (here the postischemic reperfusion injury to a renal allograft, that is, a situation where pathogens are obviously absent) and that, after activation, leads to the induction of an adaptive immune response (here an adaptive alloimmune response resulting in allograft rejection). In the center of this immune system, besides others, we proposed a role of antigen-presenting cells (later appreciated to be dendritic cells) activated by injury and subsequently leading to development of adaptive immunity, that is cells operating as a bridge between injury and adaptive immunity.

In addition, in this 1994 article, the possibility was discussed that adaptive immune response products (cytotoxic T lymphocytes, alloantibodies) induced by this system contribute – via endo-

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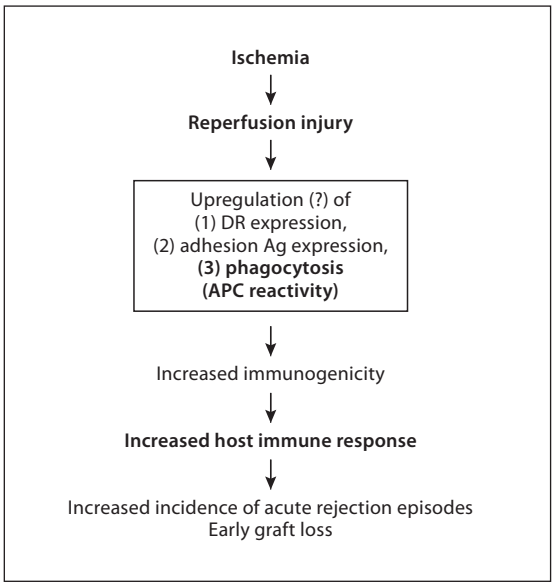

Fig. 2. This figure is traced, redrawn, and modified from figure 2 of the original 1994 article [4]. We proposed a human immune system in its own right that is activated by non-pathogen-induced tissue injury (here the postischemic reperfusion injury to a renal allograft) and that, after activation, leads to the induction of an adaptive immune response (here an adaptive alloimmune response resulting in allograft rejection). In the center of this immune system (apart from others), we proposed a role of antigen-presenting cells activated by injury and subsequently leading to the development of adaptive immunity.

thelial injuries (at that time called 'allograft endothelitis') – to chronic allograft dysfunction (at that time called chronic obliterative rejection vasculopathy).

In other words, as from where we stand today, in 1994, that is before Matzinger published her danger model [7, 8] and before the groups of Hoffmann and Beutler published the discovery of Toll and TLR4 [5, 6], we had discovered the existence of a human innate immune system activated by tissue injury and preceding adaptive immunity. We only missed to call it *innate immunity*. However, 2 years later, in 1996, in a review article, at least we briefly addressed this system as 'natural immunity' [9].

Our Injury Hypothesis – based on statistically significant clinical data – together with Matzinger's Danger Hypothesis – proposed entirely on theoretical grounds – could now extend the conceptual framework of the late Charles Janeway proposing that the immune system did not respond to all foreign antigens but only to those that are potentially associated with infection. Janeway's underlying idea was that the immune system evolved to discriminate infectious nonself from non-infectious self [10, 11]. In fact, Janeway's hypothesis turned out to be too simplistic. Most importantly, however, his model could not explain all immune responses, in particular, not the robust T cell-mediated alloimmune response leading to allograft rejection, a process in the apparent absence of microbial infection.

In fact, the danger/injury model can now explain why the innate immune system is able to mount an efficient immune response against harmful injurious pathogenic microorganisms, but not against harmless non-pathogenic microorganisms: it is the presentation of microbial antigens in the context of pathogen-induced tissue injury that triggers an efficient immune response – not simply the foreignness of microbial antigens. Likewise, the danger/injury model can also explain why the innate immune system sometimes mounts an efficient immune response against non-self foreign tissue such as transplanted alloantigens, but sometimes not, for example in case of fetal semi-alloantigens [12]. The answer is: the system distinguishes between an injured transplant (rejection) and a non-injured fetus (tolerance). Again, it is the presentation of alloantigens in the context of tissue injury that triggers an efficient alloimmune response, and not simply the foreignness of allogeneic tissue as reflected, for example, by an HLA-mismatch.

After the rediscovery of the innate immunity system, as published in first reports during the late 1990s/early 2000s, a concept of the potential impact of the innate immune events on allograft rejection was introduced by review articles to the

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transplant community in 2002/2003 for the first time [13–16]. In these reviews, in a subsequent article [17] as well as in a recently published monograph [18], the original Injury Hypothesis was extended and modified several times. Along with these modifications, we coined the terms 'innate alloimmunity' in 2002 [13] and 'damage-associated molecular patterns' (DAMPs) in 2003 [16]. Moreover, in 2003, we predicted that TLR4 mediates reperfusion injury-induced inflammatory response, a prediction that was confirmed only 1 year later by data of the group of Kupiec-Weglinski at UCLA showing that TLR4 activation mediates liver ischemia/reperfusion inflammatory response [19].

In particular, in these review articles, we proposed that oxidative stress to the brain-dead donor organism as well as the generation of reactive oxygen species during reperfusion of the allograft represent acute injurious events to the donor organ that, in turn, lead to acute rejection. By activation of donor/recipient PRR-bearing dendritic cells of the innate immune system via interaction of DAMPs with Toll-like receptors, these events lead to initiation of adaptive alloimmunity [17].

In our last published review article, evidence is provided in support of the notion that prevention of oxidative allograft injury may operate as an efficient tool in the clinical situation to present alloantigens under subimmunogenic conditions within an intragraft non-inflammatory milieu, thereby potentially generating tolerogenic dendritic cells able to induce Foxp3+ regulatory T cell-mediated innate allotolerance [20] – in fact an allotolerance-inducing principle that has been proven to be successful in elegant experiments in mice by Verginis et al. [21]. Indeed, such a concept may be discussed in view of our early clinical observation that the effect of a single intravenous injection of SOD to transplant patients is demonstrable even 8 years after its application – indicating that this free radical scavenger must have induced a fundamental long-lasting active suppressive process.

Although in earlier times heavily opposed and later on notoriously neglected by the transplant community, our Injury Hypothesis has just recently gained center stage and obviously appears well accepted by leading transplant immunologists [22, 23]. Time seems now to be ripe to think of new immunosuppressive strategies in organ transplantation such as interfering with the donor's innate immune system during organ removal and the recipient's innate immune system during allograft reperfusion, for example with the use of antioxidants, anti-IL-1β inhibitors, anticomplement agents, and polyclonal antilymphocyte preparations.

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