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Circulating biomarkers of tolerance

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A B S T R A C T

On the basis of reviewed literature here we describe models of tolerance and summarize the evidence of circulating biomarkers suitable for the assessment of immunological risk in organ transplantation.
We focused on results of evaluation of specific peripheral immune cell populations and transcripts in peripheral blood of operationally tolerant liver and kidney transplant recipients. Validation of described markers to define potentially tolerant patients before their use in clinical trials is critical.

1. Introduction

Despite the short term success to eliminate acute rejection, the long-term outcome of kidney transplantation remains unchanged as malignancies, infections, diabetes, and drug-related toxicity have frequently occurred as a consequence of immunosuppression. Contrary, inadequate control of the alloimmune response is the cause of chronic rejection, the main reason for late renal allograft loss [1,2]. Therefore, development of clinically relevant immune tolerance protocols, in which the long term immunosuppression is not necessary, seems to be viable goal of transplantation. There has been a great effort to establish tolerance through different strategies influencing central and peripheral mechanisms of tolerance [3,4] (Table 1). In fact most information about biomarkers of tolerance comes from operationally tolerant patients whose biological samples were thoroughly examined [5–7].

These biomarkers are suggested to help in identifying and monitoring of tolerant patient but also in patients with immunosuppression to guide drug minimization when needed.

In this review we describe current knowledge about circulating biomarkers of tolerance that might be implemented in monitoring of an alloimmune response in a near future.

2. Transplantation tolerance through bone marrow provision

Pilot study of centrally induced tolerance was performed by Billingham and his colleagues as early as in 1953. Immune tolerance was induced in a mice skin allograft model by administrating donor cell suspension to the host mice of a different strain early in its fetal life [8]. Thereafter, several attempts were performed towards intrathymic selection of alloantigen reactive T cells for establishing centrally induced tolerance. These animal experiments involved injection of allopeptides or donor splenocytes into the thymus along with peripheral leukocyte depletion. In human settings, similar procedures failed to induce tolerance towards alloantigens [9].

Another approach is based on administration of donor-derived hematopoietic cells simultaneously or consecutively with the transplanted organ to create chimerism [10]. Chimerism defined as the presence of donor marrow cells in the recipient induces donor-specific tolerance. Mixed chimerism, where the hematopoietic system of donor and recipient co-exists was shown to be tolerogenic and is associated with a lower risk of graft-versus-host disease (GvHD). Two forms of chimerism can be observed, micro- and macrochimerism. Microchimerism refers to the very low level of donor cells measurable in the recipient. It usually reflects trafficking of donor class II + dendritic cells from a graft into the recipient [11]. Macrochimerism is detectable by flow cytometry and reflects engraftment of the pluripotent hematopoietic stem cell [12].

After several preclinical studies dealing with mixed chimerism [13], Sachs group finally moved to clinical trials. Nonmyeloablative conditioning was shown to achieve long-term chimerism when used for patients with multiple myeloma [14]. It was thought that this type of conditioning is available for only HLA-matched donor–recipient combinations due to risk of graft-versus-host disease in HLA mismatched donor–recipient pairs. However, Kawai et al. described only transient chimerism and no GvHD in several HLA semi-mismatched kidney and bone marrow transplantation with successful immunosuppression weaning after nonmyeloablative regimen. However in one case irreversible antibody mediated rejection occurred and in some other patients donor specific antibodies were detected. Therefore in the next series, intensified rituximab dosage was used to target alloantibody production [15]. High intragraft levels of FOXP3 +, a marker of regulatory T cells, and decreased granzyme B expression, marker of inflammatory response, suggest regulatory mechanism involved in peripheral tolerance.

There are several other attempts to achieve chimerism. Scandling et al. used a combination of conditioning regimen by total lymphoid irradiation, anti-thymocyte globulin and donor hematopoietic cells infusion 10 days after in 16 patients. Eight patients were successfully withdrawn from anti-rejection therapy. These patients were monitored

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Table 1
Tolerance definition.

Central tolerance	Tolerance mediated by transplanted donor hematopoietic cells. Mechanism of central tolerance is based on the theory of recognition of donor antigens as "self" antigens.
Peripheral tolerance	Induction of tolerance without hematopoetic cell transplantation, through the use of either pharmacological immunosuppression or biological agent leading to anergy, deletion or suppression of self reactive T cells in periphery.
Operational tolerance	Tolerance observed in patients who stopped using immunosuppression for more than a year and in whom no destructive alloimmune response was noticed.

for changes in chimerism, balance of T cell subsets and responses to alloantigen. Early high ratios of CD4 + CD25+ regulatory T cells (Treg) and NKT cell versus CD4+ T cells were described in their blood [3]. In other study, eight patients received total body irradiation, nonmyeloablative chemotherapy, HLA-mismatched renal transplant from a living donor and infusion of tolerance-promoting facilitator cells and mobilized hematopoietic stem cells. In 5 patients immunosuppression was successfully weaned. Patients in this study were monitored for chimerism and for donor-specific tolerance by in vitro proliferative assays. Reduction in CD3 + Tcells/αβ-TCR+ lymphocytes early after transplant was observed. Furthermore, although there was an initial decrease in the absolute numbers of Tregs, an increase in the Treg to T effector (Teff) cell ratio was detected in durably chimeric recipients compared to recipients with only transient chimerism [4].

Taken together, transient chimerism in human transplantation was already achieved; however recent approaches do not prevent slow alloimmune response as defined by donor specific antibody production in a significant number of HLA mismatched recipients. Therefore, despite some progress, it is still premature to accept such approaches to be tested in larger cohorts.

3. Operational tolerance

Establishment of central tolerance is limited to only some cases due to reasons described above. In clinical practice, instead "operational tolerance" was achieved in patients who stopped their immunosuppression for more than a year and no destructive alloimmune response was noticed [16]. Operational tolerance was more often seen in liver transplantation, especially in the longer period after transplantation but also in some of kidney transplant recipients who decided to stop immunosuppression on their own [17]. About 10%–15% of liver transplant recipients were shown to develop operational tolerance in withdrawal trials [18].

In most cases of kidney transplant recipients, immunosuppressive withdrawal leads to the development of rejection that in some cases might occur as late as after several years [16,19]. However, "operational" tolerance or "prope" tolerance was observed in several patients free of immunosuppression. It is important to understand why these grafts were not rejected immediately and which mechanisms were involved in such status of immune quiescence vaguely defined as the absence of destructive alloimmune response.

In the last decade two international consortia, ITN (Immune tolerance network) and RISET (Reprogramming the Immune System for Establishment of Tolerance), collected data and evaluated biological material such as peripheral blood, sera, biopsies and urine [6,7].

4. Peripheral immune cells and transcripts in operational tolerance

Analysis of kidney and liver operationally tolerant patients revealed several subpopulations associated with induction and maintenance of transplantation tolerance, among them B cells [6,7,20,21], regulatory T cells [22], NK cells, γδTCR$^+$, CD8+ effector lymphocytes [23] and plazmacytoid dendritic cells [24]. Interestingly, enormous differences in biomarkers of operational tolerance in liver and kidney transplant recipients have been described.

4.1. B cells and related transcripts

Recently, many immune cell subpopulations, including B cells subpopulations were shown to be involved in the induction and maintenance of transplantation tolerance. Previously it was thought that B cells were primarily associated with development of antibody-mediated immune response, and B cell clusters in kidney graft tissue were shown to be associated with poor prognosis [25]. More recently, and contrary to this observation, several groups including ours have found immature B cells signatures to be associated with better early rejection outcomes [26–28].

Interestingly, operationally tolerant patients who had stable graft function despite the absence of immunosuppressive therapy for more than 1-year presented with distinct molecular signature. Most prominent and reproducible in different studies and cross-over validations performed in US and EU consortia (IOT, RISET, ITN) was the relation between operational tolerance and enhanced expression of B cell related transcripts as well as enhanced flow cytometric B cell counts in the peripheral blood compared to patients with chronic rejection. Sagoo et al. tested two independent groups of tolerant patients and found 174 genes common to both groups differentiating them from other comparator groups (Table 2). The majority of genes found to have significant association were enriched within B cell-related pathways [6]. These observations were confirmed by others [29,30], however frequently evaluating same patient cohorts.

In ITN derived study operationally tolerant patients showed up-regulation of 22 of B-cell specific transcripts and identified the combination of three transcripts (IGKVD1-13, IGKV4-1, IGLL1) to discriminate tolerance from controls. These 3 genes are all expressed during the differentiation of B cells from pre- to mature B cells or during B cell activation-induced transition [7]. Next, Moreso et al. evaluated the expression of IGKVD1-13 and IGKV4-1 genes in other study of kidney transplant patients treated with immunosuppression. Both genes were able to distinguish tolerant from stable patients, however in this study, samples from operationally tolerant cohorts were obtained from ITN. In CNI-treated patients' tolerance signature transcripts were upregulated while in azathioprine treated patients these markers were down-regulated in the longer follow-up [31].

Drug-free tolerant patients displayed increased numbers of B cells in peripheral blood, with decreased memory pool and increase in transitional and naïve B cell subsets. Enhanced expression of B cell differentiation and activation genes in tolerant patients was also reported [6,7,32–34]. Little is however known about the expression of B-cell signatures in stable transplant patients who are on immunosuppression. Brouard evaluated 144 stable kidney transplant patients treated with standard immunosuppression and reported only marginal similarity of peripheral transcripts with operational tolerance phenotype [35]. Our group performed a 12-month prospective observational study to monitor already known markers associated with kidney transplant operational tolerance. We demonstrated that immature and naïve B cells related and operational tolerance associated transcripts were up-regulated in peripheral blood in rejection-free kidney transplant recipients within the first year post-transplantation. In this study, patients with combined T cell- and antibody mediated rejections had the lowest immature B cell related transcripts in the peripheral blood. Moreover, higher expression levels of tolerance associated gene-1 (TOAG-1), a mitochondrial protein involved in the suppression of T cell activation [36], were observed in kidney graft tissue in rejection free patients [26].

Using gene expression and enrichment analysis of operationally tolerant patients, several B cell related transcripts were described, including those related to cell cycle (*CCNA 2, CCND2, BIRC5, CDC2, CDKN3, CKS2, PCNA*), proliferation (*CCNA2, CDC20, BUB1*) development