REVIEWS

Immunological Aspects of Cellular Transplantology

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The problem of surmounting the histocompatibility barrier and the mechanisms underlying the immune response to allo- and xenogeneic cells is discussed. Allogeneic grafts are recognized by T cells via direct and indirect pathways, while xenografts are recognized predominantly via the indirect pathway. Rejection of allografts is realized through recognition of foreign antigen by CD8 T cells, while xenografts are rejected after presentation of xenoantigens by host antigen-presenting cells to CD4 T cells. The differences in immune response to allo- and xenografts should be taken into consideration in the strategy of overcoming the histocompatibility barrier. The approaches to suppression of graft rejection are described in detail. Induction of antigen-specific tolerance proved to be the most optimal approach.

Key Words: *transplantation; immunity; tolerance; T cell*

Histocompatibility barrier is the major problem of transplantology. High level of polymorphism of the major histocompatibility complex (MHC) molecules and their codominant expression hamper the optimal choice of the donor—recipient pair. The effectiveness of medicamentous immunosuppression is not sufficiently high, since it affects both allo- and xenoreactive immunocompetent cells of the recipient, provokes immunodeficiency, and sometimes produces undesirable effects. Therefore, a strategy for selective prevention of antigen-specific destruction of transplants is necessary.

Replacing cell therapy is a prospective tool for developing such a strategy. The major advantage of this therapy is the possibility of reduction or elimination of immunogenicity of the donor cells before their transplantation. This can be done using the following approaches: modification of expression of immunogenic epitopes, removal of donor antigen-presenting cells, and immunoisolation to prevent sensitization of the recipient to implanted cells. Ectopic grafting of cell suspensions allows one to introduce cells into microenvironment favorable for their growth, differentiation, and functioning and simultaneously prevents the development of immune aggression against the graft. Induction of complete or split tolerance is the major component of the strategy, which is the same for organ and cell grafts. The choice of strategy is based on the nature of immune response to cell graft and potential consequences of immune reactions.

While considering the mechanisms underlying the response to cell and tissue grafts, it is necessary to find out whether there are principle differences between allo- and xenoreactivity. Since neovascularized cell and tissue allo- and xenografts are rejected predominantly with participation of T cells, it seems reasonable to analyze specific features of the T-cell response to allo- and xenoantigens.

At least two signals are necessary for activation of T cells [26]. One signal is provided through antigen-specific T-cell receptor, while the other is conveyed via the receptor for an antigen-specific co-

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stimulating molecule. Some cytokines produced by metabolically active antigen-presenting cells (APC) or cell interaction molecules expressed on the surface of T cells and APC serve as constimulators. CD28 and CTLA-4 on T-cells and their B7-1 and B7-2 counter-receptors on APC are the key molecules involved in costimulation [3,21]. T-cell response to graft is relaized via two pathways: 1) T cells of the recipient recognize antigens on the surface of donor cells that exhibit costimulating activity or 2) T cells of the recipient recognize antigens of the graft after their processing and presentation by APC.

The direct pathway of antigen presentation involves CD4 and CD8 T cells specific for class II and class I MHC molecules, respectively [37]. These T cells recognize MHC molecules on the donor's APC.

The indirect pathway includes processing and presentation of foreign antigens primarily in association with class II MHC molecules of the recipient with predominant activation of CD4 T cells. T cells activated via the indirect pathway are restricted by class II MHC molecules of the host and cannot directly interact with the donor cells. This model of direct (dependent on the donor APC) and indirect (dependent on the recipient APC) presentation of foreign antigens is essential for elucidation of the nature of T cell-dependent immune response to allo- and xenogeneic tissues.

The selectivity of indirect presentation of the transplant antigens in relation to class II MHC molecules is due to evolutionally conserved differences in the presentation of antigen by class I and II MHC molecules. Class I MHC molecules are antigens synthesized in the given cell. They are the host's and viral antigens. Since almost all cells in the body express class I MHC molecules, such a presentation of antigen allows T cells to recognize a hidden antigen, for instance, early products of viral genome prior to release of viral particles.

Thus, T cells recognize and lyze infected cell. Class II MHC molecules bind an antigen entering the cell from the extracelluar space and after processing present antigenic peptides on the cell surface. Class II MHC molecules are expressed by professional APC: dendritic cells, monocytes, macrophages, and B cells. Thus, the antigen becomes accessible for CD4 T cells, which receive two signals necessary for the development of cell-mediated immune response.

Let us consider specific features of immune response to allo- and xenoantigen. A very high occurrence of precursors of T cells that directly react to allogenic MHC molecules expressed by APC is a characteristic feature of alloreactivity [4]. Pronounced response of T cells has been observed even at minimal differences between stimulating and responding cells by class I or II MHC molecules. The potent allogenic response is due to a high polymorphism of MHC molecules. Although indirect recognition of processed alloantigens of APC-responding population also occurs in this case, allogenic response is realized predominantly via direct recognition [36], which determines clinical signs of acute graft rejection.

The intensity of T-cell response to xenogeneic APC shows a tendency to decrease as phylogenetic divergence between stimulating and responding cells becomes greater. The relative deficiency regarding species-specific interactions between T cells and APC is due to species-specific differences by the MHC molecules as well as by cytokines and their receptors [9,22,41]. The level of the receptor—ligand complementarity on T cells and APC correlates with the intensity of xenoreactivity. The response in xenosystems *in vitro* at a considerable phylogenetic divergence between donor and recipient is determined by recipient's APC.

It seems reasonable to analyze how direct and indirect presentation of antigen is related to phylogenetic divergence between donor and recipient. With greater extent of phylogenetic dissociation between the donor and the recipient, the manifestations of direct reactivity become less pronounced due to impaired species-specific interactions. It can be suggested that at deficiency of directly stimulated T cells antigenic differences between phylogenetically remote species become greater, i.e., the number of epitopes that can be processed and presented via the indirect pathway increases. As a result, alloreactivity is realized predominantly via the direct pathway, while xenoreactivity requires the indirect pathway. These differences are not absolute [5]. It is more important that the general scheme illustrates the differences between T-cell response to allo- and xenografts [15].

Presumably, rejection of allo- and xenogeneic pancreatic islet cells (PIC) in the treatment of diabetes mellitus depends primarily on the function of CD4 T cells [12,16].

In the process of graft rejection CD4 T cells act as 1) type I T helpers (TH_1) for CD8 T cells involved in PIC destruction; 2) type II T helpers (TH_2) for antibody-producing B cells, which generates antibodies against grafted cells and their products; 3) effector cells providing destruction of PIC by direct interaction with them or via inflammatory tissue damage by the mechanisms of delayed hypersensitivity (TH_1) [15].

Induction of immunity to allogeneic PIC is associated with CD4 T cells, while CD8 T cells are involved predominantly in allograft rejection [15,36]. However, xenoreactivity can develop in the absence of CD8 T cells restricted by class I MHC [14].

Studies of adoptive transfer of lymphocytes into immunodeficient C.B-17 *scid* mice showed that the presence of CD4 T cells is necessary and sufficient for the development of xenoreactivity to PIC, while alloreactivity requires participation of CD4 and CD8 T cells. In this model, B lymphocytes do not participate in rejection of allo- and xenografts, which can be regarded as indirect evidence for areactivity of TH_2 [13].

Thus, the major pathway of allograft rejection includes recognition of foreign antigen by CD8 T cells, while rejection of xenografts involves presentation of foreign antigens by host's APC. This way of antigen presentation leads to activation of the host CD4 T cells specific for the antigens of a xenograft in the context of class II MHC molecules of the recipient. This suggestion is confirmed by the fact that rejection of xenografts to a greater extent depends on CD4 T cells, while rejection of allografts is mediated only by CD8 T cells. Primary role of CD4 T cells in the recognition of an allograft consists in providing the function of CD8 T cells, while it the recognition of a xenograft they are involved in the development of destructive inflammatory process after interaction of CD4 T cells with antigens of the graft associated with host's MHC.

If the immunity to an allograft is realized via the direct pathway, the effector mechanism manifests itself as antigen-specific recognition of grafted cells. After xenotransplantation, the indirect pathway facilitates the antigen-nonspecific effector mechanism which is realized via toxic effects of cytokines and other mediators of inflammation, which are formed due to interaction between CD4 T cells with an antigen presented by APC.

Thus, CD4 T cells can induce xenograft rejection even if grafted cells do not express class II MHC molecules (for example, β-PIC). The same mechanism underlies PIC destruction in spontaneously developing autoimmune diabetes [38].

The differences in the nature of allo- and xenogeneic responses should be taken into consideration in the strategy aimed at reducing graft immunogenicity. Upon allotransplantation it is necessary to eliminate or inactivate donor type APC for prevention of direct recognition of foreign antigens. This is attained by culturing grafted cells *in vitro* [1,2,6,29]. Elimination of direct recognition of an allograft markedly (about 100-fold) reduces the repertoire of antigen-reacting T cells [4].

Immunogenicity of grafted cells can be reduced by their culturing at low temperatures, application of antibodies to class II MHC antigens or markers of dendritic cells, ultraviolet irradiation, exposure to low pH, and the use of fetal cells. These approaches have been employed to decrease immunogenicity of thyroid, parathyroid, and ovarian cells, hepatocytes, keratinocytes, and PIC [18,20,24,25,32,34].

In xenotransplantation, the necessity of removing donor type APC is determined by phylogenetic divergence of donor and recipient. Allo- and xenotransplantation of fetal PIC have been successfully used in Russia for the treatment of autoimmune diabetes mellitus without medicamentous immunosuppression. Transplantation of pooled PIC obtained from several donors provides immunological tolerance to the graft free from donor type APC [1,2].

Immune ignorance as a result of overload of host's APC by a broad spectrum of antigens which are individually expressed on the APC surface may be responsible for immune resistance to allogeneic PIC grafts. Since PIC do not express class II MHC molecules, PIC destruction should be provided by short-distance transmitters produced upon interaction of TH_1 with antigen presented by APC in immediate proximity to the graft. On the surface of APC class II MHC molecules present class I MHC allopeptides or a set of xenopeptides, including MHC peptides. In the first case, there is a competition for the binding sites for class II MHC molecules of host's APC between quantitatively prevailing monomorphic (identical for all donors and recipient) and polymorphic (determining allospecificity) MHC peptides. In the second case, xenoantigen peptides from all donors compete for these sites. In both cases a broad spectrum of individual antigens belonging to several genetically different donors does not create the conditions for presentation of the necessary amount of homogeneous antigenic epitopes of each APC for T helpers with certain specificity.

Immunoisolation of transplanted cells is another approach to the solution of the histocompatibility problem. Microincapsulation of graft should meet the following demands: prevent immune recognition of grafted cells and provide release of their metabolites that correcting the function of damaged system in the recipient [35].

In allotransplantation this approach makes the contacts between grafted cells and host's T cells impossible, i.e., provides blockade of the direct recognition pathway. The effectiveness of this approach is problematic in xenotransplantation, since antigens exfoliated from grafted cells may penetrate the capsule wall and be recognized by the host's APC [18]. This problem can be resolved by application of capsules impermeable for mediators of inflammation.

Immunological tolerance by the central mechanism can be induced by transplantation of allo- or xenogeneic cells into the thymus. As a result of thymic selection, maturing T-cells recognize foreign

alloantigen as own [33,43]. Specific tolerance can be used by creating true hemopoietic chimerism, when hemopoietic cells are transferred against the background of immunosuppression [19,25,43].

Peripheral immunological tolerance can be attained by many methods, since immunoreactivity is determined by a variety of factors. Reaction of T cells to antigen depends on their maturity and on microenvironment, in which they receive antigen-specific and costimulating signals. Immunity and tolerance can be considered as different results of a double-signal process. In this context, costimulation is a functional term without precise molecular definition. This is illustrated by some methods of inducing T-cell reactivity in transplantation.

Immunization with alloantigens after pretreatment with anti-CD4 antibodies leads to stable areactivity of alloantigen-specific T cells. The antigen-specific nature of the tolerance is determined by small population of CD4 T cells escaping cytotoxic effect of antibodies and contacting the antigen via T-cell receptor in the absence of costimulating signal from CD4 molecule, which promotes their transformation into TH_2. The population is expanded under the action of TH_2 cytokines (interleukin-4); these cells start regulating maturation of CD4 T cells into TH_2. This abolishes cellular alloreaction mediated by TH_1. This example illustrates the important role of regulatory CD4 T cells in the induction phase of tolerance [7].

The second activating signal for T cells can be successfully blocked with CTLA-Ig chimeric protein [27,33] which possesses high affinity for B7.2 molecule on APC.

Here we do not regard the possibility of abrogating immune confrontation through suppression of T cell activation by peptide antigens or anti-idiotypic reagents, since many clones of antigen-reactive cells are involved in immune response.

Substantional contribution of the microenvironment in immunoreactivity has been confirmed by the development of immunological tolerance after peroral, intrathracheal, and intraportal administration of antigen.

Peroral or intragastral administration of antigen induces specific tolerance [17] in experimental animals due to modulation of the cytokine profile of T cells toward the prevalence of transforming growth factor-β. *Per os* administration of the major myelin protein leads to the emergence of T cells with an irregular cytokine profile in mice with experimental autoimmune encephalomyelitis. These cells (TH_3) secrete interleukin-4, interleukin-10, and transforming growth factor-β. TH_3 are identical to encephalogenic clone of T cells.

Thus, essential cytokine transformation is possible with one clone of T cells upon confrontation with an antigen under conditions of modified microenvironment [8]. Peroral administration of antigen has been used for the treatment of the following autoimmune diseases: rheumatoid arthritis [28] and multiple sclerosis [39]. In patients with multiple sclerosis, the occurrence of autoreactive T cells (specific for the major myelin protein and proteolipoprotein) producing transforming growth factor-β increases [11].

Infusion of allogeneic cells via the portal vein reduces the intensity of antigen-specific reaction or results in stable immunological tolerance. Intraportal infusion of cells derived from allogeneic bone marrow or spleen prolongs functioning of heart, kidney, liver, and PIC grafts [10]. Localization of donor homeopathic stem cells in the recipient liver is necessary for induction and maintenance of tolerance [44]. Liver CD4-CD8 T cells are responsible for intraportal tolerance [23]. These cells induce tolerance after being transferred into an allogeneic recipient [30]. In mice, cells of the same phenotype CD4-CD8-Nk1.1$^+$ restricted by CD1d1, a nonclassical class I MHC molecule, are the first to produce interleukin-4 after antigenic stimulation [42]. These cells may be crucial for induction of TH_2 with subsequent abrogation of immune response to allo- and xenoantigens mediated by TH_1.

Such a pretreatment of the recipient is a prerequisite of intraportal transplantation of cells. Favorable microenvironment is provided for intraportally grafted PIC, since Kuppfer cells and liver endotheliocytes produce hepatocyte growth factor HGF/SF [29] which induces proliferation of human fetal PIC [31].

In the context of cellular transplantology, immunological confrontation can be of positive significance under certain conditions. This is confirmed by successful application of allogeneic leukocytes for the treatment of chronic miscarriage. Although these results have no theoretical basis, they indicate that immunological confrontation can be used for restoration of functional systems in the body. Presumably, the potential of immune cells in reparation and morphogenesis is rather high [40]. Immunocompetent cells produce a broad spectrum of cytokines, many of which exert pleiotropic effects, modifying functions of cells and organs outside the immune system. The ideal variant of directed influence on T cells consists in abrogation of the aggression against the graft and simultaneous selective induction of T-cell factors promoting the graft growth. The ability of T cells to produce cytokines varies according to type and dose of the T cell receptor ligand (i.e., antigen),

which manifests itself as a great variety of cytokines or as a dissociation between production of cytokines and cellular proliferation. In addition, T cell reaction depends on the quality of costimulating antigen-nonspecific signals.

The reprogramming of T cells is most probable via the microenvironment, where they interact with grafted cells, and under the influence of transplanted cells. It is necessary to create conditions for interaction between immunocompetent cells, graft, and tissue compartment in which associations between these components are established as a result of information exchange to provide viability and functioning of the graft.

The desired result can be achieved by employing an external factor, for instance, monoclonal antibodies to costimulating molecules of immunocompetent cells, antagonists of cytokines and their receptors, cytokines, or transfection of certain genes into transplanted cells.

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