A REVIEW OF CURRENT STRATEGIES TO OVERCOME REJECTION IN XENOTRANSPLANT

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Summary

Currently, improved immunosuppressive protocols used in allotransplant ensure successful interventions in most cases, but the limited number of donors for allogeneic cells, tissues and organs makes the transplant possible to only 15-59% of patients. Reduced availability implications on human health and the progress of transplantation immunology in recent decades determined more and more researchers to focus on xenogeneic sources. Given that since the first results were more complex obstacles and even other than those registered in allotransplant was argued that clinical xenografting success will depend, at least in part, by finding methods to induce tolerance in the purpose to remove the xenogeneic barriers rather than relying entirely on non-specific immunosuppressive drugs.

This article is a review of the main strategies applied in experimental research for delaying and prevention of xenografts rejection, especially for the hyperacute one, xenotransplant currently maintaining within the boundaries of basic research.

Key words: xenotransplant, strategies to overcome rejection

The use of animal organs for transplantation in humans is seen as a potential solution to the short supply of human donor organs available for clinical transplantation. However, while several attempts at clinical xenografting have been made over the last ninety years, xenotransplantation has not matched the success of allotransplantation, because of a vigorous rejection response. Xenografts' rejection is mediated by mechanisms that differ from those involved in alloreactivity and which are inadequately controlled by conventional immunosuppressive agents.

Therefore, xenotransplantation requires the development of specific strategies to overcome rejection, through modification of the host immunity or production of genetically engineered organs.

Several approaches have been proposed or used to prevent or reduce the xenogeneic immunologic rejection response, including immunosuppression, genetic engineering, complement inhibitors, and physical barriers.

Immunosuppressive chemotherapy

Immunosuppression, such as that used in allotransplantation, provides some protection from cell-mediated rejection of the concordant xenografts. Thus, therapy with Cyclosporine A delayed rejection of heart xenografts in rat-to-mouse combination (16).

These therapies, however, do not affect preexisting antibodies or complement. Immunosuppression also increases the risk in the host to opportunistic infections, which may be particularly problematic in patients undergoing xenotransplantation procedures (7).

Complement inhibitors

One approach to decreasing the immediate hyperacute rejection (HAR) is to administer complement inhibitory molecules. For example, soluble complement receptor 1 (9) or cobra venom factor (9, 18), both inhibiting complement activity, have been used to obtain prolonged survival of the xenotransplantation product in animal-to-animal models. Because it blocks both activation pathways, these factors are more efficient than C1 inhibitor (18), but the results below to those obtained in allotransplant.

Anti-C5 monoclonal antibody (mAb) also inhibits both complement activation pathways, but can not stop C3a and C3b formation. This limits its applicability to the prevention of hyperacute rejection in the early hours which follow transplantation (14).

Depletion of natural antibodies

Hitherto several more or less selective disposal methods of xenogeneic natural antibodies (XNA) were experimentally applied legacy infusion, plasmapheresis and xenogeneic organs perfusion representing the most common approaches.

Plasmapheresis involves the exchange of recipient plasma by plasma substitutes such as albumin. Thus, in addition to XNA, non-immunoglobulin plasma proteins such as complement and coagulation factors are also depleted by this approach. To avoid these drawbacks, an antibody-based immunoadsorption (immunopheresis) has been recently described, which allows depletion of total plasma IgG and IgM through immunoaffinity columns (2).

Immunoadsorption offered some good results in pig-to-baboon combination, lowering the anti-pig IgG (54-486 folds) and IgM (9-54 fold) antibody titers (23).

However, both plasmapheresis and immunopheresis methods are hampered by the removal of immunoglobulins not specific for xenoantigens that could increase recipient sensitivity to infections in the post-transplant period (2).

Xenogeneic organs perfusion involves passing recipient blood through an organ of one animal belonging to the same species like the xenografts' donor. Following this intervention, xenogeneic preformed antibodies bind specifically to endothelial cells of the perfused organ. The blood thus obtained will contain all plasma proteins except xenogeneic natural antibodies and will be reintroduced in the recipient circulation without problems.

Organ perfusion allows antibody removal for a longer period (4 days) than plasmapheresis where antibodies return 12-24 h after antibody depletion (2). However, the efficacy of organ perfusion is limited by the number of endothelial cells available in the perfused organ. Thus, the antibodies absorption rate is lower in kidney (25-50%) than in liver (60-80%), which is explained by significantly higher

vascularization of the latter organ (3, 24). A drawback of the organ perfusion method is the sequestration of recipient blood cells, leading to anemia. This can be avoided by perfusion of the recipient plasma instead of the whole blood (2).

An alternative method for selective removal of natural antibodies, with fewer drawbacks, is the use of columns carrying specific xenoantigens. Thus, positive results were obtained from blood passage of the animal recipient xenografts through a cartridge containing Galα1-3Gal antigen bound to a matrix (26).

The presented methods prolongs xenograft survival, but there are limited by the return of anti-donor antibodies after a few days. This indicates that depletion of antibodies applied as the sole method of inducing tolerance to xenografts is not enough, imposing itself complementary strategies to reduce the level of antibodies to a longer period and also to prevent acute vascular rejection. In this respect, it was appealed to splenectomy for decreasing the number of B lymphocytes (4), to depletion of IgM-producing B cell clones (5) or to immunosuppressive agents which greatly reduce the level of antibodies by the effect on B lymphocytes (13).

Physical barriers

Physical barriers, such as capsules, semipermeable membranes, or diffusion chambers, are thought to protect transplanted cells (eg, pancreatic islets or lung cells) from cells immune attack, but not from humoral response (6, 30).

For example, diffusion chambers (such as TheraCyte® device consists of two semi-permeable membranes with pores of 0.45 to 5μm) allowed maintenance of the lung xenografts in mouse-to-rat combination for three weeks. However, these results were well below those achieved in lung allotransplant (one year), and immune response was violent, with severe accumulation of inflammatory cells and a decrease in local vascularization (6). In contrast, the pigs' pancreatic cell isolated and encapsulated in alginate-polylysine-alginate membranes provided remission of diabetes in BALB mice for a period of 85 days (30). Applying the same protocol, without immunosuppression, for combination pig-to-macaque resulted in restoration of spontaneous diabetes for up to three months (31).

Thymus and bone marrow transplant (mixed chimeras)

Xenogeneic thymus transplantation was the first approach that has proved useful to induce tolerance in the discordant pig-to-mouse combination, the recipient mice of the pigs' fetal thymic grafts being arreactive in mixed lymphocyte reaction and accepting the subsequent skin xenografts. Experimental protocol consists in initial thymectomy of the immunocompetent mice and treatment with anti-T cells mAbs, procedure that allows subsequent grafting of the pig' thymus in recipients' kidney capsule (22, 33).

Basically, swine thymus replaces the recipient murine thymus, allowing reconstitution of T-cell populations and also the subsequent xenografting. These populations of T cells that repopulate secondary lymphoid organs are active and provide protection against infection while being tolerant to pigs' xenoantigens (33).

Negative selection in grafted thymus is mediated by murine and pig antigen presenting cells (APC) (33, 34) and positive selection exclusively by pigs' APC (35).

Administration of anti-T cells and NK cells mAbs associated with a reduced dose of total irradiation (3 Gy) allowed bone marrow transplant in rat-to-mouse combination and also achieving mixed hematopoietic chimeras with specific tolerance to xenoantigens (28).

Regardless of the protocol applied to obtain mixed chimeras in rat-to-mouse combination, specific tolerance to donor cells at both T and B compartments was observed.

Genetic engineering

A great advantage of xenotransplantation comparing to allotransplantation is the fact that the donor of xenotransplantation is known long before the operation and, therefore, it can be modulated to promote the successful of the graft. Thus, knowledge of molecular biology techniques has allowed experimental medicine to genetically manipulate the donor species (15).

On this line, genetic engineering (transgenesis to add genes or replace an endogenous gene with another) has been suggested as a means to provide better immunologic compatibility between the xenotransplantation product and the human recipient, especially for reducing the strength of complement-mediated hyperacute rejection (7, 27). In efforts to prevent humoral rejection can be mentioned obtaining the transgenically engineered pigs that express human complement regulatory proteins like hDAF (human decay accelerating factor, CD55) (10), CD59 (membrane attack complex inhibitor) (8, 10), and hMPC (human membrane cofactor protein, CD46) (1). The results showed that effective prevention of hyperacute rejection is only when there are several transgenes.

Although these approaches have been successful in terms of xenografts hyperacute rejection, were not viable solutions for preventing acute humoral and cellular rejection (19, 21). Some encouraging results were obtained when applying complex protocols, including, for example, prior to kidney xenotransplant in macaque, transfection of hDAF in donor pigs and splenectomy combined with immunosuppressive therapy (Cyclosporine A, Cyclophosphamide and steroids) in recipient, with rejection delaying to 80 days (11).

Galα-1-3Gal epitope, one of the antigens predominantly recognized by human immune system, can be eliminated by enzymatic treatment, but the molecules will regenerate quickly (17, 25). In mice and, more recently, in pigs it was already managed to obtain lines that are characterized by the absence of the gene responsible for synthesis of Galα-1-3Gal epitope – α-1,3 galactosyl transferase knockout mice and pigs (12, 20, 32).

Also, masking epitopes with other carbohydrates (12) and use of antisense RNA to inhibit α-1,3 galactosyl transferase (29) there are another methods for proposed for preventing rejection.

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