Transplantation Immunology

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The rapid development of surgical procedures in recent years made organ transplantation somehow to be possible in human. The transplantation of the kidney, heart and liver has been used as a remedy for disease[1]. In the otolaryngological field the transplantation of the larynx has been studied for the purpose of restoring the normal functions of the larynx following laryngectomy[2][3]. The first application of the larynx transplantation to human was performed in February 1969[4]. However, the adoption of transplantation is not widespread because the transplanted organ or tissue is threatened by immunologic events which are called "graft rejection". The chief obstacle to success of the transplantation is the rejection reaction which is not yet fully understood.

This paper will discuss rejection phenomena and immunosuppressive regimens which are to guarantee the viability and function of the transplants.

REJECTION

The rejection or acceptance of transplants between individuals of the same species is governed by histocompatibility antigens which have been investigated extensively in mice and humans.

In mice, the specificities of transplantation antigens are under the control of genes at the large number of loci. Among them one complex locus, known as H-2 locus, houses the genes that control the appearance of antigens playing an important role in transplantation immune responses. Antigens designated by the H-2 locus are H-2 antigens. The H-2 antigens are distributed widely in the tissue of mice. Lymphoid cells are especially rich in H-2 antigens, brain and skeletal muscles are very poor, and liver, lung and kidney seem to have intermediate amounts.

In humans, there is a number of independent loci whose genes are involved in determining the specificities of transplantation antigens. One complex locus, designated as HL-A, is analogous to the H-2 locus in mice in many respects. The tissue and cellular distribution of the HL-A antigens are the same as that of the

H-2 system except for erythrocytes in which HL-A antigens do not lodge.

The H-2 and HL-A antigens are main transplantation antigens, and the differencies of these antigens provoke strong rejection reactions. These antigens are complexes of proteins, carbohydrates and lipids, residing chiefly on the surface of lymphoid cells.

When transplantation is performed between individuals who have same histocompatibility antigens, e.g., the exchange between monozygotic twins, a successful acceptance of the transplant occurs. In the case between monozygotic twins, the genes in the histocompatibility loci of the donor do not determine any antigens different from those present on the tissues of the recipients. Namely, the transplantation antigens of both the donor and recipient are same. However, larger differencies in histocompatibility antigens between donor and recipient evoke stronger rejection reactions. Antigens of the donor absent in the recipient represent for foreignness of the graft.

The presence of a foreign transplantation antigen will not result in the rejection process unless this presence is recognized by the host. For example, poor lymphatic connections between the graft and host may prevent the recongnition of the donor's antigen by the host. So, the sensitization process may be inhibited. The anterior chamber of the eye, the brain and the cheek-pouch in hamster are the privileged sites for this situation.

There are two concepts for the host sensitization. One of these is that the sensitization occurs "centrally" within the host, particularly in regional lymph nodes[5]. In this concept the lymphatic connection between the graft and host is of importance to recongnize the host antigens. The brain, which is lymphatic-free but which has a rich vascular supply, and the cheek pouch of the hamster seem to act as privileged sites for the growth of transplanted tissues[6][7]. Moreover, an artificially privileged site can be created by raising skin flaps in which the vascular supply is preserved but the lymphatics are severed[8]. The other is a concept proposed by Medawar[9] that the sensitization occurs "peripherally" within the graft. According to Medawar, immunologically competent cells which are present constantly in peripheral blood could come specifically sensitized as they pass through the graft.

The mode of sensitization of the recipient may differ on the types of transplants. Whole organ transplants is connected by the anastomosis of large vascular vessels at the surgery, although the development of lymphatic communications is delayed.

There is a considerable amount of evidences which indicates that the allograft rejection is a cell-mediated process, very similar to that which occurs in delayed hypersensitivity: a heavy concentration of lymphocytes in the graft is a chief change of the graft rejection, patients with congenital aplasia of the thymus gland

—DiGeorge syndrome—, who are found to have normal functions of immunoglobulins formation but the impairment of allograft rejection, have been reported, and neonatal thymectomy prolongs the survival of the allograft transplants. However, the humoral immunity can not be excluded in the rejection reactions. For example, the rejection occurs in certain cases in which the cellular infiltration is absent. Kissmeyer-Nielson and his associates[11] reported the occurrence of hyperacute rejection in patients where lymphocytotoxic antibodies against donor cells could be detected.

The early microscopic evidence of rejection in the graft is the adherence of small lymphocytes to the endothelium of capillaries and venules. The cell infiltration leads disturbance of the blood flow, thrombus, and finally necrosis. In the later stage of rejection neutrophiles and macrophages participate to the cell infiltration, forming a nonspecific inflammatory change. On the other hand, histologic changes in the recipient are that large cells with pyroniophilic cytoplasma are frequently observed around the splenic arterioles and post-capillary venules of the regional lymph nodes, and that an enlargement of the germinal centers of these lymph nodes is seen. The peak of the rejection process is associated with marked proliferation of lymphocytic cells in the regional lymph nodes.

The structural aspects of rejection are summarized as follow : the immunologically competent lymphocytes which recognized the donor's transplantation antigens proliferate in the regional lymph nodes, and then a number of sensitized lymphocytes infiltrate to small vessels in the graft. The cell infiltration of blood vessels causes the disturbance of the blood flow, anemia, thrombus and necrosis.

As shown in figure 1 the recognition of foreign transplantation antigens by immunologically competent cells of the host initiates the graft rejection. Small lymphocytes that recognized graft antigens divide into many sensitized lymphocytes. Humoral antibodies are also produced. Sensitized lymphocytes and humoral antibodies accumulate to the graft, triggering an inflammatory process which further progresses to the graft necrosis.

IMMUNOSUPPRESSION

There are four approaches to control the graft rejection : these are selection of a donor and recipient with least possible antigenic differences, nonspecific immunosuppression, antilymphocyte serum (ALS), and immunologic tolerance (see Table 1).

Methods for the selection of donor and recipient are known as tissue typing or histocompatibility test. The tissue typing is still in the developmental stage, although the usefulness of matching donor and recipient by tissue typing has been
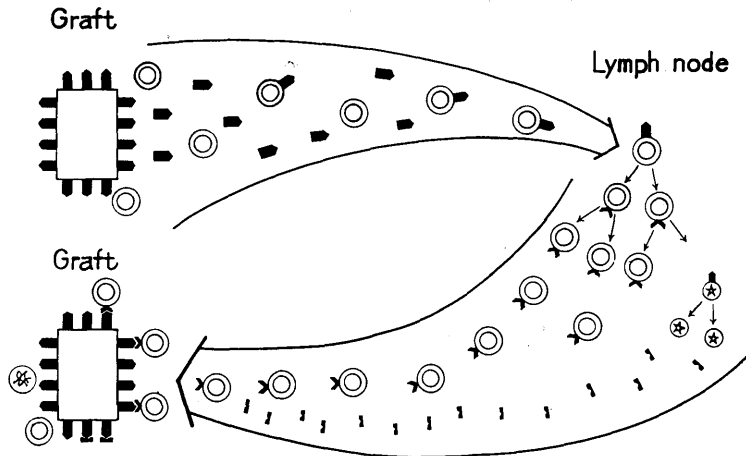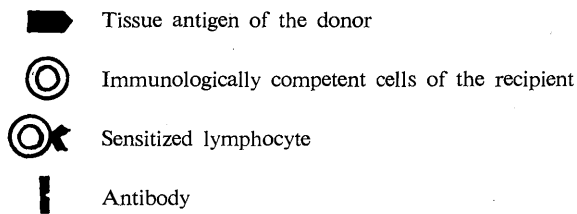
ALLOGRAFT REJECTION

Fig. 1.   The Mechanism of Allograft Rejection

➤   Tissue antigen of the donor

◎   Immunologically competent cells of the recipient

◎✗ Sensitized lymphocyte

❘   Antibody

Antigens of donor's tissue are recognized by immunologically competent cells of the recipient. This recognition responds with both sensitized lymphocytes and antibody formation which react with antigens of donor's tissue.
Subsequently, mononuclear cells accumulate, and the graft destruction becomes evident.

proven by long survival of properly typed grafts.   The rejection of foreign tissue is expression of genetically determined differences between individuals.   If a donor's tissues are antigenically the same as the recipient's tissues, no rejections occur. However, it is very difficult to select a completely matched pairs other than monozygotic twins.   Therefore, tissue typing offers the hope for minimal difference and for minimal severity of rejection.

Several techniques have been used for tissue typing as shown in Table 2.   Among them the mixed leukocyte culture test and serotyping of leukocytes are believed to be most available.

When blood lymphocytes from two normal individuals dissimilar genetically each other are mixed and cultured in in vitro, certain cells undergo morphological

Table 1. Immunosuppression

Tissue Typing or Histocompatibility Test
Nonspecific Immunosuppressive Methods
Antilymphocyte Serum (ALS)
Immunological Tolerance

Table 2. Tissue Typing or Histocompatibility test

Third Man Test
Normal Lymphocyte Transfer (NLT) Test
Mixed Leukocyte Culture Test
Irradiated Hamster Test
Serotyping of Leukocytes

transformation into blast. Antigens of leukocytes from the donor not present in the recipient's leukocytes stimulate leukocytes of the recipient. If leukocytes are from monozygotic twins, blastoid transformation does not occur, the degree of blastoid formation is estimated by the number of cells transformed or by uptake of the H_3-thymidine.

Sera from multigravidas and from patients who have received multiple transfusion of whole blood may contain antibodies against the lymphocyte isoantigens. Multigravidas are evidently immunized during gestation by fetal antigens whose specificities are determined by paternally derived genes not represented in the mother's genome. A number of these antisera are tested against the cells of a panel of randomly selected persons for cytotoxicity and leukoagglutination.

Each of these antisera is capable of recognizing one or more isoantigens on the surface of lymphocytes obtained from donors and recipients.

Irradiation, surgical methods such as thymectomy, splenectomy, and thoracic duct fistula, and chemicals are categolized to nonspecific immunosuppressive methods (see Table 3). These methods act as lymphoid-cell ablation.

Whole-body irradiation has been discarded because of its dangers, while the efficacy of both the local irradiation and extracorporeal irradiation in the graft prolongation has been demonstrated.

Chemical immunosuppressive regimens are most common in clinical transplantation. Most of the useful drugs have derived from cancer chemotherapy. Calne et al[12] reported that azathioprine, known as Imuran is a most valuable agent.

Table 3. Nonspecific immunosuppressive methods

Irradiation	Chemicals
total body irradiation	6-mercaptopurine (6MP)
extracorporeal irradiation	Azathioprine (Imuran)
local irradiation	Steroid
Surgical Methods	Actinomycin C
thymectomy	methotrexate
splenectomy	
thoracic duct fistula	

Antilymphocyte serum (ALS) is made by immunizing animals of one species with lymphoid cells from animal of another species. Since 1963, when Woodruff and Anderson[13] reported prolonging skin allograft survival in rats by use of heterologous antilymphocyte serum, this substance has come to the forefront as a promising new form of immunosuppressive therapy.

Mogi, et al[14]−[18] have studied horse anti-dog thymocyte plasma with the intention of using it to promote the survival of transplanted larynx. ALS is a potent immunosuppressive agent capable of enhancing the survival of grafts. Moreover, it is known that the cell-mediated immunity is much more attenuated by ALS than the humoral immunity. However, the mode of action of ALS remains obscure. Several hypotheses have been proposed to explain the mechanism of action of ALS: the non-selective lymphoid depletion theory postulates that ALS depresses lymphoid cells non-selectively as do non-specific immunosuppressive methods, while the selective lymphoid depletion theory explains that the target of ALS is thymus dependent and long lived immunologically competent cells. Levy and Medawar[19] proposed two hypotheses. The first was "the blindfold hypothesis", according to which ALS may coat lymphocytes and blindfold them in such a way that they are no longer able to recognize and interact with transplantation antigens. The second hypothesis was "the sterile activation hypothesis" which postulates that ALS stimulates lymphocytes to an activity unconnected with transplantation antigens.

It is drawback that ALS is a mixture of heteroglous proteins which are possible to raise antibodies against themselves in the host. Antibodies to ALS not only might cause harmful side effects, such as anaphylaxis and kidney damage, but also might interfere with the activity of ALS. Mogi, et al[16] detected precipitation antibodies against ALS-immunoglobulins, which arose before the grafts were rejected. It is considered that these precipitation antibodies interfere with the activity of ALS, resulting in poor allograft survival.

The induction of specifically immunologic tolerance to the donor antigens responsible for the rejection has the greatest promise, theoretically. However, the method is practically the least well developed.

SUMMARY

The graft rejection, which is the chief obstacle to obtaining effective and harmless transplantation, was discussed. This phenomenon is still not yet fully understood. Several approaches to suppress the graft rejection have been attempted, but these methods are still less than a perfect guarantee of the viability of transplants for an extended or indefinite period of time.

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