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LYMPHSPIRATION

A PLAN TO SUPPRESS OR PREVENT THE IMMUNE REJECTION OF ALLOGRAFTED ORGANS

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ABSTRACT

Progress in the resolution of allograft rejection and associated complications has been hampered by the nonselective nature of current immunosuppressive therapy. In the following protocol, we propose instead of broadly suppressing immunoreactivity to remove selectively from the bloodstream supplying the transplanted organ only those T lymphocytes and/or antibodies capable of reacting with and destroying the allograft. This goal is to be accomplished by offering them (T cells and/or antibodies) a decoy of antigenically identical tissue. The proposed procedure has the added advantage that it may activate suppressor T cells specific for the allogenic tissue, thereby further promoting acceptance of the graft while providing the host immune system the opportunity to recognize the allograft as autochthonous.

It is generally accepted that the primary mechanism for the destruction of allografted tissues or organs is through the action of a class of thymus-dependent lymphocytes, called T lymphocytes or T cells. Within any individual, many distinct clones of T lymphocytes exist with surface structures (termed the T cell receptor for antigen) specific for a very narrow range of antigenic determinants. Conversely, in the total population of a species, such as humans, a complex array of distinct antigenic patterns exist in any tissue type. Thus, each individual has several distinct genetically determined patterns on all eukaryotic body cells with the MHC (major histocompatibility complex) proteins as most important antigenic system. If the MHC pattern of the donor tissue does not closely match the pattern of the host, the tissue is recognized as foreign by the immune system and those specific clones of T cells capable of reacting with it mount an immunogenic response. It is this genetic determination of tissue antigen patterns that explains why transplants between identical twins are successful, between close relatives less frequently so, and between unrelated individuals virtually impossible without some means of immunosuppression.

Two general mechanisms are responsible for tissue rejection (1). In the first, allogenic tissue with surface class I MHC antigens interact with cytotoxic CD8 positive T cells, which are activated by interleukin 2 (IL-2) to produce agents toxic for these target cells (the donor tissue). In the second process, allogenic class II MHC antigens interact with helper CD4-bearing T cells which then recognize the foreign tissue as "presenting cells" and trigger the activation of anti-allogenic B cells to release antibody specific for the donor tissue. Other non-specific phagocytic cells are then called in by these helper T cells to cooperate in the

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process of destroying the foreign tissue. The second helper T cell response, however, is the most important in the rejection of foreign tissue as patients with matched class II antigen display much better allograft tolerance than those matched for class I antigens. Nonetheless, the proposed plan (see below) should protect against both mechanisms.

Current means of immune system suppression (i.e., drugs, irradiation) are largely non-selective. All clones of T lymphocytes are serially suppressed, as are other immune defenses, such as antibody formation, leaving the host at risk to other alien "invaders" such as microorganisms or malignant neoplastic cells. The most widely used immunosuppressive agent in the past decade has been cyclosporine. Its success can be traced to a more selective mode of action relative to other drugs (2,3). Cyclosporine is a T cell-specific suppressor that minimally inhibits other components of the immune system, and is generally inactive towards other host tissues. However, cyclosporine still blocks IL-2 mediated T cell functions such as proliferation and activation of cytotoxicity, and patients accordingly remain immunocompromised. Much promise has been afforded type-specific monoclonal antibodies as more tissue-specific agents in recipient immunosuppression (4). Whereas some initial progress has been made in linking toxins to monoclonal antibodies, at the moment most research is geared to host-specific immunosuppression, as for example with anti-IL-2 receptor antibodies, again leading to a generalized inhibition of T cell function. The ideal use of monoclonal antibodies appears to lie in the production of an anti-idiotypic antibody, directed against precisely that class of T cells which is responsive to the allogenic donor tissue (5). Such antibodies would effectively block both T cell and antibody responses to the donor tissue. Nonetheless, currently available technology cannot as yet overcome the complex antigenicity of the donor tissue to permit the development of passive administration of anti-idiotypic antibodies.

Except for tissue type matching and use of immunosuppressive drugs, there has been little success thus far with alternative approaches to enhance acceptance of foreign donor tissue. This paper proposes a novel approach which averts compromise of the host immune system. Rather than suppressing immunoreactivity, the proposal aims to remove selectively from the bloodstream supplying the transplanted organ only those T cells and/or antibodies capable of reacting with and destroying the graft. This goal is to be accomplished by offering them (T cells and/or antibodies) a substitute (i.e., decoy) of antigenically identical tissue.

The plan envisions the removal of a small piece of tissue from the organ to be transplanted at the time of removal from the donor, macerating it, and growing it in a tissue culture medium. At the time of allotransplantation a hollow cylinder of suitable nonreactive material is inserted into each artery carrying blood to the transplanted organ (e.g. the renal artery in kidney transplantation). The chief strategy is to load the cylinder as soon as possible with allografted tissue grown *in vitro*. This decoy or sacrificial tissue, having the same antigenic structure as the newly transplanted organ, would presumably be the first foreign substance that the donor-specific T cells and antibodies would meet in coursing through the bloodstream. Those T cells and immunoglobulins having that specific pattern of determinants to react with the foreign tissue in the cylinder would attack and bind to it; the other immune cells and protein complexes would pass through leaving the host practically as immunologically competent against other threatening non-self factors as before the transplant.

There are three components of this proposal which need further expansion. First is the growth and supply of tissue for the cylinder; second is the assembly of the cylinder, and third is the recharging of the cylinder.

Tissue culture techniques

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Many human cells can reproduce in tissue culture for a finite number of generations. Much experimental work indicates that this limit may be extended by the addition to the culture medium of such components as selenious acid, transferrin (bound with ferrous iron), growth factors, insulin, unsaturated fatty acids, vitamin E or hydrocortisone.

With the first allotransplant there is usually a 10-14 day interval before the host's immune system begins to destroy the transplant. This "grace" period should be sufficient time to amass an adequate quantity of antigenically identical cells (with plenty of residual cells to continue growing in the culture) for the purpose of the proposed plan. If not, conventional immunosuppressant therapy may be *temporarily* initiated until the quantity of growing tissue is sufficient.

Most cells of a given individual are antigenically identical, at least for the HLA series of determinants. If the original tissue bank growing in culture happens to exhaust itself before the transplant is finally "accepted," new cultures can be started from other readily available tissues of the original donor (e.g., skin, connective tissue) without further appreciable harm. Because allogenic MHC antigens are primarily responsible for the immune response against the donor tissue, and most cells derived from the donor contain these antigens, then it follows that most donor cells cultured can provide cellular material for the cylinder. Alternatively, when a cadaver is used as a donor source, a large amount of tissue from the other paired organ could be frozen for later use.

Cylinder assembly

The exposure to foreign antigens needs to be maximized in the cylinder unit. Both surface area and flow rate are maintained at the highest level through the use of a tissue immobilization medium such as the 300μm diameter Sepharose 6MB macrobeads or collagen-coated Cytodex 3 beads, both obtainable from Pharmacia. The large bead size permits smooth flow through the apparatus, while maintaining high surface area for maximum tissue presentation. Even higher flow rates can be achieved using activated membrane filter media or chemically derived paper rolled several times parallel to the direction of flow. This latter maneuver reduces the available surface area; nonetheless, it should still be sufficient to bind an adequate portion of donor tissue. In each of these instances, either cytoplasmic membrane preparations from donor tissue, or preferably irradiated whole cells can be chemically linked to the support. For example, integral membrane proteins on irradiated cells can be connected via a disulfide bond and short linkages to amino groups on the collagen surface of the Cytodex 3 beads with the cross-linking reagent N-succinimidyl-3-(2-pyridyl-dithio)propionate (SPDP). Alternatively, diazophenylthio-ether cellulose paper can be directly coupled covalently to the same cells. There are a variety of approaches that have been described for linking proteins, and hence cells, to solid supports including carbohydrate-specific chemistry as well as both amino and carboxylate chemistry.

This cylinder would then be inserted into each artery entering the donor organ by transecting such arteries and coupling the cylinder between the cut ends. Preferably the cylinder is housed outside the body wall (for easy recharging) and plastic tubing is used as required to connect with the cut arterial ends. Afferent blood thus flows through the cylinder before reaching the transplanted organ. Because the effectiveness of the procedure depends on the continued flow of blood to the allograft, pressure transducers should be inserted within the cylinder to permit constant monitoring of the pressure differential and, hence indirectly, the maintenance of blood flow.

Cylinder recharging

After a certain period of time the supply of decoy tissue in the cylinder would, of course, become exhausted and the elements hostile to the transplant would start getting

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through to attack the graft. In addition, large agglomerates of T cells and accessory cells would likely begin to impede blood flow. Before this point in time (which must be determined empirically), the cylinder would have to be emptied and "recharged" with fresh material from the continuously growing tissue culture. This process would need to be continued indefinitely (perhaps even for the rest of the host life), or until the transplant gradually gains acceptance as non-foreign and ceases to be rejected.

The promise of averting morbidity and mortality by organ transplantation has prompted considerable research over the past several decades and has led to a much greater understanding of the human immune system. While the promise is still there, the practical results remain suboptimal. Except in the rare instance of a perfectly matched donor, intense immunosuppressive therapy must be administered to facilitate transplant survival. If the transplant survives, the patient continues to have an increased risk of premature death due to the immunotherapy required to maintain the transplant. This paper suggests a method to maintain the patient in an immunologically competent state by selectively removing from the peripheral blood only those immune components likely to attack and destroy the allotransplant.

The merits of the proposal need testing in a model system to determine its validity. The availability of isogeneic and allogeneic murine strains as well as standardized procedures for assessment of murine immune system status makes this an attractive model for study. There are several parameters which need to be evaluated in this model system. First, temporal analyses of T cell classes following transplantation and cylinder insertion should be carried out. Not only should helper and cytotoxic classes decline in the allograft, but in time, suppressor T cells should begin to appear. Second, the presence of antiallograft antibody needs to be assessed. Whereas antibody and complement fixation probably play a less prominent role in allograft rejection, so-called "enhancing" antibodies may nonetheless lead to immune suppression. Third, the presence in the cylinder of accessory cells should be monitored. Blood flow through the cylinder is a critical parameter, will be influenced by invasive cell populations, and must be monitored for build up of a pressure differential using a standard transducer. Indeed, the process of cylinder design should be guided empirically to maximize both blood flow and internal surface area.

The suppressor T cell subset is likely to play a major role in eventual acceptance of the allograft as "self" or autochthonous. The mechanism(s) by which the immune response is regulated by suppressor cells is still unclear, but in the network hypothesis of Jerne (6), a complex interplay of all immune components serve to modify each host response. Ideally, the indwelling cylinder as outlined would favor immunosuppression through a delay in direct graft cytotoxicity, as cytotoxic helper T cells would be activated and trapped by the decoy tissue in the cylinder. After many months, when suppressor T cells become sufficiently active that they are able to neutralize most cytotoxicity, then theoretically the cylinder may be permanently removable without notable risk of graft rejection.

It has been recently suggested that the HLA G histocompatibility determinants, which are not polymorphic and are found exclusively in placental tissue may play a pivotal role in prevention of maternal rejection of the allogeneic fetus (7). Conceptually, HLA G action involves trapping in the placenta key cytotoxic maternal lymphocytes, thereby immunologically isolating and protecting the fetus from the mother's immune system. Although the issue of HLA G receptors on lymphocytes has not been adequately addressed, the possibility exists of exploiting this natural phenomenon in the context of the aforementioned scheme outlined to facilitate allotransplantation. Thus, if the cylinder were loaded with placental tissue, then perhaps it may be equally as effective as being loaded with donor tissue.

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Moreover, placental tissue would represent an advantage over donor tissue because of the never-ending supply of newborn human placenta.

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Editorial Comment:

An idea which at first glance sounds utopic may in fact someday be a legitimate option. Accordingly, a new and imaginative look at the biologic issues involved with allograft rejection may be refreshing even if the concepts emanate from chemists rather than transplantologists. In brief, Jacoby and Brock suggest designing and constructing a biological absorption device capable of selectively removing donor-antigen specific cells and antibodies before they gain access to an allogeneic transplanted organ by a decoy tissue cylinder which "traps" recipient inflowing blood elements.

How reasonable is the idea in light of current knowledge in allograft biology? The answer should be divided into two considerations–one the mitigation of allograft rejection and the other addressing the nonspecific process evoked by the contact of perfusing blood with surfaces of uncovered cells and foreign materials.

The exposed major histocompatibility (MHC), organ specific and adherence molecules on cells in the "preorgan" (my designation for the donor cylinder) should be able to absorb at least a portion of incoming specific antibodies and block donor-specific and hordes of nonspecific lymphocytes and monocytes from reaching the graft. The surface area of the indwelling device, however, seems crucial. After several hours a decrease of cells and antibodies in peripheral blood is likely to occur. Thus, removal and elimination of these "undesirable" cellular and protein moieties is certain to be accompanied by release of large amounts of cytokines and other enzymatic elements from both attacker and target cells. Without inactivation, these vasoactive and immunomodulators are likely to act as mediators for graft rejection. Moreover, the efficiency of absorption by the "preorgan" in terms of attenuation of the rejection phenomenon is of course unknown and will require extensive experimentation.

Perhaps of even more concern are the problems connected to events at the blood-cell and foreign material interfaces. The surface of cells previously not in contact with plasma and the reactions within the cylinder are likely to initiate a cascade of coagulation and complement activation which, in turn, will generate heavy deposition of platelets and white blood cells (e.g. polymorphonuclear leukocytes). In experiments in our laboratory with intravascular transplantation of non-lymphoid cellular elements (even in a syngeneic setting) we have observed rapid agglutination of granulocytes and clot formation ultimately attaching to the vein wall. Heparin is minimally effective in arresting this process. Perhaps the key question, however, is will fibrinogen and platelets block the surface of recipient organ

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cells (e.g., hepatocytes, renal epithelium) to such an extent as to prevent donor-specific lymphocytes and antibodies in attaching to the specific epitopes? Could this process be regulated? Parenchymal cells only have contact with whole plasma in conjunction with white blood cells during local inflammation. In this respect, they differ from circulating blood cells and endothelium. Elucidation of this difference in reactivity may be the clue for designing optimal methods for prevention of clotting and complement activation after exposure of isolated cells to the bloodstream.

Nonetheless, despite these theoretical considerations, the idea is novel and worthy of serious experimentation. After all, original and unexpected observations may arise which could recharge current stereotypic thinking including the role of drug immunosuppression in transplantation biology.

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