Pediatr Transplantation 2009: 13: 814–819

Pediatric Transplantation
DOI: 10.1111/j.1399-3046.2009.01143.x

Lessons from cardiac transplantation in infancy

Platt JL, Cascalho M, West L. Lessons from cardiac transplantation in infancy.
Pediatr Transplantation 2009: 13: 814–819. © 2009 John Wiley & Sons A/S.

Abstract: Besides correcting devastating pathophysiology, cardiac transplantation in infancy offers an incomparable model for exploring the structure and function of the immune system. Infants and young children have relatively few memory B cells and T cells. Hence, the response of the young to allotransplantation better represents a primary immune response and may be better suited to testing spontaneous tolerance. Those who undergo cardiac transplantation in infancy are also unique because they are often subjected to thymectomy and depletion of mature T cells. These subjects can have a dramatic contraction of T cell diversity, allowing the testing of how diversity contributes to function.

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Key words: cardiac transplantation – thymus – cellular immunity – tolerance – MHC – ABO blood groups

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Accepted for publication 17 December 2008

Cardiac transplantation in the newborn and in young infants corrects the devastating pathophysiology of congenital heart disease and cardiomyopathy, averting the morbidity and mortality they might cause. While transplantation in the young ''cures'' cardiac disease, it might also provide insights not otherwise available into the structure and operation of the immune system. The infant undergoing cardiac transplantation is probably naïve immunologically and the response ensuing or lack thereof is unalloyed by the function of memory lymphocytes that accumulate with age. The procedure of cardiac transplantation is often accompanied by removal of the thymus, by depletion of mature T cells and followed by life-long immunosuppression. These manipulations in concert should deprive the recipient of some measure of immunological competence.

Abbreviations: FACS, fluorescence activated cell sorting; MHC, major histocompatibility complex; TCR, T-cell receptor; TREC, T-cell receptor excision circle.

We have undertaken a collaboration to investigate the structure and function of the immune system in those who undergo cardiac transplantation in infancy. Here, we communicate the current understanding of immune physiology that sparked our interest in this subject and the lessons, some quite surprising, which emerged.

The immune response to allotransplantation in the young

Considering the very limited genetic diversity among members of a species and the likelihood that the immune system evolved to protect against microbial organisms, one would predict that immune responses to transplants would be idiosyncratic and weak in comparison to immune responses to microorganisms. But, such a prediction would prove wrong. The immune system responds more reliably, faster and more powerfully to allotransplantation than it does to infection. Whether this difference reflects fundamental differences in immune recognition and effector functions or whether it might be

explained by conventional immune physiology is uncertain. As we shall explain, pediatric transplantation and particularly cardiac transplantation in infancy might offer key insights on this subject.

We describe the immune reaction to allotransplantation in mature individuals as universal (it occurs in every un-manipulated recipient), rapid (it occurs in days) and quite severely destructive (it completely destroys the target) (1). The reaction is dominated by responses to allogeneic antigens encoded in the MHC. In contrast, immune responses to bacteria, viruses, fungi, vaccines and other antigens, including minor histocompatibility antigens occur sporadically (e.g., the rate of response to BCG is 50% at best), over periods of weeks or even months and do not generally destroy the targeted cells, at least not quickly. The difference between the immune response to allografts and to all other antigenic challenges could reflect the peculiar way in which major histocompatibility antigens are presented or it could reflect unanticipated immunological memory, memory responses like allogeneic responses being universal or nearly so, rapid and highly effective (2, 3).

The mechanism by which T cells recognize allogeneic cells might explain the universal, rapid and highly destructive allogeneic response. T cells can recognize allogeneic cells ''directly''; that is, T cells recognize intact allogeneic MHC expressed on allogeneic antigen-presenting cells. By direct recognition a T cell can potentially engage a large fraction of a given MHC on antigen-presenting cells (because peptide plays a nominal role) (4) or because the receptor can recognize many peptides (5). Hence, direct recognition activates up to 30% of T cells (4, 6) and responses occur always or nearly always so that the responses can be used to test for MHC class II and to map MHC (7). In contrast, T cells recognize other antigens, such as toxins, bacterial and viral proteins, as degraded foreign peptides associated with MHC on autologous antigen-presenting cells. When T cells recognize antigen on autologous antigen-presenting cells, ''indirectly'' as such, only a small fraction of MHC complexes on the autologous cells contain a given peptide. Hence, indirect recognition activates only a small fraction of 1% of T cells; in some cases no activation ensues.

Although the difference between direct and indirect recognition has been the subject of much comment, and may be important for the biology of allografts in some cases, this difference does not fully explain why allograft rejection is universal, rapid and severe. Grafts consisting of allogeneic cells and tissues are fed by blood vessels of the recipient and the immunological reaction seems to be directed mainly against these blood vessels (8, 9); recognition of these blood vessels must involve the indirect pathway, that is peptide of the donor presented by MHC of the recipient. Yet, these grafts are rejected universally, rapidly and severely. Nor does eliminating allogeneic MHC from the surface of all donor cells prevent or even slow the course of allograft rejection (10).

If peptide of allogeneic MHC presented indirectly can eventuate powerful rejection reactions, then the immune system might recognize peptide of allogeneic MHC differently than peptide of other proteins. Before association of peptide with MHC had been proven, Jerne (11) speculated that the immune system is predisposed to respond aggressively to allogeneic MHC. Heightened immunogenicity of MHC derived peptides must reflect, at least in part, that peptides from MHC efficiently loaded on MHC complexes (12). We found that T cells may be selected by peptides such as immunoglobulin, encoded by the immunoglobulin supragene family (13). MHC with MHC-derived peptide also interacts distinctly with TCR. Besides efficient loading, peptides from MHC might be recognized in some special way. This idea may explain the distinct structure of TCR bound to MHC–MHC peptide complexes.

As still another explanation for the universal, rapid and severe response to allotransplantation, one might postulate that the allogeneic response is actually a manifestation of immunological memory. Generally, a protective response occurs weeks and sometimes months after the first exposure to antigen if it occurs at all and full protection is only exhibited when upon re-exposure to antigen. On the other hand, responses on first exposure to cells bearing allogeneic MHC can be detected within a few days. The speed and intensity of the allogeneic response thus resembles the speed and intensity of the response on re-exposure to antigen. Consistent with this possibility, many of the T cells that respond to allogeneic cells in human adults are memory T cells (14). This explanation would place alloimmune responses within the framework of conventional immune responses. Some T cell clones for peptides of cytomegalovirus and perhaps intestinal flora also respond to allogeneic MHC (15, 16). Consistent with this concept, but also subject to other explanations, allogeneic grafts in the newborn sometimes generate immunity and sometimes do not (17). Also consistent with the concept, newborn mice do not reject tumor grafts

acutely but can still be primed to generate second set responses (18). One might reason then that only the newborn and young infants, lacking immunological memory, might offer clues to whether the alloimmune response truly differs from conventional responses.

On the other hand, the lessons taught by transplantation of the newborn are confounded by the possibility that transplants might engender tolerance. Billingham et al. (19) found that young rabbits reject skin as vigorously as mature rabbits, but Billingham and Brent (20) found that newborn mice exhibit a period of non-responsiveness. As newborn mice are relatively immuno-deficient, this experience would seem to indicate that the vigorous immune response to allotransplantation does not require immunological memory (we caution however that even a very small number of memory cells might cause rejection, given high frequency of alloreactive cells).

We have begun to explore the response of infants to allogeneic antigens. Subjects with the hypoplastic left heart syndrome and other complex cardiac anomalies can be treated surgically by the Norwood procedure, in conjunction with which a cuff of allogeneic tissue is inserted and blood is transfused. Our preliminary analysis reveals that all of these subjects are sensitized either to MHC classes I or II antigens (21). While further analysis is needed, the high rate of response is consistent with the concept that allogeneic responses differ from conventional immune responses.

Tolerance vs. immunity to transplantation in the young

Although exposure of young infants to allogeneic tissue can induce immunity, transplantation early in life might instead be able to lead to acquired immunological tolerance. In the classical experiment, Billingham et al. (17) found that administration of living haematopoietic cells to mice early in life changes the recipient in such a way that subsequent grafts of allogeneic tissue are retained.

We explored the possibility that introduction of allogeneic antigen early in life might induce tolerance rather than immunity by studying infants with severe defects in cardiac function who underwent ABO-incompatible cardiac transplantation (22). We found that infants can safely receive ABO-incompatible cardiac transplantation because they do not have antibodies against histo-blood groups A and B substances, as would normal adults (23). Even more striking however was our observation that none of the 16 subjects investigated developed detectable antibodies against the blood group of the donor but all (of 14 of blood group O) developed antibodies against the blood group not present in the graft. Lack of antibody specific for donor blood groups was not evidently because of binding of antibodies in the graft as graft biopsies showed no evidence of bound antibody or complement. Rather, the absence of antibody specific for donor blood groups reflected acquired immunological tolerance as B cells of the recipient cultured in the absence of donor cells or antigen produced no anti-donor blood group antibody and because ELISPOT analysis showed a paucity or absence of B cells capable of recognizing donor blood group. Normal infants of the same age did produce such antibodies against the histo-blood group antigens they lacked because this was a reflection of specific B-cell tolerance was further supported by the absence of detectable donor-antigen-specific B cells by FACS analysis.

Our results show that delivery of blood group antigens early in life can induce specific immunological tolerance. Importantly, this form of tolerance does not require the administration of lymphohaemopoietic cells of the donor but apparently can be induced by parenchymal cells containing donor antigen. This clinical experience mimics animal models of neonatal tolerance. Tolerance in this setting occurs by elimination of donor-reactive B lymphocytes and depends upon continuing expression of antigen. These findings suggest that intentional exposure to non-self A/B antigens may prolong the window of opportunity for ABO-incompatible transplantation, and have profound implications for clinical research on tolerance induction to T-independent antigens relevant to xenotransplantation.

Accommodation after transplantation

We discovered accommodation more than 20 years ago as an unexpected observation following transplantation of kidneys across blood group barriers in children (24, 25). Kidneys expressing blood groups A or B antigen were transplanted into recipients lacking those antigens and having as a result antibodies directed against those antigens. The anti-blood group antibodies were depleted and the spleen removed from the recipients. The kidney transplants functioned well in many cases. Particularly striking was that antibodies specific for the blood group antigens returned to the circulation of some recipients yet the graft appeared not to be compromised in function or in structure. Biopsies of the transplants revealed that the blood group

antigen of the donor was expressed after transplantation as it was before.

We reasoned that the failure of anti-blood group antibodies to injure the transplanted organ after the antibodies returned to the circulation reflected an acquired resistance of the organ to injury by the antibodies or change in the pathogenicity of the antibodies. We later excluded the possibilities that alloreactive antibodies lost pathogenicity by isotype switching (26), although such a change might occur in other circumstances (27, 28). We referred to this condition of acquired resistance to injury (which could reflect a change in the pathway by which injury occurs as true resistance) as "accommodation" (29).

Although the definition for accommodation we originally put forward (the condition in which a graft continues to functions in a recipient with antibodies directed against the graft) would seem clear, this definition is difficult to apply. Because normal organs can absorb large amounts of antibody, we have observed accommodation in experimental models in which an organ absorbs all donor-reactive antibodies. Without donor-reactive antibody, one might find distinguishing accommodation from tolerance challenging. Also unclear is whether donor-reactive antibody induces accommodation or whether other factors such as cytokines or T cells might do so.

ABO-incompatible transplantation in newborn and young infants offers what may be the best opportunity to address those questions. If the recipient has no antibodies against the donor and tolerance arises spontaneously as described below, one might determine whether donor-reactive antibodies are necessary for the development of accommodation (in those subjects who later develop antibodies). Our studies to date reveal no evidence of accommodation in subjects lacking donor-reactive antibodies. We are also keen to learn whether tolerance precludes accommodation and vice versa. If tolerance precludes accommodation, as our preliminary studies suggest, then donor-reactive antibodies may be essential for accommodation. Conversely, if those which accommodation cannot have tolerance (as they make antibodies against the graft), then absence of accommodation might be taken as evidence of tolerance [providing accommodation is a common outcome of organ transplantation as we have suggested (30, 31)].

Impact of transplantation on the development of immunity in the young

Immunity, in a classic sense, refers to heightened resistance to infection in those previously exposed to an infectious agent, toxins or vaccines. Immunity is conferred by either "memory T cells" or by antibodies. Defining immunity in this way, one puts aside for the moment "natural" or "innate" resistance conferred by complement, phagocytes and other components of the innate immune system, the other components of what is now considered to be innate immunity. The developing of immunity to infectious agents or their products depends on several components and interconnected events. First, the infectious agent must provide a source of antigen that can be recognized by T cells, i.e., antigenic peptide presented in association with MHC classes I or II molecules on antigen-presenting cells and delivery of effective co-stimulatory signals by those cells. Secondly, the rare T cells capable of recognizing those peptides must be brought into contact with the antigen-presenting cells, as occurs typically in regional lymph nodes and spleen where the T cells are activated and expansion of protective clones occur. Thirdly, antigens on the surface of infectious agents and toxins activate B cells committed to the production of protective antibodies in conjunction with delivery of signals by helper T cells that recognize antigenic peptide. Finally, following the second or subsequent encounter with the microorganism or its products, the expanded clones of "memory" T cells and B cells act quickly to control viral replication or eradicate bacteria and viruses that enter the body. All of these events, and especially the second, third and the final listed should be compromised severely in those who have undergone cardiac transplantation in infancy.

Removal of the thymus to facilitate exposure of the heart prevents the recipient of a cardiac transplant from generating new T cells. Treatment of the recipient with anti-CD3 or with thymoglobulin depletes many mature T cells. T lymphopenia induced by depletion of T cells causes the remaining T cells to undergo homeostatic proliferation. In the absence of a thymus, only homeostatic proliferation can restore T-cell compartment to the original numbers. Because new T cells cannot be made, and many mature T cells have been depleted, the T-cell compartment of the infant transplant recipient should be markedly less diverse than the T-cell compartment of a normal individual. To the extent that T cell diversity determines immune fitness, the recipient of a cardiac transplant in infancy should have defective responses. The defect in function should be worsened profoundly by the immunosuppressive therapy that so effectively controls alloimmunity.

Impact of cardiac transplantation in infancy on the structure of the T-cell compartment

We tested the assumptions mentioned above (32). Following removal of the thymus, cardiac transplantation and depletion of T cells, the number of T cells in the blood was restored over a period of months. Restoration of the T-cell compartment reflected homeostatic proliferation at least in part because more T cells expressed markers of memory such as CD45RO. As expected, removal of the thymus in whole or part abolished or profoundly reduced the production of new T cells as demonstrated by a profound decrease, and in some cases, absence of TREC in the blood. TREC are small pieces of DNA excised from the TCR locus during formation of the receptor. TREC do not replicate in mitosis and hence the level of TREC in the blood is a direct function of the output of T cells by the thymus and an inverse function of homeostatic proliferation.

If output of the thymus is decreased, as the profound decrease in TREC suggests, and if some fraction of mature T cells had been depleted, the diversity of the T-cell repertoire should be highly contracted. To test that idea, we used a novel assay of TCR diversity (33). This analysis demonstrated that diversity of TCR in the blood of those who underwent cardiac transplantation in infancy was contracted by orders of magnitude (normal people have roughly one billion different T cells; these subjects had as few as 1000 different T cells). One might expect that those who undergo cardiac transplantation in infancy would have markedly defective immunological fitness.

Impact of cardiac transplantation in infancy on immunological competence

While much is known about how the immune system functions, this knowledge is not reflected in reliable and quantitative assays of immune fitness, particularly of the T-cell compartment. T-cell function and cell-mediated immunity are typically assayed by determining the number of T cells in the blood, the ability to mount delayed-type hypersensitivity responses to *Candida* or other common environmental stimuli and response of T cells to mitogens (34). None of these assays is likely to detect immunodeficiency caused by contraction of the T-cell repertoire – the number of T cells is restored by homeostatic proliferation, delayed-type hypersensitivity tests for memory, and the capacity to mount a primary response and mitogen responses should be normal.

However, one might explore immune physiology more discerningly in those undergoing transplantation early in life. One could measure the T-cell-dependant antibody responses to common vaccines or the levels of viruses that commonly infect the young. Both approaches were taken and defects in immunity were observed. Importantly, those cardiac transplant recipients with no evidence of thymic function and contracted T-cell repertoires had 10-fold higher levels of HHV-7 than those with detectable thymic function (32). Thus, from the study of those who had undergone cardiac transplantation in infancy, we could report for the first time impact of T-cell diversity on immune physiology.

Concluding remarks

This communication provides what might be considered an interim report. We continue to investigate the structure and function of the immune system in those undergoing cardiac transplantation and we expect new insights will emerge. Yet, we think certain conclusions can be drawn at this point. First, we consider the subjects of our investigation and other who undergo transplantation early in life extraordinary models of immune physiology. These models allow testing of functions, such as primary immune responses, that cannot be tested in adults. Secondly, while we can associate profound contraction of T-cell diversity and treatment with immunosuppressive agents with abnormalities in cell-mediated immunity, we are struck that T-cell repertoire contraction and immunosuppression are not evidently associated with opportunistic infection characteristic of inherited or acquired immunodeficiency. Kulikowska et al. (35) did find that those with cardiac transplantation in infancy had higher rates of pyogenic infection, as one might expect if T-cell-dependant antibody responses are impaired. However, neither the subjects reported by Kulikowska et al. (35), nor the subjects we investigated, had disseminated viral infections or infections with unusual organisms or unusual cancers. Either the immune system has the capacity to compensate for loss of diversity and impairment in signalling well beyond what is currently envisioned or the requirements for immune fitness are far from being well understood. We suspect both.

Acknowledgments

This study was supported by the National Institutes of Health (HL79067).

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