Studies with T Cell Lines and Clones and Their Implications for the Understanding of Mechanisms in Pediatric Graft Rejection and Tolerance

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Today, transplantation of the kidney is widely recognized as the treatment of choice for children with end-stage renal disease. Similarly, grafting of the liver has become an accepted therapy for cholestatic and metabolic liver disease, and heart transplantation has emerged during recent years as a successful treatment for myopathic and complex structural heart disease. Despite improvement in 1-year graft survival, long-term results after pediatric transplantation have improved slowly during recent years. As in adults, acute and chronic rejection remain major problems. These immune responses comprise a complex series of effects of various alloantigen-specific cells, non-specific cells, cytokines and adhesion molecules. T cell lines and clones from transplant recipients can be used to define cellular and molecular mechanisms of rejection and tolerance. In this review, we analyze studies in which T cell lines and clones were used to study mechanisms of rejection and tolerance for an allograft.

ABBREVIATIONS

APC	antigen-presenting cell
CTL	cytotoxic T lymphocyte
ELISA	enzyme linked immunosorbent assay
HLA	human leukocyte antigen
MHC	major histocompatibility complex
PBL	peripheral blood lymphocyte
PBMC	peripheral blood mononuclear cell
RPA	RNAse protection assay
RT-PCR	reverse transcriptase–polymerase chain reaction
TCR T	cell receptor
Th1 T	helper cell type 1
Th2 T	helper cell type 2

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Introduction

Renal transplantation is widely recognized as the treatment of choice for children with end-stage renal disease.[1] In contrast to children on dialysis, children with a functioning renal transplant can grow normally and can have adequate psychomotor development and scholastic achievement.[2] However, acute and chronic rejection of the first renal graft, leading to graft loss in 17% and 30%, respectively, still remain major problems.[3] Therefore, despite improvement in 1-year graft survival (91% for living related and 82% for cadaver donors), post-1-year graft survival has not changed much in the last decade (5-year graft survival: 78% for living related and 64% for cadaver donors).[3,4] However, post-1-year pediatric renal allograft survival is equal or even better compared to adults.[5] Interestingly, a recipient aged less than 2 years is a significant contributor to the risk of renal graft failure, but only in the first 3 years following transplantation. With increased follow up, the disadvantage in graft survival associated with very young recipients has diminished. In addition, there is evidence that among recipients with a functioning graft at 1 year, the youngest recipients had the longest estimated graft half-lives.[6]

Transplantation of heart, liver or lung likewise ultimately leads to chronic rejection and subsequent graft loss. Although immunosuppressive therapy has an improved short-term outcome, it comes with a cost as it has significant side effects, especially in children, where it can result in growth failure. Furthermore, as in adults, side effects of immunosuppression are nephrotoxicity, hypertension, hyperlipidemia, diabetes mellitus, osteoporosis, post-transplant lymphoproliferative disease and other malignancies.[2] Therefore, the ultimate goal for children with end-stage renal, liver, heart or lung disease is to achieve a state for tolerance to their first transplant without the need for lifelong immunosuppression.

Recent understanding of graft rejection is much more complex than the classical concept of a straightforward T cell–mediated immunological phenomenon. Acute and chronic rejection processes comprise interrelated series of effects of various alloantigen-specific cells, non-specific cells, cytokines, inflammatory mediators and adhesion molecules. The expression of host responsiveness is also dependent upon several factors involved in the event of engraftment, including the circumstances surrounding donor organ retrieval and preservation, early function, histocompatibility differences between donor and host and effects of attempted initial and maintenance immunosuppression.[7,8] T cell recognition is the central and primary event that ultimately leads to allograft rejection, although several other factors (e.g., ischemia, infection) may contribute.[9,10]

It is now clear that there are two non-mutually exclusive pathways of allorecognition, the so-called direct and the indirect pathway. In the direct pathway, alloreactive T cells recognize intact allo–major histocompatibility (MHC) molecules on the surface of donor cells.[11] In the indirect pathway, T cells recognize processed alloantigen in the form of peptides after processing and presentation by self antigen-presenting cells (APCs). The direct pathway may be responsible for the vigorous response in acute rejection, whereas the indirect pathway is thought to be the dominant mechanism in chronic rejection.[11-13] Indirect allorecognition leads to activation of $CD4^+$ T cells, whereas the exact mechanism and role of $CD8^+$ T cells remains unknown. $CD4^+$ T cells, as helper T cells, are responsible for the production of most of the cytokines necessary to stimulate an immune response.[11] There are two types of $CD4^+$ T helper cells, type 1 (Th1) and type 2 (Th2) cells. Acute allograft rejection is predominantly a Th1 response with production of cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ).[14] However, controversy exists about the role of Th2 cells, which produce IL-4, IL-5, IL-10 and IL-13 and have been demonstrated in tolerance as well as in chronic rejection.[15,16]

One way to learn more about mechanisms of rejection and tolerance is from T cell lines and clones generated from transplant recipients. Here we review studies in which T cell lines and clones were used to study mechanisms of rejection and tolerance for an allograft. Almost all data on T cell lines and clones were obtained from cells derived from adult transplant recipients, therefore possible conclusions for the situation after pediatric transplantation can so far only be drawn using data from adults.

Clinical Cases to Illustrate the Value of T Cell Lines and Clones in Elucidating Mechanisms of Chronic Rejection and Tolerance

A patient received an HLA-DR*0101 mismatched living donor renal allograft. She had gradually declining renal function with a serum creatinine of 2.9 mg/dl 3 years after transplantation. Renal biopsy demonstrated typical histological features of chronic rejection with focal interstitial infiltrate and signs of glomerulosclerosis and arteriosclerosis. Another patient received an HLA-DR*1501 mismatched cadaver renal allograft and had a stable renal function with a serum creatinine of 1.6 mg/dl for more than 2 years. T cell lines were propagated from both patients from peripheral blood lymphocytes (PBLs) via the indirect pathway of allorecognition, with repeated stimulation using a mismatched peptide together with APCs (Fig. 1). Subsequently, T cell clones were generated by limiting dilution and were phenotyped using flow cytometric analysis, while cytokines from the culture supernatants were measured by enzyme linked immunosorbent assay (ELISA). T cell clones from the patient with chronic rejection were found to be $CD4^+$ and proliferated specifically to the mismatched peptide. Clones from the patient with stable renal function were also $CD4^+$. Interestingly, the cytokines produced by the clones from the patient with chronic rejection had a Th1 profile (IL-2, IFN-γ), whereas the cytokine profile from the patient with stable renal function was Th2-like (IL-4, IL-10). This study demonstrated for the first time at the clonal level that chronic allograft rejection is associated with the presence of Th1 phenotype cytokines, whereas stable renal function is associated with a Th2 phenotype.[14]

Studies With T Cell Lines and Clones in Acute Rejection

Acute rejection is primarily a cell-mediated process. Leukocytes enter the interstitium after interaction and adhesion to up-regulated selectins on the endothelium, in particular as a consequence of injured endothelial cells. Increased expression of adhesion

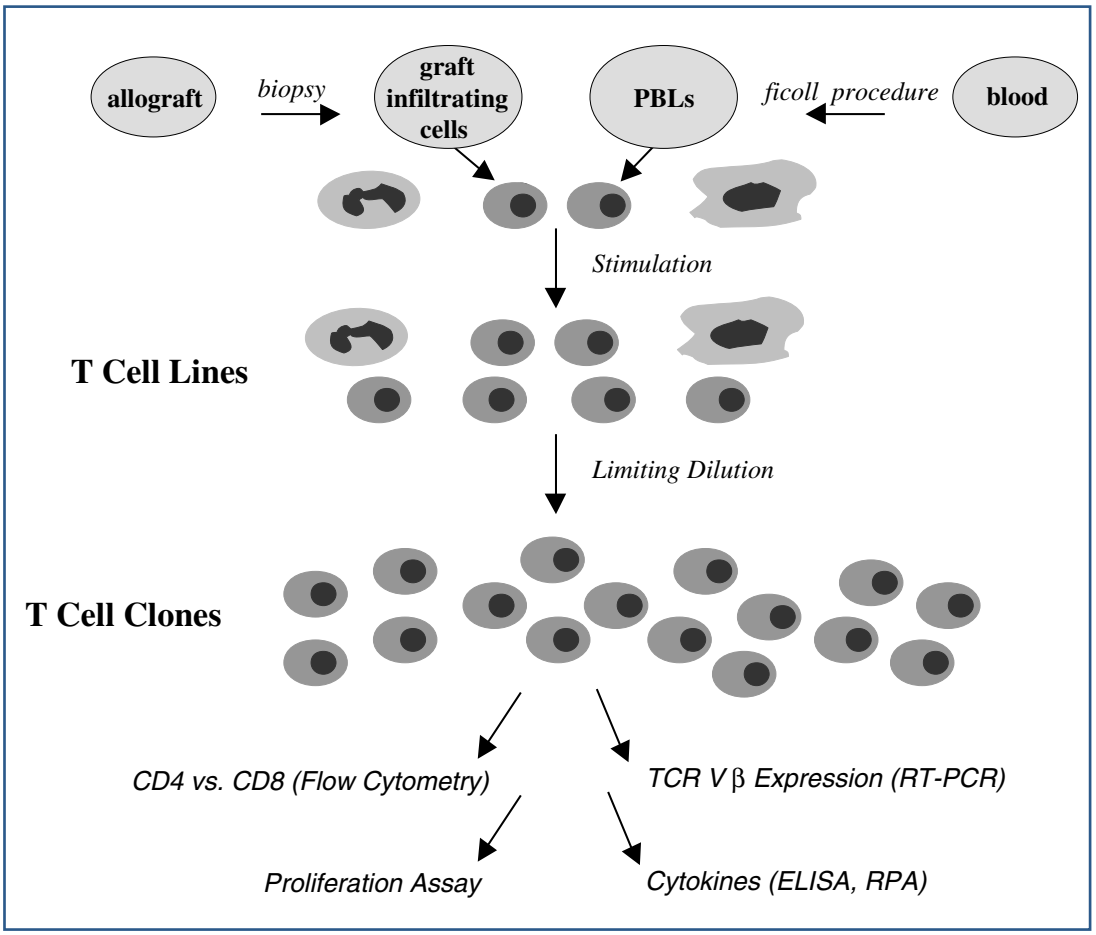

Figure 1. Generation of T cell lines and clones. Cells propagated from graft infiltrating cells or peripheral blood lymphocytes (PBLs) are repeatedly stimulated with donor cells or with self antigen-presenting cells (APCs) together with an allopeptide. T cell receptor Vβ (TCR Vβ); reverse transcriptase polymerase chain reaction (RT-PCR); enzyme linked immunosorbent assay (ELISA); RNAse protection assay (RPA).

molecules and enhanced vascular permeability follow, resulting in infiltration of the graft by host macrophages and lymphocytes.[17,18] Both $CD4^+$ and $CD8^+$ T cells are present in allografts being acutely rejected. Recent experimental data indicate that $CD4^+$ cells and their cytokines (IL-2, IFN-γ and TNF-α) play a key role in this process. Several studies in kidney, heart, liver and pancreas allografts were performed to analyze the role of these cells.

Renal Transplantation

The first data based on isolated cells from a rejected human kidney allograft were described almost 30 years ago.[19] Donor-specific cytotoxic lymphocytes obtained from rejected human kidneys demonstrated enhanced cytotoxicity in vitro compared to peripheral blood lymphocytes, indicating that infiltrating cells constituted a population distinct from the circulating lymphocytes.[20] Based on these early observations, many studies have focused on propagating T cell lines and clones from kidney graft infiltrating cells from nephrectomized kidneys, renal biopsies and fine-needle aspirates from patients with acute rejection.[21-24] In vitro expansion of T cells generated from graft infiltrating cells was associated with the severity of acute rejection as determined by histologic grading and subsequent graft outcome.[22,25,26] For instance, T cell clones with

specific recognition of HLA determinants were generated from a patient with irreversible acute rejection. Whereas all of the anti-HLA class I clones were $CD8^+$, all clones specific for HLA class II were either $CD4^+$ or $CD8^+$.[27] In this context, it has been demonstrated that not only $CD8^+$ but also $CD4^+$ cells can act very efficiently as cytotoxic lymphocytes (CTLs).[28] Interestingly, another group reported that donor-specific T suppressor cells from a renal transplant recipient could be induced in vitro despite the fact that this patient developed irreversible rejection.[29] In T cell clones successfully generated from renal biopsies from patients with acute rejection, more than 10% were CTLs recognizing HLA class I antigen on donor-specific kidney epithelial cell lines. However, these T cell clones could recognize HLA class I antigen on Epstein Barr virus–transformed B-lymphoblastoid cell lines, indicating that peptides, recognized in the context of donor MHC, were tissue specific.[30,31] There is evidence that both β-chain selection and clonal dominance are operating during acute graft rejection, resulting in the appearance of predominant β-chain rearrangements in T cell clones generated from patients with acutely rejecting renal allografts.[32] T cell clones generated from graft infiltrating cell lines from a patient with acute rejection all contained $CD8^+$ cells with α/β TCR (T cell receptor) specificity. In these T cell clones, cytotoxicity was demonstrated to be class I restricted.[33] However, γδ TCR T cells were also frequently present in acutely rejecting renal allograft T cell clones and in some cases represented the predominant T cell population, suggesting that these γδ+ T cells had a direct cytolytic activity against renal epithelium.[34]

To summarize, T cell lines and clones could be generated from renal graft infiltrating cells, although the success was associated with the severity of acute rejection. The resulting T cell clones were either $CD4^+$ or $CD8^+$, with specific recognition of HLA class I and II peptides and predominant TCR Vβ chain rearrangements. Some of the clones demonstrated tissue-specific cytotoxicity.

Heart Transplantation

During the last decade, cardiac transplantation has emerged as a reasonably durable modality for the treatment of myopathic and complex structural heart disease in children. Currently, more than half of the pediatric recipients of a heart transplant are in the first 3 months of life.[17] After heart transplantation, tissue-infiltrating lymphocytes can be derived from endomyocardial biopsies, performed either routinely or upon suspicion of acute rejection. The majority of cultured lymphocytes from graft infiltrating cells from heart biopsies expressed a $CD3^+$ phenotype and contained the αβ T cell receptor. $CD4^+$ and $CD8^+$ molecules were heterogeneously expressed among T cell lines. Regarding cytotoxic surface markers, a significant percentage of cells were found to be $CD56^+$. Evaluation of CD45 isoforms showed that both "naïve" and "memory" cells were present among heart tissue–infiltrating lymphocytes.[35] For instance, T cells derived from a heart biopsy with acute rejection demonstrated a strong cytolytic activity against donor cells. No cytolytic T cell clones, on the other hand, could be established from biopsies without rejection. T cells responsible for this cytolytic activity, as detected by T cell cloning and TCR analysis, could not be found in earlier routinely performed biopsies, indicating that these cytolytic cells were recently recruited toward the endocardium.[36] In addition, lymphocytes cultured from endomyocardial biopsies which were obtained at two different time points after transplantation, during acute rejection and at quiescence, were not only reactive to donor heart–derived endothelial cells but also appeared to be specific for these cells.[37] Interestingly, T cell lines generated from heart biopsies used, like T cell lines from the kidney, a restricted Vβ gene repertoire. This was in contrast to the heterogeneous expression in peripheral blood T cells of the same patient.[38] T cell lines derived from human heart allograft infiltrate expressed γ/δ TCR poorly compared with T cell lines derived from human kidney allografts.[34,39] Furthermore, it has been shown that donor-specific cytotoxic T cells can contribute to the spectrum of locally produced cytokines. However, the cytokine expression of these cytotoxic T cells seems not to be limited to a special profile.[40]

In conclusion, T cell lines and clones from patients with acutely rejecting heart allografts, such as T cell lines and clones from acutely rejecting renal allografts, were $CD4^+$ or $CD8^+$ and demonstrated cytotoxicity in vitro. The cell lines and clones showed a restricted TCR Vβ repertoire. Production of cy-

tokines by T cell lines and clones from acutely rejecting heart allografts were found to be non-specific.

Liver Transplantation

Pediatric patient and graft survival after orthotopic liver transplantation have improved dramatically in recent years as a consequence of improved surgical techniques, advances in immunosuppression and astute management of early postoperative complications. However, the induction of donor-specific tolerance in children remains perhaps the most challenging event, particularly as several reports of long-term acceptance without signs of chronic rejection and without immunosuppression are known from adults after liver transplantation. In the small number of studies performed in this field, T cell lines were established from liver biopsies and were subsequently analyzed for their allospecificity. No consistent relationship between a predominance of $CD4^+$ or $CD8^+$ cells specific to HLA class I or II antigens was observed.[41] Although cytotoxicity could be detected against both class I and class II antigens, T cells which demonstrated a greater magnitude of donor-directed cytotoxicity appeared to be directed against class I antigens. Furthermore, as in T cell clones from renal and heart transplants, cultured lymphocytes from liver biopsies demonstrated an anti-donor-specific reactivity in vitro which was significantly correlated with cellular rejection.[41] It has been suggested that donor-derived soluble HLA class I antigen may have a tolerogenic effect in the transplant setting. To investigate this mechanism, HLA-specific T cell lines were used. It was demonstrated that soluble HLA antigen derived from sera of liver transplant recipients inhibited CTL activity in an allele-specific fashion, which could be explained by allele-specific apoptosis.[42]

Although less data are available on T cell lines and clones derived from patients with an acutely rejecting liver allograft, these specific cells appeared not to be phenotypically different clones generated from the kidney or the heart. However, soluble HLA antigen derived from sera of liver transplant recipients could suppress CTL activity of T cell lines.

Pancreas Transplantation

Pancreas transplantation is performed for the treatment of type 1 diabetes mellitus. In recent years, it differed from many other types of organ transplantation in terms of a greater uncertainty that this procedure would become fully justified, medically or economically. Pancreas transplantation is not life sustaining; its value must therefore be determined by comparison to the alternative therapy. Because insulin treatment is extraordinarily effective, even over relatively long periods of time, pediatric pancreas transplantation was carried out only in rare cases. Many studies using β cell transplants were performed in order to improve the quality of life imposed on patients by intensive insulin treatment. Only in exceptional cases were T cell lines generated from pancreas graft infiltrating cells. These cells appeared to have the same phenotype with respect to their CD4:CD8 ratio, cytokine production (IFN-γ, no IL-10) and allospecificity as compared with cell lines from kidney graft infiltrating cells during acute rejection after simultaneous pancreas-kidney transplantation.[43] However, differences were seen in a decreased lysis by some T cell clones derived from pancreas graft infiltrating cells of proximal tubular epithelial cells and donor spleen cells as compared to T cell lines derived from kidney infiltrating cells, suggesting that tissue-specific antigens might play a role.[43]

To summarize, T cell lines from kidney and pancreas graft infiltrating cells from patients with acute rejection after simultaneous pancreas-kidney transplantation showed similar characteristics but differed in cytotoxicity, indicating that tissue-specific antigens may be important in mechanisms of acute rejection.

Studies With T Cell Lines and Clones in Chronic Rejection and Tolerance

Despite improved immunosuppression, chronic rejection is still a major problem and results in constant attrition of many types of organ allografts. The mechanisms of chronic rejection are poorly understood and seem to be more complex than those of acute rejection. Whereas acute rejection is histologically characterized by an increasing inflammatory, cell-destructive process within days, chronic rejection represents a proliferative process leading, from months to years, to allograft arteriosclerosis and interstitial fibrosis. This phenomenon occurs in about half of all transplant patients and is the major cause of late graft loss. Induction of donor-

specific tolerance with preservation of otherwise normal immune responses is therefore a major goal of research in the field of transplantation.

Renal Transplantation

Using the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) database to determine clinical correlates of chronic rejection in pediatric renal transplants, it has been shown that chronic rejection was the most common cause of graft failure. More than two acute rejection episodes and late acute rejection (>365 days after transplantation) were the most common correlates for the development of chronic rejection.[44] The indirect pathway of allorecognition seems to play a major role in this process. Several studies were performed to clarify the role of indirect alloreactive T cells.

T cell clones were generated from peripheral blood of a chronically rejecting HLA-A2 negative patient of an A2-positive kidney transplant using a mixture of peptides corresponding to the polymorphic regions of the A2 sequence. The resulting T cell clones were specific for a single peptide of HLA-A2 and restricted by HLA-DRB*1502. Donor and recipient were DRB*1501 and DRB*1502, respectively. Although the large majority of clones responded to the synthetic A2 peptide presented by both alleles, only 3 out of 10 clones responded to cells expressing both DRB*1501 and A2. This suggests that sharing of an identical DR allele between donor and recipient might be beneficial and favors the induction of tolerance via the indirect pathway of allorecognition.[45] Recently, these T cell clones were used to study the effect of peptide analogues of HLA-A2 on the proliferative response of the clones. Presentation of the analogous peptide together with the wild-type peptide inhibited T cell responses, indicating that the use of altered peptides might be a new strategy to prevent the activation of T cells and therefore allograft rejection in vivo.[46] In another report, it was observed that the indirect pathway of allorecognition, initiated by soluble MHC antigens, could also be suppressed by high doses of synthetic peptides corresponding to the dominant alloepitope.[47]

T suppressor cells. Although the concept that T suppressor cells can down-regulate the immune response has long been accepted, the existence of a distinct population of lymphocytes that mediates suppression has not been convincingly demonstrated. T suppressor cell lines propagated from the circulation of a stable renal transplant patient demonstrated, for instance, class I restriction and functionally inhibited T cell proliferation.[48] In another patient with a long-term surviving renal allograft, both TCR αβ and γδ CTLs specific for class I or class II HLA donor antigen were generated. Interestingly, despite the relatively high frequency of these CTLs, no signs of graft rejection were observed in this patient, indicating that these specific CTL clones did not seem to be operational in vivo.[49] Suciu-Foca's group used human T cell lines to analyze the suppressive effects of $CD8^+$ $CD28^-$ T cells in allopeptide specific responses. $CD8^+$ $CD28^-$ T cells inhibited proliferation of $CD4^+$ T helper lymphocytes with cognate antigen specificity. These $CD8^+$ $CD28^-$ T cells displayed the critical functional characteristics of T suppressor cells.[50] More recently the same group demonstrated that $CD8^+$ $CD28^-$ T cells directly inhibit the CD40 signaling pathway of APCs, by a contact-dependent mechanism that renders bridging APCs incapable of inducing $CD4^+$ T helper activation. The effects of T suppressor cells on the functional state of APCs support the concept that the order in which both T suppressor and T helper cells interact with cognate APCs determines the functional outcome of immune responses.[51]

T cell lines and T cell clones phenotype. TCR αβ T cells infiltrating chronically rejected kidney allografts were reported to exhibit a strongly altered TCR Vβ gene repertoire. In contrast, a study of 18 patients with long-term surviving kidney allografts who experienced minor changes of chronic rejection without infiltrative activity suggests that only few antigenic determinants might have stimulated the immune system of the recipients.[52] Alloreactive $CD4^+$ and $CD8^+$ T cell clones were established from peripheral blood of another patient, who received a kidney from his mother and received no immunosuppression for the last 7 years with long-term stable renal function without chronic rejection. Unexpectedly high levels of donor HLA-specific T cell clonotype mRNA in peripheral blood were ob-

served in this patient during tolerance. It was suggested that some of these anti-donor HLA-specific T cells might contribute to the maintenance of peripheral tolerance to an allograft.[53] Recently, we demonstrated for the first time at the clonal level, using $CD4^+$ MHC allopeptide–specific T cell clones via the indirect pathway of allorecognition, that chronic allograft rejection is associated with the presence of Th1 phenotype cytokines whereas stable renal function is associated with a Th2 phenotype.[14]

In summary, T cell clones from patients with chronic allograft rejection were donor allopeptide specific and produced Th1 type cytokines whereas clones from patients with stable renal function were of Th2 phenotype. In addition, sharing of an identical DR allele between donor and recipient might favor the induction of tolerance, as seen from T cell lines and clones derived from renal allograft recipients with stable renal function. Furthermore, presentation of an analogous peptide, together with the wild-type allopeptide, inhibited T cell responses, indicating that the use of altered peptides might be a new strategy to prevent allograft rejection in vivo. Interestingly, suppressor $CD8^+$ $CD28^-$ cell lines, isolated from patients with stable renal function, displayed the functional characteristics of T suppressor cells. Moreover, the order in which both T suppressor and T helper cells interacted with cognate APCs appeared to determine the functional outcome of immune responses. How the latter finding will translate to the clinical setting is not yet clear.

Heart Transplantation

Most T cell clones generated from routinely performed heart biopsy specimens from patients after transplantation showed donor-specific reactivity. However, a small but distinct frequency (2-10%) of the T cell lines did not reveal anti-donor or third-party reactivity in vitro. Addition of these non-donor-reactive T cells to autologous peripheral blood mononuclear cells (PBMCs) markedly suppressed the donor-specific, but not third-party proliferative, response and prevented the generation of anti-donor but not third-party cytotoxicity. These suppressor cells were of $CD3^+$, $CD8^+$, CD45RO phenotype and expressed the $\alpha\beta$ TCR.[54]

Limitations of T Cell Lines and Clones

In general, T cell lines are obtained from peripheral blood or tissue biopsy specimens and only occasionally from rejected organs. Based on the literature and our own experience, it seems that generating T cell lines and clones, particularly from cells of tolerant patients, is much more difficult probably due to the lower frequency and proliferation rate in vitro of these cells, compared to cells derived from acutely or chronically rejecting patients.[14] Although in vitro propagated T cell lines and clones represent a biased population of cells that are selected for growth in an artificial environment with IL-2 and/or other cytokines or growth factors like phytohemagglutinin, they provide a unique tool to dissect at the clonal level the cellular mechanisms of rejection and tolerance.

Conclusion: What Can We Learn About Mechanisms of Pediatric Graft Rejection From T Cell Lines and Clones?

T cells remain quiescent and are recirculating within the lymphoid tissues until they recognize their specific antigen. Interaction of the TCR with its cognate antigen results in consecutive activation of a large number of intracellular signaling pathways. This results in de novo expression of a range of genes, including those encoding cytokines and new cell surface proteins. The signaling pathways involved in this process have been increasingly well characterized and are now the target of some of our most potent immunosuppressive drugs. Enormous interest has been attracted to study how depriving the T cell of costimulation results in an unresponsive and even regulatory fate, because harnessing and applying this mechanism could be of major use in preventing graft rejection. It was clearly demonstrated that T cells receive important signals during activation through binding of soluble proteins, the cytokines, to specific cell surface counterreceptors. It is possible that through up-regulating the expression of receptors for other cytokines, cytokines derived primarily from macrophages, such as IL-1 and IL-12, sensitize T cells to the proliferative and differentiative effects of the T cell–derived cytokines, such as IL-2 and IL-4. Particular interest has been focused on the possibility that, while a Th1 driven response may inevitably be damaging

and result in graft rejection, a Th2 driven response may not have this effect and in consequence may be associated with the induction of tolerance to an allograft. It was observed from these studies that tolerance or reduced anti-donor specific reactivity is associated with a decrease in expression of the Th1-associated cytokines IL-2 and IFN-γ. This decrease may be accompanied by the expression of regulatory Th2 cells, and there was indeed some evidence that the expression of cytokines such as IL-4 was preserved during the development of tolerance. However, cells other than Th2 cells can also produce these cytokines, so that their detection does not necessarily infer the presence or action of a distinct Th2 population.

Both humoral and cellular mechanisms can affect graft destruction. Therefore, it is likely that any type of immunity, Th1 or Th2 driven, can result in graft rejection. Generally, $CD4^+$ cells seem to play a key role in directing graft rejection, but there is data showing that $CD8^+$ cells are also required for rejection. CTLs may be involved in graft rejection, although they are present only at low levels in grafts undergoing rejection. Moreover, graft destruction may also occur in the absence of demonstrable CTL activity, and the presence of such cells within a graft may not always lead to graft destruction. Other experiments have demonstrated the presence of CTLs within a graft that was not rejected. These results remain intriguing, as they provide direct evidence of cytotoxic effector cells within an organ graft that is not ultimately rejected. Even if CTLs themselves do not mediate the tissue damage that results in graft loss, they may still be important in the immune response to the graft. Through the elaboration of high levels of IFN-γ, they are able to recruit and activate cells involved in delayed-type hypersensitivity lesions, thus initiating acute or chronic rejection.

Various factors may account for the high incidence of graft failure following an index acute rejection episode. Although this finding is controversial, infants and young children, as compared to adults, may have a heightened immune response that may be inadequately suppressed with current immunosuppressive protocols. Additionally, failure to reverse the index acute rejection episode may be attributable to a delay in diagnosis since serum creatinine levels are a poor factor indicating an acute rejection episode when a large kidney is placed into a small child. Other diagnostic indicators such as molecular markers of acute rejection may need to be utilized in infants and young children to indicate the presence of rejection if the diagnosis is to be made before extensive damage to the graft occurs. But the only true solution to this problem seems to be the development of strategies to induce donor-specific tolerance in the recipient. Better understanding of the mechanisms of rejection and tolerance through T cell lines and clones may lead in the future to novel therapeutic strategies. Since acute rejection appears to be the most critical element in the genesis of chronic rejection, it is imperative that maximal effort be made to prevent first rejection episodes. Pediatric organ recipients are perhaps the most likely group to derive long-lasting advantages from the vigorous research efforts currently underway to avoid long-term immunosuppression and eventually achieve donor-specific tolerance.

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