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The role of alemtuzumab in facilitating maintenance immunosuppression minimization following solid organ transplantation

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ABSTRACT

National registry data indicate a trend towards the incorporation of lymphocyte depletion antibody induction therapy into immunosuppressive regimens for solid organ transplantation. Depletional induction has been shown to reduce the risk of early acute rejection, but increase the risk of immune incompetence. As such, it recently has been paired with reduced maintenance immunosuppression in an effort to curb excessive immunosuppression without sacrificing low rejection rates. Alemtuzumab is a humanized CD52-specific monoclonal antibody that has been used in the setting of maintenance immunosuppression minimization. Although not specifically indicated for organ transplantation, it is now used off-label as an induction agent in approximately 10% of transplant recipients in the United States. In general, alemtuzumab is well tolerated and substantially reduces the risk of acute rejection in the first 6 months post-transplant in non-sensitized recipients. There is little evidence to support the notion that it uniquely promotes tolerance, and growing evidence that it is ineffective in the setting of allosensitization. Alemtuzumab-treated patients clearly remain dependent on maintenance immunosuppression. Long-term outcome data will be required to determine the magnitude and type of maintenance therapy that makes best use of alemtuzumab's depletional effects.

1. Introduction

In the past two decades the field of organ transplantation has witnessed unparallel advances in short-term patient and graft survival [1]. Much of this success can be attributed to the development of potent immunosuppressive agents to include brief courses of lymphocyte-depleting antibodies [2]. The result has been marked reduction in graft loss due to acute rejection [1,3] and an unprecedented variety of effective immunosuppressive agents available to clinicians [4–6]. Indeed, no fewer than 30 combinations of immunosuppressive drugs are reportedly used clinically in North America [3].

Unfortunately, despite exceptional success in early graft and patient survival, late results remained plagued by excessive patient morbidity and mortality [1]. Importantly, the immunosuppressive agents responsible for admirable early transplant results are not benign and their chronic administration is associated with significant immunosuppression-related complications. Notably, herpesvirus reactivation, post-transplant diabetes mellitus, and cardiovascular disease have been shown to claim many chronically immunosuppressed patients despite excellent allograft function, and in particular, lymphocyte-depleting antibody therapies used for induction have been shown to increase infectious and malignant morbidity, especially when paired with standard multi-drug immunosuppressive regimens [7–14]. Thus, there is growing interest in the development of strategies that reduce ones dependence of chronic immunosuppression without sacrificing the freedom from acute rejection now typical of modern transplant regimens.

Immunosuppression regimens can be fundamentally divided into induction, maintenance, or rescue therapies. Induction therapy is characterized by an intense prophylactic therapy initiated at the time of transplantation based on the tenet that powerful immunosuppression is required early on to prevent acute rejection. Over time, the risk of rejection diminishes and therefore induction therapy is replaced by maintenance regimens. These are often of less potency and adapted to an individual's needs and pharmacological responses. Rescue therapy is similar to induction therapy in that it represents an aggressive yet brief course of immunosuppression designed to reverse established rejection episodes.

As evidenced by the many regimens in routine clinical use, every transplant center develops its preferred immunosuppression regimens based on institutional and anecdotal experiences. Despite these differences, the principle of induction therapy is time-honored and routinely employed. Strategies may include high doses of maintenance agents (bolus glucocorticosteroids or intravenous calcineurin inhibitors; CNIs), or specific induction drugs such as antibody therapy. The use of antibody induction therapy has been steadily growing in the United States and exceeds 50% of patients for all organs except liver [2,15]. Many of these specialized induction agents have been studied in randomized trials and have proved to be efficacious in combination with standard maintenance immunosuppression. No

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agent has yet to distinguish its superiority in the clinical setting. These agents when considered collectively have been shown to reduce the incidence of early acute rejection in renal allograft recipients with the greatest advantage in those who are at high risk of rejection [16,17]. The role of antibody induction therapy in cardiac transplantation is unclear and there is limited evidence showing any benefit in liver transplantation [18]. In the setting of simultaneous kidney pancreas transplantation, there is modest evidence of their efficacy in reducing acute rejection [19,20].

Specific induction antibody therapy can be divided into T cell depleting agents or non-depletion therapies based on whether their use leads to bulk reduction of peripheral lymphocytes. Examples of non-depleting antibody therapies include the CD25-specific antibodies. Both daclizumab and basiliximab have been shown to reduce acute rejection with minimal patient intolerability in kidney, liver, heart and islet cell transplantation [21–24]. Unfortunately, these agents have not been shown to as successfully lead to CNI avoidance or monotherapy maintenance immunosuppression compared to their depletional counterparts [25,26].

Depletional induction therapy can be further subdivided into those that are polyclonal anti-T lymphocyte/thymocyte preparations (thymoglobulin [ATG-R], ATGAM, ATG-Fresenius) and monoclonal preparations specific for common lymphocyte antigens such as CD3 (muromonab), CD20 (rituximab) and CD52 (alemtuzumab). These agents typically lead to rapid lymphocyte depletion that can be prolonged [27]. There have been uncontrolled pilot reports of the use of polyclonal antibody preparation induction with monotherapy maintenance therapy with comparable patient and graft survival to standard therapy in selected patients [28,29]. However, despite the theoretical benefits of reduced maintenance immunosuppression associated with these regimens, their results have not adaptable to general clinical populations. Recently, the pairing of depletional induction with maintenance minimization has taken place with alemtuzumab. This manuscript will provide an overview of the role of alemtuzumab in immunosuppression minimization regimens and summarize the current clinical evidence of its efficacy towards this goal.

2. History

Alemtuzumab originated with the introduction by Waldmann in 1984 of complement-fixing antibodies specific for CD52, a glycoprotein expressed on most lymphocytes, natural killer cells, monocytes, and thymocytes [30,31]. The initial preparations included both rat IgM (Campath-1M) and IgG (Campath-1G) isotypes. Early clinical trials with these rat-derived agents demonstrated their lymphocyte-depleting potential, but in combination with full dose maintenance or rescue regimens, were plagued by infectious complications [32,33]. For perspective, these trials were performed prior to the currently available potent antiviral prophylactic agents. In addition, these agents were found to be potentially immunogenic limiting their clinical utility [34]. Further work revealed human IgG1 as the best choice for complement lysis and antibody dependent cellular cytotoxicity and advancements in bioengineering allowed the development of a humanized IgG1 CD52-specific monoclonal antibody, Campath-1H, which was developed for clinical use [35–37]. In 1999, the FDA approved Campath-1H as alemtuzumab for the treatment of lymphoid malignancies and its role in transplantation has steadily grown, especially in concert with immunization minimization trials.

3. Mechanistic insights

CD52 is a 12-amino acid glycosylphosphatidylinositol (GPI) anchored glycoprotein uniquely suited for use as a therapeutic target [38]. It is a high-density, non-modulating molecule expressed on lymphocytes and monocytes that is absent on lymphoid progenitors. CD52's small size and proximity to the cell membrane facilitates efficient complement activation and membrane attack complex deposition upon alemtuzumab binding [39]. Antibody dependent cellular cytotoxicity (ADCC) is also believed to account for its efficacy and is aided by the persistence of CD52 on the cell surface despite antibody binding (unlike CD3).

The presumed dominant mechanism by which alemtuzumab reduces the risk of allograft rejection is lymphocyte death and a commensurate reduction in allospecific T cell precursor frequency. A single dose of alemtuzumab can lead to >99% peripheral blood lymphocyte depletion within 1 h of administration. There is also significant lymph node lymphocyte depletion within two to four days post-therapy [40]. While this is clearly mediated by compliment-mediated lysis and ADCC, other mechanisms have been reported. Nuckel et al. reported that alemtuzumab may facilitate apoptosis of leukemic lymphocytes via the classical caspase-dependent cell death possibly secondary to activation of CD52 dependent signaling pathway associated with increased caspase 3 and 8 expression [41]. Alemtuzumab's pro-apoptotic effect was augmented in the presence of cross-linking anti-Fc antibody which promoted cell clustering suggesting a role of ADCC. Stanglmaier et al. have proposed that alemtuzumab may also lead to enhanced lymphocyte apoptosis via a non-classical caspase-independent death [42].

While depletion is profound in alemtuzumab-treated patients, the nuances of the drug's effect may have substantial influence on its efficacy. Pearl et al. have demonstrated that alemtuzumab leads to heterogeneous lymphocyte depletion showing specifically that antigen-experienced memory T cells are less susceptible to depletion compared to naïve cells, a trait also shared by polyclonal agents [43]. The mechanisms by which this effect occur remain a matter of speculation, but may relate to survival pathways inherent to long-lived memory T cells, or differential distribution of antigen-experienced cells that sequester them from antibody exposure.

The inhomogeneous depletion seen in alemtuzumab-treated patients suggests that a recipient's pre-transplant heterologous allospecific immune memory dictates relative resistance or sensitivity to therapy and have been evoked as a potential mechanism of long-term efficacy. Trzonkowski et al. recently reported on their pilot study of alemtuzumab induction, reduced maintenance approach in 13 kidney allograft recipients [44]. The authors presented novel evidence demonstrating that the relative resistance of CD28−CD8+ T cells correlates with protection against acute rejection. These cells in an *in vitro* setting appear to compete with the recovery of CD4+ cells through either cell-to-cell contact or IL10 dependent mechanisms and in doing so limit CD4 cell help for a de novo alloimmune response.

There is growing preliminary evidence suggesting that the residual post-alemtuzumab T cell population may be biased towards T cells with regulatory potential. Noris et al. recently reported on the results of a randomized, prospective trial with alemtuzumab induction and either sirolimus (SRL) or cyclosporine (CsA) with mycophenolate mofetil (MMF) maintenance therapy in kidney transplant recipients. The authors noted the emergence of T regulatory cells defined by CD4+CD25+ FoxP3+ with *in vitro* regulatory function in setting of SRL but not CsA. There have been corroborating reports with similar findings involving preliminary experiences with alemtuzumab induction therapy in kidney transplant patients [27,45]. Once again, alemtuzumab induction compared to Thymoglobulin or daclizumab led to a shift towards increased percentage of post-depletional T cells with a regulatory-like surface phenotype (CD4+CD25+) and more prominent FoxP3 mRNA expression. Though it should be noted that these observations of increased or disproportionate T regulatory cells have not been uniformly observed [43]. These discrepancies may be attributable to the influence of the maintenance immunosuppression on homeostatic repopulation and not unique to alemtuzumab.

4. Clinical application of alemtuzumab in renal transplantation

Clinical trials have focused efforts on strategies minimizing exposure to either corticosteroids or CNIs. These efforts can be summarized by two