Conventional immunosuppression and co-stimulation blockade

Andrew B. Adams, Thomas C. Pearson and Christian P. Larsen*

The Carlos and Marguerite Mason Transplantation Research Center, Department of Surgery, Emory University School of Medicine, 5105 Woodruff Memorial Research Building, 1639 Pierce Drive, Atlanta, GA 30322, USA

Organ transplantation has become an accepted and successful therapeutic intervention for many patients with end-stage organ disease. Current conventional immunosuppressive regimens achieve very good short-term allograft survival but long-term outcomes are less than adequate. Furthermore, non-specific immunosuppression has its attendant side-effects including increased risks of malignancy and infection as well as drug-specific sequelae. With recent advances in the field of immunology, promising new therapies have arisen that could potentially eliminate lifelong drug therapy and promote indefinite acceptance of the donor tissue. Identification of co-stimulatory signals essential for T-cell activation has provided exciting new possibilities for controlling the alloimmune response. The compatibility of these new agents with proven conventional therapeutics has yielded mixed results. When used in combination, their immunosuppressive properties appear synergistic. However, if the goal of therapy is sustained, specific T-cell hyporesponsiveness, many conventional agents antagonize the effects of co-stimulatory blockade in several immune tolerance models.

Keywords: transplantation; co-stimulation blockade; immunosuppression; immune tolerance

1. INTRODUCTION

Therapeutic manipulation of T-cell co-stimulatory pathways has emerged as an important avenue of investigation in the field of transplantation. It is widely accepted that naive T cells require more than one cellular signal for optimal activation, proliferation and cytokine production. The first signal is acquired by the interaction between the T-cell receptor (TCR) and antigenic peptide bound to the major histocompatibility complex (MHC). 'Signal 1', as it is called, imparts specificity to the immune response and is necessary but not sufficient for optimal T-cell activation. 'Signal 2', the co-stimulatory signal, is provided by a variety of molecules present on antigen-presenting cells, the best studied of which include CD80, CD86 and CD40 (Jenkins 1994; Lafferty & Gill 1993). Receipt of both signals leads to activation and effector maturation. If, however, a T cell receives 'signal 1' without a co-stimulatory signal, it will either become 'anergic', i.e. unable to respond to further stimulation, or undergo apoptosis (Janeway & Bottomly 1994). Over the past decade a number of laboratories have exploited this observation to biologically block co-stimulatory signals at the time of transplantation in the hope of specifically inactivating or eliminating alloreactive T cells. Indeed, many have shown that regimens employing co-stimulation blockade can achieve long-term allograft survival in experimental models of organ and tissue transplantation. The majority of these studies have used cytotoxic T-lymphocyte antigen 4 immunoglobulin (CTLA-4-Ig), a fusion protein that binds CD80 and CD86, and/or a blocking monoclonal

antibody (mAb) to CD154 (CD40 ligand (CD40L)). The promising results achieved with co-stimulation blockade in animal models have prompted enthusiasm for their potential clinical use.

Current immunosuppressive therapies in transplantation achieve excellent results, especially in terms of relatively short-term patient and allograft survival rates. Ethical consideration will probably preclude complete abandonment of these agents when testing the efficacy of co-stimulation blockade-based strategies in clinical transplantation. Consequently, clinical trials using co-stimulation blockade will require a combination of these therapies to ensure an adequate margin of safety in patients. Therefore, an assessment of compatibility of co-stimulation blockade with conventional immunosuppressants is essential. In this review we provide our perspective on what is known and not known about the interactions of co-stimulation blockade and current immunosuppressive modalities.

2. SYNERGISTIC IMMUNOSUPPRESSIVE ACTIVITY

T-cell activation involves a number of cyclosporin A (CsA)-sensitive mechanisms including the Ca^{2+} -dependent intracellular signalling cascade initiated by MHC binding of the TCR-CD3 complex (Siekierka & Sigal 1992). An additional co-stimulatory signal through CD28, initiated by the binding of a member of the B7 family, is also required for optimal activation. This signal has been reported to be calcium independent and relatively resistant to inhibition by CsA (June *et al.* 1987; Ledbetter *et al.* 1990). Van Gool *et al.* (1994a,b) reasoned that the combination of CsA and an anti-B7 mAb, which

*Author for correspondence (clarsen@emory.org).

blocks CD28–B7 interactions, would act more efficiently to inhibit T-cell activation. Alone, neither CsA nor anti-B7 mAb was able to completely block alloantigen-induced T-cell activation *in vitro*, while dual therapy inhibited both proliferation and cytotoxicity. Others have demonstrated additive effects of low-dose CTLA-4-Ig and low-dose CsA. For example, Bolling *et al.* (1994) suggested a role for co-stimulation blockade in lowering the dose of CsA to maximize immunosuppression while minimizing side-effects associated with this agent. These findings are in apparent conflict with earlier work by Jenkins and colleagues (Jenkins *et al.* 1987, 1988; Jenkins & Schwartz 1987) who found that CsA blocked the induction of non-responsiveness in T-cell clones. They reported that a calcium-dependent signal, mediated via the TCR, was essential for induction of anergy. While there were experimental design differences between these studies, which may explain the discrepant results, the dilemma remains as to the actual impact of calcineurin inhibitors on co-stimulation blockade-based strategies.

In vivo, the immunosuppressive activities of calcineurin inhibitors and blockade of the CD28–B7 pathway appear to be additive. For example, Hale *et al.* (1997) reported that when CsA was combined with CTLA-4-Ig, skin graft survival of MHC class I-disparate mice was significantly improved. Others have also demonstrated that CsA and CTLA-4-Ig are compatible and lead to improved allograft survival in rodent models (Azuma *et al.* 1996; Bolling *et al.* 1994; Perico *et al.* 1995). We have shown that there are additive immunosuppressive effects of CsA and CTLA-4-Ig in large animal models as well (Pearson *et al.* 1997). Treatment of rhesus monkeys undergoing renal transplantation with CTLA-4-Ig revealed possible synergy between CTLA-4-Ig and CsA in delaying acute rejection and prolonging graft median survival time (MST). A course of CTLA-4-Ig treatment alone at the time of transplantation was associated with modest prolongation of allograft survival (MST of 20 days versus 10 days in control). However, when a regimen of CsA and steroids was combined with CTLA-4-Ig, allograft survival was remarkably improved (MST of 150 days versus 22 days in a group receiving CsA and steroids alone). In this model we have recently observed that administration of BMS-224818, a high-affinity mutant form of CTLA-4-Ig, is immunosuppressive (MST of 45 days versus 8 days) and acts synergistically when combined with either a regimen including mycophenolate mofetil (MMF) and steroids (MST of 157 days versus 30 days) or Simulect® (anti-interleukin 2 receptor mAb) (MST of 125 days versus 8 days) (Pearson *et al.* 2001). It has also recently been reported that the combination of CsA and an anti-B7 mAb acts to extend skin graft survival in a non-human primate model (Ossevoort *et al.* 1999). Thus in models where the goal of co-stimulation blockade treatment is immunosuppression, the addition of conventional immunosuppressants, particularly CsA, affords additional immunosuppressive activity when compared with either agent alone.

3. ABROGATION OF TOLERANCE INDUCTION

In contrast to these findings when co-stimulation blockade is used as a method to induce a state of tolerance

or durable hyporesponsiveness, the effects of calcineurin inhibitors appear to be quite different. For example, we were surprised to discover that concomitant CsA administration abrogated the beneficial effects of a regimen combining CTLA-4-Ig and anti-CD40L mAb that prolonged skin graft survival in mice (Larsen *et al.* 1996). These results imply that the induction of unresponsiveness in T cells deprived of co-stimulatory signals is an active process requiring signalling via the TCR–CD3 complex and/or other CsA-sensitive pathways, consistent with the results reported by Jenkins several years earlier.

A mechanistic basis for these observations emerged with the report that CsA may affect the expression of co-stimulatory molecules, particularly CD40L (Fuleihan *et al.* 1994; Grammer *et al.* 1999). The CD40–CD40L interaction is essential for T-dependent B-cell activation and subsequent Ig isotype switching. Fuleihan *et al.* demonstrated that activation of protein kinase C and calcineurin are required for CD40L gene expression and that CsA inhibits CD40L gene expression and CD40L-dependent T-cell function. They specifically observed that CsA analogues inhibited CD40L expression with a potency relative to their ability to inhibit calcineurin activity, indicating that the phosphatase plays a key role in CD40L gene expression. Blair *et al.* (2000) have suggested that anti-CD40L mAb may be exerting its beneficial effects by a means other than simply blocking CD40–CD40L interactions. They report abortive T-cell activation and enhanced apoptosis following *in vitro* stimulation with anti-CD40L and anti-CD3, suggesting anti-CD40L may exert some of its effects by delivering a 'negative' signal through CD40L. Treatment with CsA abolished these effects, although it is unclear whether this was due to the absence of signal 1 or decreased expression of CD40L. More recently, work by Li *et al.* (1999) has elucidated another possible mechanism whereby calcineurin inhibitors prevent the induction of hyporesponsiveness. These studies convincingly demonstrated that cell-cycle-dependent apoptosis was required for co-stimulation blockade-dependent peripheral tolerance induction. CsA abrogated the effects of co-stimulation blockade by inhibiting proliferation and apoptosis of alloreactive T cells. In contrast to CsA, sirolimus (rapamycin), which does not alter preliminary signalling through the TCR, was very compatible with co-stimulation blockade.

Kirk *et al.* (1999) recently reported that treatment with anti-CD40L mAb (a total of five months) in a non-human renal allograft model achieved long-term survival in the absence of immunosuppression. Once again, the addition of conventional immunosuppressants seemed to hinder the beneficial effect of a co-stimulation blockade-based strategy. When transplant recipients were treated with anti-CD40L mAb and an extended course of either steroids and MMF or the calcineurin inhibitor tacrolimus, allograft survival was significantly reduced as compared with those animals treated with anti-CD40L alone. These results also support the hypothesis that the induction of peripheral hyporesponsiveness is an active process requiring signals, some of which are calcineurin dependent.

Recently non-myeloablative regimens using donor bone marrow and co-stimulation blockade to promote mixed chimerism and transplantation tolerance have been

reported (Durham *et al.* 2000; Wekerle *et al.* 2000). Whether protocols that promote stable haemopoietic chimerism will be affected with the addition of conventional immunosuppressants remains to be determined.

4. CONCLUSIONS

The effects of conventional immunosuppressants, in particular calcineurin inhibitors, on treatment regimens including co-stimulation blockade seem to depend on the therapeutic goal. The experimental results to date suggest that calcineurin inhibitors may produce beneficial effects when combined with co-stimulation to achieve immunosuppression. In contrast, calcineurin inhibitors may block 'tolerogenic' effects of co-stimulation blockade. Future studies will be required to define which conventional immunosuppressive agents can be effectively combined with the various co-stimulation blockade agents in ethically acceptable clinical trials in transplantation. These efforts hold the promise for improved short-term allograft survival rates, a decrease in negative side-effects, and ultimately transplantation tolerance.

REFERENCES

- Azuma, H., Chandraker, A., Nadeau, K., Hancock, W. W., Carpenter, C. B., Tilney, N. L. & Sayegh, M. H. 1996 Blockade of T-cell costimulation prevents development of experimental chronic renal allograft rejection [see comments]. *Proc. Natl Acad. Sci. USA* **93**, 12 439–12 444.
- Blair, P. J. (and 10 others) 2000 CD40 ligand (CD154) triggers a short-term CD4(+) T cell activation response that results in secretion of immunomodulatory cytokines and apoptosis. *J. Exp. Med.* **191**, 651–660.
- Bolling, S. F., Lin, H., Wei, R. Q., Linsley, P. & Turka, L. A. 1994 The effect of combination cyclosporine and CTLA4-Ig therapy on cardiac allograft survival. *J. Surg. Res.* **57**, 60–64.
- Durham, M. M., Bingaman, A. W., Adams, A. B., Ha, J., Waitze, S.-Y., Pearson, T. C. & Larsen, C. P. 2000 Administration of anti-CD40 ligand and donor bone marrow leads to hemopoietic chimerism and donor-specific tolerance without cytoinductive conditioning. *J. Immunol.* **165**, 1–4.
- Fuleihan, R., Ramesh, N., Horner, A., Ahern, D., Belshaw, P. J., Alberg, D. G., Stamenkovic, I., Harmon, W. & Geha, R. S. 1994 Cyclosporin A inhibits CD40 ligand expression in T lymphocytes. *J. Clin. Invest.* **93**, 1315–1320.
- Grammer, A. C., McFarland, R. D., Heaney, J., Darnell, B. F. & Lipsky, P. E. 1999 Expression, regulation, and function of B cell-expressed CD154 in germinal centers. *J. Immunol.* **163**, 4150–4159.
- Hale, D. A., Gottschalk, R., Maki, T. & Monaco, A. P. 1997 Use of CTLA4-Ig in combination with conventional immunosuppressive agents to prolong allograft survival. *Transplantation* **64**, 897–900.
- Janeway Jr, C. A. & Bottomly, K. 1994 Signals and signs for lymphocyte responses. *Cell* **76**, 275–285.
- Jenkins, M. K. 1994 The ups and downs of T cell costimulation. *Immunity* **1**, 443–446.
- Jenkins, M. K. & Schwartz, R. H. 1987 Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. *J. Exp. Med.* **165**, 302–319.
- Jenkins, M. K., Pardoll, D. M., Mizuguchi, J., Chused, T. M. & Schwartz, R. 1987 Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones. *Proc. Natl Acad. Sci. USA* **84**, 5409–5413.
- Jenkins, M. K., Ashwell, J. D. & Schwartz, R. H. 1988 Allogeneic non-T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically modified antigen-presenting cells. *J. Immunol.* **140**, 3324–3330.
- June, C. H., Ledbetter, J. A., Gillespie, M. M., Lindsten, T. & Thompson, C. B. 1987 T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol. Cell. Biol.* **7**, 4472–4481.
- Kirk, A. D. (and 15 others) 1999 Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates [see comments]. *Nature Med.* **5**, 686–693.
- Lafferty, K. J. & Gill, R. G. 1993 The maintenance of self-tolerance. *Immunol. Cell Biol.* **71**, 209–214.
- Larsen, C. P. (and 11 others) 1996 Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature* **381**, 434–438.
- Ledbetter, J. A., Imboden, J. B., Schieven, G. L., Grosmaire, L. S., Rabinovitch, P. S., Lindsten, T., Thompson, C. B. & June, C. H. 1990 CD28 ligation in T-cell activation: evidence for two signal transduction pathways. *Blood* **75**, 1531–1539.
- Li, Y., Li, X. C., Zheng, X. X., Wells, A. D., Turka, L. A. & Strom, T. B. 1999 Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. *Nature Med.* **5**, 1298–1302.
- Ossevoort, M. A., Lorre, K., Boon, L., Van den Hout, Y., de Boer, M., De Waele, P., Jonker, M. & Van de Voorde, A. 1999 Prolonged skin graft survival by administration of anti-CD80 monoclonal antibody with cyclosporin A. *J. Immunother.* **22**, 381–389.
- Pearson, T., Routenberg, K., Abrams, J., Linsley, P. & Larsen, C. 1997 Prolongation of renal allograft survival with CTLA4-Ig in rhesus macaque. In *Programs and abstracts of the 17th American Society of Transplant Physicians Annual Meeting, Chicago* 10–14 May 1997.
- Pearson, T. (and 11 others) 2001 Prolongation of renal allograft survival with blockade of the CD28 pathway using a novel mutant CTLA4-Ig fusion protein in non-human primates. (In preparation.)
- Perico, N., Imberti, O., Bontempelli, M. & Remuzzi, G. 1995 Toward novel antirejection strategies: *in vivo* immunosuppressive properties of CTLA4-Ig. *Kidney Int.* **47**, 241–246.
- Siekierka, J. J. & Sigal, N. H. 1992 FK-506 and cyclosporin A: immunosuppressive mechanism of action and beyond. *Curr. Opin. Immunol.* **4**, 548–552.
- Van Gool, S. W., Ceuppens, J. L., Walter, H. & de Boer, M. 1994a Synergy between cyclosporin A and a monoclonal antibody to B7 in blocking alloantigen-induced T-cell activation. *Blood* **83**, 176–183.
- Van Gool, S. W., de Boer, M. & Ceuppens, J. L. 1994b The combination of anti-B7 monoclonal antibody and cyclosporin A induces alloantigen-specific anergy during a primary mixed lymphocyte reaction. *J. Exp. Med.* **179**, 715–720.
- Wekerle, T., Kurtz, J., Ito, H., Ronquillo, J. V., Dong, V., Zhao, G., Shaffer, J., Sayegh, M. H. & Sykes, M. 2000 Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoinductive host treatment. *Nature Med.* **6**, 464–469.