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OVERVIEW

# TRENDS IN THE USE OF GLUCOCORTICOIDS IN RENAL TRANSPLANTATION[1]

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Over the past 40 years, corticosteroids have played a key role in the evolution of successful organ transplantation. As early as 1951, Billingham and colleagues (*1*) demonstrated that the immune-modulating effects of corticosteroids prevented rejection and promoted survival of rabbit skin homografts. In 1963, Goodwin et al. (*2*) described the effectiveness of large doses of corticosteroids in suppressing and reversing rejection episodes in one of the first technically successful human renal allograft transplants. The use of corticosteroids to control acute allograft rejection was a breakthrough that allowed renal transplantation to become a routine procedure. During the late 1950s, the immunosuppressive activity of an AZA metabolite also was recognized (*3*, *4*). Although AZA was used alone in the early days of cadaveric transplantation, the combination of AZA and prednisone soon thereafter became the conventional maintenance immunosuppressive regimen in organ transplantation and remained so for nearly 2 decades (*5*).

Despite the numerous and well-recognized side effects of corticosteroids, reliance on these agents has persisted since the introduction of CsA (*6*). Even before the availability of CsA, a trend had begun toward lowering steroid dosage both for maintenance immunosuppression (*7-9*) and for treatment of acute rejection (*10*, *11*) in order to minimize side effects. These "low-dose" regimens were initiated at a time when our understanding of the immunosuppressive mechanisms of steroids and of the immunologic consequences of altering steroid dosage was rudimentary. CsA has proved to be even further steroid sparing, and its widespread use during the past decade has been accompanied by continued interest in steroid reduction regimens. This past decade also has witnessed a rapid expansion in knowledge of the cellular and subcellular mechanisms of corticosteroid immunosuppression. Despite their toxicities, corticosteroids are still prescribed in combination with AZA and/or CsA as maintenance immunosuppression for most allograft recipients. In addition, corticosteroids remain the agents most commonly used for initial treatment of acute rejection (*12*). However, the emergence of CsA as the primary immunosuppressant for organ transplant recipients has been paralleled by a growing interest in steroid-free immunosuppression. In this review, we summarize the current understanding of the mechanisms by which corticosteroids suppress the rejection response. In addition, we present an overview of historical trends in the use of corticosteroids for the prophylaxis and treatment of rejection in renal transplantation, and we consider the benefits and risks of steroid-free immunosuppression in the modern era.

## PHARMACOLOGY OF GLUCOCORTICOIDS IN PREVENTION OF ALLOGRAFT REJECTION

### *T Cell Activation*

T cell-dependent immune responses, which require T cell activation, are the predominant factors accounting for acute allograft rejection. T cell activation is a coordinated, preprogrammed process that ensues upon recognition by T cells of foreign antigen expressed in association with MHC class II molecules on the surface of professional APCs. Engagement of TcRs (first signal) and the costimulatory signal provided by the LFA3-CD2 interaction initiates the calcium-dependent phase of T cell activation (*13*, *14*). Association of *src*-like tyrosine kinases (such as $p56^{lck}$, $p60^{fyn}$, and ZAP-70) with intracellular domains of CD3 and CD4/CD8 molecules results in the phosphorylation of several cellular proteins, including phospholipase C (*15*, *16*). Phospholipase C, in turn, catalyzes the breakdown of inositol bisphosphate leading to generation of inositol trisphosphate and diacylglycerol. Inositol trisphosphate mobilizes calcium from intracellular stores while diacylglycerol binds to and induces the translocation of the calcium- and phospholipid-sensitive protein kinase C to membrane-bound compartments (*13*, *17*). The terminal phase of intracellular signal transduction requires activation/expression of DNA-binding proteins (transcription factors) that trigger gene transcription. Calcineurin, a calcium-dependent phosphatase, plays a key role in activation of factors required for IL-2 gene transcription (*13*, *18*).

An ordered sequence of transcriptional events follows (*13*). Genes encoding IL-2 and the low-affinity IL-2 receptors

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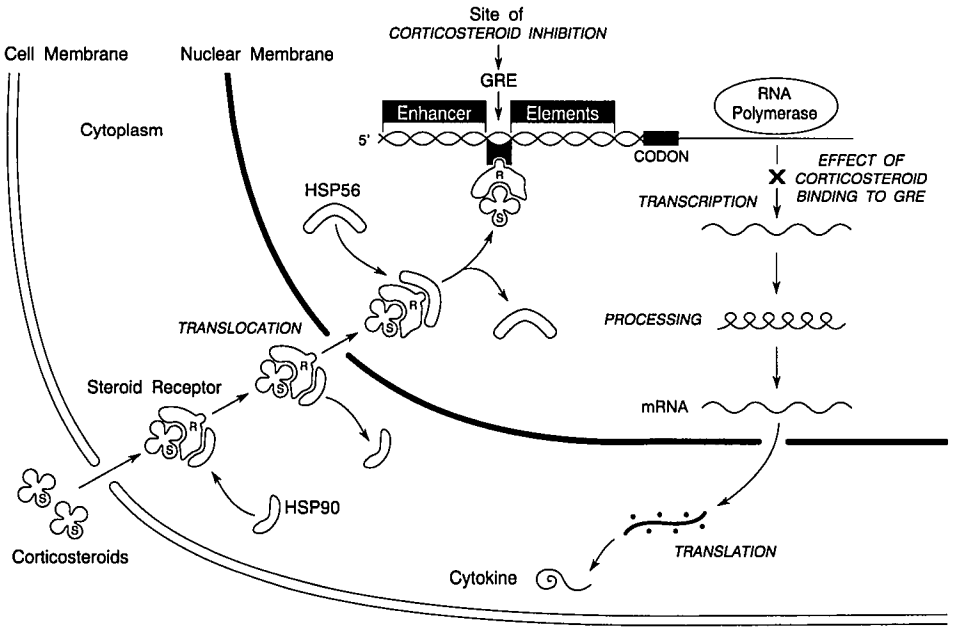


FIGURE 1. Glucocorticoids exert their immunosuppressive activity by blocking the expression of several cytokine genes. S, glucocorticoid; R, glucocorticoid receptor; GRE, glucocorticoid response elements; HSP, heat shock protein; X, inhibition by corticosteroids. (Figure originally drawn by D.R. Salomon, Scripps Research Institute.)

(IL-2R α-chain, p55, Tac) are activated (*13*, *19*, *20*). IL-2 interaction with IL-2R induces/amplifies the expression of IL-3, IL-4, IL-5, IL-6, IL-7, and IFN-γ (*17*, *21*). IL-2 also amplifies the expression of serine esterases (granzymes) by cytotoxic T cells, thereby enhancing their cytotoxic activity against histoincompatible donor cells (*22*).

### *Glucocorticoid Binding and Mechanism of Transcription Inhibition*

In exerting their immunosuppressive effects, glucocorticoids do not: (1) block the early signal transduction events that are elicited as a result of T cell activation via the TcR/CD3 complex; (2) block IL-2R-mediated signal transduction (activation of protein tyrosine kinases, elevation in intracellular calcium, and activation and translocation of protein kinase C); or (3) directly induce suppressor cell activity (*23*). Instead, glucocorticoids appear to exert their immunosuppressive effects by blocking the expression of several cytokine genes (*24*) (Fig. 1 [originally drawn by D.R. Salomon, Scripps Research Institute]). Owing to their hydrophobic nature, glucocorticoids diffuse intracellularly and bind to cytoplasmic receptors (*25*, *26*), which exist in association with the 90-kDa heat shock protein (*27*). Binding of glucocorticoids induces the dissociation of the 90-kDa heat shock protein from the complex (*27*) and the translocation of the ligand-receptor complex into the nucleus, where it binds DNA to sites collectively referred to as glucocorticoid response elements (GRE).* DNA sequences consistent with the GRE motif (TGTTCT) have been localized in the promoter region of several cytokine genes (*28*). Binding of the glucocorticoid-receptor complex to GRE DNA elements may inhibit transcription of cytokine genes either by: (1) acting on distant genes to induce expression of transcription inhibitory factors (*trans*-acting mechanism), or (2) acting on adjacent genes (*cis*-acting mechanism) (*23*, *29*) and possibly altering the chromatin structure (*30*).

* Abbreviations: AP-1, activating protein-1; GRE, glucocorticoid response elements; HSP, heat shock protein; NFAT, nuclear factor of activated T cells.

### *Effects of Glucocorticoids on Cytokine Production*

One means of prolongation of allograft survival might involve blocking cytokine/cytokine-receptor expression that occurs as a consequence of T cell activation. Glucocorticoids inhibit T cell activation by blocking the expression of T cell-derived and APC-derived cytokines. Glucocorticoids inhibit the expression of IL-1 (*31*, *32*), IL-2 (*29*, *33*, *34*), IL-3 (*35*), IL-6 (*36*, *37*), TNF-α (*38*), and IFN-γ (*39*, *40*) at the transcriptional and posttranscriptional levels. Glucocorticoid-induced immunosuppression results, in part, from the direct blockade of IL-1 and IL-6 gene expression by APCs (*36*, *37*); IL-2 expression by activated T cells requires co-stimuli provided by accessory cells (*41*, *42*). These signals can be provided by T cell interaction with APC surface-anchored molecules (e.g., LFA-3, B7) or by APC-derived monokines, in particular IL-1 and IL-6 (*13*, *43*). On the other hand, glucocorticoids directly block the expression of IL-2 and other T cell-derived cytokines in vitro. Recent evidence demonstrates that during transcription, ligand-activated glucocorticoid receptors selectively impede the synergistic effects of DNA-binding proteins that stimulate IL-2 gene transcription: the nuclear factor of activated T cells (NFAT) and activating protein-1 (AP-1) (*29*). Glucocorticoids do not appear to block the appearance of NFAT and AP-1 (*33*), but rather block the consequences of NFAT and AP-1 binding to the IL-2 gene. Supporting the notion that glucocorticoids exert their effects by inhibiting cytokine expression is the finding that glucocorticoid-mediated inhibition of mitogen- and alloantigen-induced T cell proliferation is abrogated by the combination of the recombinant cytokines rIL-1, rIL-6, and rIFN-γ, but not by high concentrations of these and other individual cyto-

kines (*28*). Glucocorticoids may also inhibit IL-2R expression at a posttranscriptional level (*44*).

Glucocorticoid inhibition of the expression of T cell-derived and APC-derived cytokines is reversible. Moreover, sudden withdrawal of glucocorticoids often precipitates an immune-rebound phenomenon exemplified by an accelerated rejection of transplanted graft. Recent in vitro studies demonstrate that glucocorticoid withdrawal is associated with vigorous T cell proliferation, possibly via up-regulation of cytokine/cytokine-receptor expression (*45*). Exposure of T cells to corticosteroids has been found to up-regulate osteoblast-like cell IL-1 receptors (*46*), human monocyte IFN-$\gamma$ receptors (*47*), and hepatic-cell IL-6 receptors (*48*). The expression of receptors for IL-2, TNF-$\alpha$, and IFN-$\gamma$ were also significantly elevated when T cells were preincubated with dexamethasone (*45*). Therefore, when instituting dosage reduction or complete withdrawal of steroids to avoid systemic toxicity, a regimen that prevents the sudden surge in cytokine/cytokine-receptor expression should be considered.

### *Effective Glucocorticoid Concentrations*

In vitro data clearly establish that glucocorticoid blockade of T cell activation and cytokine expression at the transcriptional and posttranscriptional levels is concentration dependent, with a 50% effective concentration of 1–10 nM for dexamethasone (*49*). Knudsen et al. (*31*) also have demonstrated that blockade of IL-1$\beta$ by dexamethasone is also concentration dependent. Dexamethasone concentrations ranging from 10 to 100 nM effected a complete block in IL-1$\beta$ mRNA accumulation after toxic shock supernatant stimulation in vitro, whereas greater than 500 nM was necessary to completely block IL-1 expression by prestimulated cells. Thus, in vitro, low doses of corticosteroids appear to be sufficient to block cytokine transcription in resting cells, while high doses are necessary to block translation in activated cells. The in vivo data have not been as conclusive, in part as a result of the multiplicity of biological effects mediated by glucocorticoids and because of the confounding effects of other concomitantly administered agents.

### *Anti-inflammatory Effects*

Because inflammation plays a lesser role in the final expression of acute allograft rejection (*50*), the anti-inflammatory effects of steroids constitute a minor aspect of their efficacy in the prevention and treatment of rejection (*5*).

### *Relative Potencies and Duration of Action of Glucocorticoids*

The commonly prescribed glucocorticoids differ with respect to potency and duration of hypothalamic-pituitary-axis suppression (*51*) (Table 1). Prednisone, prednisolone, and methylprednisolone exhibit intermediate durations of action (12–36 hr) and are the agents most commonly used for the prevention and treatment of allograft rejection. Prednisone must be converted to the bioactive moiety prednisolone by the liver enzyme 11-$\beta$-hydroxydehydrogenase. However, the systemic bioavailability of prednisolone after administration of prednisone, prednisolone, or methylprednisolone is quite comparable (*52*), and no clinically significant differences in immunosuppressive efficacy have been discerned among these agents.

TABLE 1. Relative potencies and duration of action of commonly prescribed corticosteroids[a]

| Drug | Equivalent anti-inflammatory dose (mg) | Relative anti-inflammatory potency | Relative mineralocorticoid potency | Duration of action[b] (hr) |
|---|---|---|---|---|
| Hydrocortisone | 20 | 1 | 1 | 8–12 |
| Cortisone | 25 | 0.8 | 0.8 | 8–12 |
| Prednisone | 5 | 4 | 0.8 | 12–36 |
| Prednisolone | 5 | 4 | 0.8 | 12–36 |
| Methyl-prednisolone | 5 | 5 | 0.5 | 12–36 |
| Dexamethasone | 0.75 | 25 | 0 | 36–72 |

[a] Ref. *51*. Adapted with permission from Gilman et al., eds. The Pharmacological Basis of Therapeutics. New York: Pergamon Press, 1990: 1431.

[b] Hypothalmic-pituitary-adrenal axis suppression.

## CORTICOSTEROID INDUCTION AND MAINTENANCE IMMUNOSUPPRESSION FOR RENAL TRANSPLANTATION

### *The Pre-CsA Era*

In the early to mid-1970s, major transplant centers in the United States and Britain used empiric high-dose corticosteroid therapy—usually in conjunction with AZA—for immunosuppression after renal allograft transplantation. Doses as high as 200 mg of oral prednisone (*53*) or 1 g of intravenous methylprednisolone (*54*) were used for induction of immunosuppression and then tapered at fixed intervals over a few weeks to 3 months to daily maintenance doses of 0.25–0.5 mg/kg/day of prednisone (*53, 55-58*) or 0.4–1.5 mg/kg of methylprednisolone (*54, 59*). High doses were reinstituted briefly for acute rejection episodes. In some centers, initial daily doses (during the first month after transplantation) were almost as high as those used by other centers for the treatment of acute rejection (*8, 60–62*).

*Low- versus high-dose corticosteroids.* McGeown et al. (*60*) first reported that low-dose steroid therapy (20 mg prednisone daily) instituted immediately after transplantation was associated with patient and graft survival rates comparable to those of historical controls. Subsequently, several investigators compared the impact of low- versus high-dose steroid maintenance therapy on the outcome of renal transplantation. Some centers suggested that low-dose steroid therapy was associated with a greater incidence and severity of acute rejection episodes (*63, 64*). Others reported that low-dose steroid therapy was less successful in patients receiving suboptimal doses of AZA (*65*) or in patients younger than 45 years of age (*66*). However, these early studies generally demonstrated that low maintenance corticosteroid doses did not jeopardize allograft survival (in patients followed for up to 2 years) and, in addition, were associated with a lower incidence of steroid-related side effects (*8, 9, 62–68*). Moreover, Buckels et al. (*9*) reported a significantly lower mortality rate in patients receiving low doses of steroids.

*Alternate-day dose regimens.* Alternate-day steroid therapy represented the next logical step in minimizing steroid-related side effects after renal transplantation. In early, uncontrolled trials (*7, 58, 69–71*), alternate-day corticosteroid therapy was initiated at various times, ranging from immediately after transplantation to as long as 32 months later. Daily maintenance doses of 15–30 mg of prednisone or 16–32 mg of methylprednisolone were gradually converted (usually over 4 months) to alternate-day doses. Acute rejection epi-

sodes were reported in 4–36% of allograft recipients maintained on alternate-day steroid therapy (*7*, *58*, *69–72*). The majority of episodes were successfully reversed by treatment with high-dose methylprednisolone and/or return to daily steroid therapy (*7*, *58*, *69–72*). With relatively short periods of follow-up, renal function, as measured by blood urea nitrogen, serum creatinine (*69*, *70*), 24-hr creatinine clearance, and protein excretion (*58*), generally remained stable. However, Breitenfield et al. (*73*) suggested that conversion to alternate-day steroid therapy was associated with subsequent deterioration of renal function in approximately 20% of patients.

In 2 large randomized controlled trials, alternate-day corticosteroid dosing was as effective in maintaining graft function and as well tolerated as daily dosing (*74*, *75*). In a group of 91 cadaveric renal recipients, 5-year actuarial patient survival was 97% and 83%, and allograft survival was 85% and 67% in the alternate-dose and daily-dose treatment groups, respectively, and there were no significant differences in the incidence of acute rejection (*75*). In another series (*74*), of 38 patients on alternate-day therapy, serum creatinine concentrations, frequency of acute rejection, and prevalence of chronic rejection were similar to those of 38 patients on daily steroids.

*Effects on complications.* A number of investigators have compared the effects of long-term alternate-day steroid therapy with those of daily steroids on specific complications. Some (*7*, *70*, *71*) but not all (*75*) studies suggest that alternate-day therapy reduces the incidence of gastrointestinal hemorrhage, cataracts, infectious complications, obesity, and posttransplant diabetes mellitus. Dumler et al. (*74*) showed that the incidence of hip osteonecrosis was reduced significantly (2.6% vs. 18.6%). Curtis et al. (*76*) reported that 75% of 74 patients on alternate-day therapy (for a mean of 3.9 years) were normotensive. Factors associated with the occurrence of hypertension in the remaining patients were impaired renal function (creatinine greater than 2 mg/100 ml), the presence of native kidneys, and cadaveric donor source. Conflicting data have been obtained on the effect of alternate-day steroid therapy on hyperlipidemia in renal allograft recipients. In a prospective, controlled trial (*77*), dose spacing (by alternate-day therapy) and not the total dose of corticosteroids appeared to be the factor lowering the risk of hypercholesterolemia and hypertriglyceridemia in 14 patients with pretransplant hyperlipidemia. In a similar study (*78*), alternate-day, equal-dose prednisone therapy was accompanied by a significant reduction in both triglyceride production rate and mean plasma triglyceride concentration (129 mg/dl vs. 174 mg/dl during daily dosing). In contrast, 2 prospective studies evaluating the impact of alternate-day therapy on serum lipids found no beneficial effects on hyperlipidemia in either adults (*79*) or children (*80*), despite attempts to control influential variables such as diet. The disparate results of these studies suggest that factors other than steroid therapy may play a role in the pathogenesis of posttransplant hyperlipidemia.

Results of a number of studies (*70–72*) indicate that alternate-day therapy may be particularly beneficial in the maintenance of growth and achievement of sexual maturation in pediatric patients with stable renal allografts. However, in one retrospective comparison (*81*) of 28 growth-retarded, renal allograft recipients with 15 matched historical controls, catch-up growth in children less than 10 years of age was similar in patients receiving alternate-day and daily steroid regimens during a mean treatment period of 3.2 years. In this trial, conversion to alternate-day therapy increased growth significantly only during the second year after initiation of the regimen.

### *The CsA Era*

The introduction of CsA in the early 1980s permitted effective immunosuppression with even lower corticosteroid doses than those used previously. Few clinicians would disagree that the use of CsA has been accompanied by a reduction in the cumulative dose of steroids administered to renal transplant recipients. Some might argue, however, that the decline in steroid-induced morbidity has not been proportional with the reduction in dosage to currently used "low" levels. Since the introduction of CsA, cardiovascular disease has emerged as the major cause of late posttransplant morbidity and mortality (*82*, *83*). The observations that steroids and CsA independently promote the development of hypertension (*84–86*), hyperlipidemia (*87*, *88*), and glucose intolerance (*6*, *89*, *90*) raise the concern that even low doses of steroids may continue to increase the risk of cardiovascular disease in transplant recipients simultaneously receiving CsA. As a corollary, it remains to be proved that limiting or eliminating steroid therapy will mollify any negative impact of CsA on cardiovascular risk. Complete withdrawal of corticosteroid therapy was rarely attempted in the pre-CsA era (*91–94*). The availability of CsA has rekindled an interest in steroid-free immunosuppression for renal transplant recipients—achieved either by avoidance of steroids from the time of transplantation or by withdrawal of steroids at an arbitrary point thereafter.

*Steroid-free immunosuppression: corticosteroid avoidance.* The early experience with CsA monotherapy (*95*, *96*) demonstrated that renal transplantation could be performed successfully in some patients without the use of steroids. Three subsequent randomized prospective studies failed to demonstrate any advantage in graft or patient survival when the combination of CsA and steroids was compared with CsA alone (*97–99*). However, the high doses of CsA used in early monotherapy protocols were complicated by dose-related nephrotoxicity that was difficult to distinguish from acute rejection. Moreover, 15–79% of patients treated with CsA monotherapy ultimately have required the addition of steroids after the development of acute rejection episodes (*97–103*). As a result, standard doses of CsA were reduced greatly during ensuing years and steroids often were added to induction immunosuppression regimens for their "CsA-sparing" effect.

Steroid avoidance also has been attempted in patients maintained on AZA and CsA from the time of transplantation. Bry et al. (*104*) have shown, however, that approximately 50% of patients treated in such a fashion ultimately require renewal of steroid therapy—a percentage remarkably similar to that described in the experience with CsA monotherapy. Overall, it appears that long-term steroid-free immunosuppression can be achieved in fewer than 50% of renal transplant recipients in whom steroids are avoided from the time of transplantation.

*Steroid-free immunosuppression: corticosteroid withdrawal.* An increasingly popular approach to steroid-free im-

munosuppression in the CsA era has consisted of using steroids in combination with AZA and/or CsA during the early posttransplant period, followed by gradual elimination of steroids at some arbitrary time point after transplantation. During the past 10 years, this strategy has been used by an increasing number of transplant centers (*105–116*). The widely varying success of steroid withdrawal in reported series of patients receiving CsA-based immunosuppression probably reflects differences in patient selection, the timing and rapidity of steroid withdrawal, residual immunosuppression (CsA alone or CsA plus AZA), duration of follow-up, and the criteria for defining successful withdrawal of steroids or for renewing steroids in patients undergoing attempted withdrawal. Although failure to withdraw steroids occasionally has been attributed to AZA-induced leukopenia, recurrent glomerular disease, other systemic diseases, or adrenal insufficiency (*105, 106, 109*), acute allograft rejection is the major risk associated with complete withdrawal of steroids and continues to be the most common reason for renewing steroids in patients deemed to have failed steroid withdrawal.

*Risk of rejection.* Assessing the risk of rejection associated with steroid withdrawal has been difficult because much of the published literature on withdrawal of steroids after renal transplantation has been reported within the context of uncontrolled trials (*105–112*) (Table 2). However, a meta-analysis of 7 randomized, prospective controlled trials (*100, 102, 103, 113–116*) suggested that steroid-free immunosuppression was associated with a significantly higher incidence of acute allograft rejection without influencing either patient or graft survival (*117*). Overall, steroid-free immunosuppression was deemed to be "successful" in only 323 of 681 patients (47%). However, 4 of the 7 studies incorporated into this analysis were steroid avoidance studies (*100, 102, 103, 115*) and, in 2 of the 3 steroid withdrawal studies (*113, 116*), prednisone was withdrawn rapidly less than 3 months after transplantation. In an uncontrolled study limited to living related allograft recipients, Stratta et al. (*109*) similarly reported that 21 of 46 patients (46%) withdrawn from steroid therapy during the first month after transplantation sustained rejection episodes requiring at least temporary renewal of prednisone. Thus, results of both controlled (*113–116*) (Table 3) and uncontrolled trials and of a meta-analysis converge to suggest that steroid avoidance or early, rapid discontinuation of steroids is associated with a high incidence of acute rejection that frequently prompts renewal of steroid therapy.

Recent evidence from uncontrolled trials suggests that later and slower withdrawal of steroid therapy may reduce the risk of acute rejection. Kupin et al. (*105*) suggested that the risk of rejection was lower among patients in whom steroids were slowly tapered over a period of 5 months or more. Hricik et al. (*118*) compared their experience with early withdrawal of steroids (within 3 weeks of transplantation) (*115*) to later and slower withdrawal of prednisone (at least 6 months after transplantation over a period of 3–4 months) in patients subsequently maintained on CsA and AZA. Fifty-nine percent of the patients undergoing early withdrawal of prednisone sustained rejection episodes requiring renewal of steroid therapy. By contrast, later withdrawal of prednisone was successful in approximately 80% of patients followed for up to 5 years. The optimal timing of "late" steroid withdrawal remains to be determined. Furthermore, the rapidity with which steroids are withdrawn may be even more important than timing after transplantation. Almawi et al. (*45*) have shown recently that rapid elimination of steroids in vivo leads to a rebound in T cell proliferation that could be a correlate of acute rejection after rapid discontinuation of steroids.

Aside from timing after transplantation, there are few clinical variables that reliably predict the outcome of attempted steroid withdrawal. Hricik et al. (*118*) showed that,

TABLE 2. Results of prospective open studies evaluating the effects of corticosteroid withdrawal in renal allograft recipients during the CsA era[a]

| Ref./posttransplant maintenance drug regimen | Evaluable patients | Transplant source | Mean mos posttransplant to completion of steroid withdrawal | Percent acute rejection | Percent prednisone withdrawal success (mos follow-up) |
|---|---|---|---|---|---|
| Kupin et al. (*105*)<br>AZA, CsA, MPred, or MPred-free | 25 | Cadaver | 5[b] | 24 | 76 (15) |
| O'Connell et al. (*106*)<br>AZA, CsA, PNL (low dose) or PNL-free | 84 | Cadaver | 4–6[c] | 73 | 70[d] (8) |
| Pirsch et al. (*107*)<br>AZA, CsA, Pred, or Pred-free | 131 | LRD | 0.4 | 44 | 40 (NR) |
| Reisman et al. (*108*)<br>CsA, Pred, or Pred-free (with AZA in 1 case) | 16[e] | Cadaver<br>LRD | 7–60[c] | 56 | 44 (19–41[c]) |
| Stratta et al. (*109*)<br>AZA, CsA, Pred, or Pred-free | 52 | LRD | 0.5 | 50 | 64 (8) |
| Tejani et al. (*110*)<br>CsA, Pred, or Pred-free | 53[e] | Cadaver<br>LRD | NR | 74 | 26 (32) |
| Venkat et al. (*111*)<br>AZA, CsA, MPred, or MPred-free | 71 | Cadaver | 11–15[c] | 39 | 46 (31) |
| Walker et al. (*112*)<br>AZA, CsA, PNL, or PNL-free | 11[e] | Cadaver | 4–6 | 45 | 60 (3–19[c]) |

[a] Abbreviations used in table: AZA, azathioprine; CsA, cyclosporine; LRD, living related donor; MPred, methylprednisolone; NR, not reported; PNL, prednisolone; Pred, prednisone.

[b] Actual time.

[c] Range.

[d] Thirty-six of 51 patients.

[e] Pediatric patients.

TABLE 3. Results of comparative trials evaluating the effects of corticosteroid withdrawal in renal allograft recipients during the CsA era[a]

| Ref./posttransplant maintenance drug regimen | Evaluable patients | Transplant source | Mean mos posttransplant to completion of steroid withdrawal | Percent acute rejection | Percent graft survival (mos follow-up) |
|---|---|---|---|---|---|
| Gulanikar et al. (*113*) | | | | | |
| CsA, Pred withdrawal/placebo | 41 | LRD, cadaver | 3 | 24[b] | 83 (60) |
| CsA, Pred | 44 | LRD, cadaver | — | 6.8 | 84 (60) |
| Maiorca et al. (*114*) | | | | | |
| CsA, MPred withdrawal | 35 | Cadaver | 7[c] | 46 | 94 (27) |
| CsA, MPred | 31 | Cadaver | — | 10 | 100 (27) |
| Schulak et al. (*115*) | | | | | |
| CsA, AZA, Pred withdrawal | 32 | LRD, cadaver | 6–20 days[d] | 81[e] | 90 (18)[f] |
| CsA, AZA, Pred | 35 | LRD, cadaver | — | 54 | 94 (18)[f] |
| Sinclair (*116*) | | | | | |
| CsA, Pred withdrawal/placebo | 260 | LRD, cadaver | 3 | — | 73 (60)[f,g] |
| CsA, Pred | 263 | LRD, cadaver | — | — | 85 (60)[f] |

[a] Abbreviations used in table: AZA, azathioprine; CsA, cyclosporine; LRD, living related donor; MPred, methylprednisolone; Pred, prednisone.

[b] Pred versus Pred-free ($P<0.005$).

[c] Actual time.

[d] Range.

[e] Pred versus Pred-free ($P\leq0.02$).

[f] Actuarial.

[g] Pred versus Pred-free ($P=0.03$).

independent of HLA-mismatching, donor-recipient racial mismatching (most prevalent in black patients) was a significant predictor of acute rejection when steroids were withdrawn. In addition, the degree of renal insufficiency before withdrawal was related independently to the risk of acute rejection. Neither age, sex, HLA-match, pretransplant panel-reactive antibody, source of the allograft, nor the presence of diabetes mellitus was predictive of outcome. Venkat et al. (*111*) suggested that the development of acute rejection was related to the dose of CsA with higher rates of rejection in patients receiving less than 3.5 mg/kg/day at the time of attempted steroid withdrawal.

The risk of chronic allograft dysfunction associated with steroid-free immunosuppression has not been assessed adequately, in part because of the relatively short duration of follow-up in most reported series. To the extent that corticosteroids inhibit the function of fibroblasts, it is tempting to speculate that the absence of steroids may promote the development of interstitial fibrosis characteristic of both chronic rejection and chronic CsA nephrotoxicity. In this regard, recent findings from a large randomized prospective trial reported by the Canadian Multicentre Transplant Study Group (*116*) are of concern, suggesting a significantly lower rate of graft survival among patients withdrawn from steroid therapy after 5 years of follow-up. In this study, steroids were tapered rapidly 90 days after transplantation in patients subsequently maintained on CsA monotherapy, making it difficult to extrapolate the results to those of studies in which steroids are withdrawn later and more slowly in selected patients maintained on CsA and AZA. Moreover, because the causes of graft loss in the steroid-free group were not delineated, this study shed little light on the risk of chronic rejection associated with steroid-free immunosuppression. Isoniemi et al. (*119*), on the other hand, evaluated histologic indices of chronic allograft damage in renal biopsies obtained 2 years after transplantation in 128 cadaveric allograft recipients randomized to receive a variety of immunosuppressive protocols. Patients maintained on CsA and AZA exhibited a higher index of chronic allograft damage compared with those receiving steroid-based immunosuppression. Results of this study underscore the need for additional long-term observations to further assess the relationship between steroids, steroid withdrawal, and chronic rejection.

*Effects on complications.* Withdrawal of steroid therapy clearly accelerates growth in pediatric renal transplant recipients maintained on CsA (*110*, *112*). In adults, studies of the effects of steroid withdrawal have focused on cardiovascular risk factors, including hyperlipidemia, hypertension, and glucose intolerance. Withdrawal of steroids substantially alters the lipid profile of patients maintained on CsA. Total serum cholesterol levels tend to decrease by 13–18% (*87*, *105*, *107*), but there is a proportional decrease in both low-density lipoprotein and high-density lipoprotein cholesterol levels such that total to HDL cholesterol ratios remain unchanged (*120*). Withdrawal of steroids results in significant reductions of blood pressure and in the number of antihypertensive agents required by patients maintained on CsA (*84*, *105*, *107*). A number of investigators have demonstrated that glucose intolerance improves when steroids are withdrawn from patients maintained on CsA and AZA (*121–123*). Furthermore, Hricik et al. (*121*) reported that a majority of patients with posttransplant diabetes mellitus were able to discontinue insulin or oral hypoglycemic therapy within 4 months of successful steroid withdrawal.

Complete elimination of steroid therapy thus appears to be accompanied by a number of metabolic effects that may decrease the risk of cardiovascular disease in renal transplant recipients, despite the continued administration of CsA. It should be noted, however, that the benefits of completely eliminating corticosteroid therapy in CsA-treated patients have never been satisfactorily compared with similar benefits derived from low-dose steroid regimens such as alternate-day therapy. Moreover, whether the short-term benefits of eliminating steroids will result in decreased rates of cardiovascular morbidity and mortality or in increased patient longevity remains to be proved.

## CORTICOSTEROID USE IN THE TREATMENT OF RENAL ALLOGRAFT REJECTION

Even before their routine use in maintenance immunosuppression regimens, corticosteroids were found to be effective in reversing acute renal allograft rejection (*2*). However, the best route and dosage for steroid treatment of renal allograft rejection remain to be determined. Whether steroids should continue to be the agents of first choice for treatment of acute rejection also is a subject of increasing controversy.

In the 1960s and 1970s, therapeutic approaches to the treatment of acute rejection centered on administration of high doses of oral glucocorticoids in combination with either AZA or actinomycin C. Doses of prednisone or prednisolone ranging from 150 mg to 600 mg/day were tapered over 1–3 weeks (*56*, *124*, *125*). However, large oral doses of steroids were associated with devastating complications, such as severe infections and gastric erosion, then attributed to the long serum half-life of oral agents (*53*). Bell et al. (*53*) first demonstrated the efficacy of administering a single, large (1 g) dose of intravenous prednisolone in reversing acute renal allograft rejection. Thereafter, others reported the benefits of administering intravenous bolus (pulse) doses of prednisolone or methylprednisolone for several days. Theoretic advantages of using intravenous steroids include a very rapid lympholytic effect that persists for up to 2 days and a short serum half-life—thus minimizing the side effects associated with longer-acting oral preparations (*55*, *61*). In early reports, doses used for "pulse" therapy ranged from 1 g of prednisolone daily for 3 days (*53*) to 2 g of methylprednisolone daily for 10 days (*55*), resulting in reversal of acute rejection in 86–100% of cases (*53*, *55*, *126*).

### *Comparative Trials of Intravenous and Oral Corticosteroids*

Comparative trials have failed to demonstrate an advantage of intravenous methylprednisolone over oral steroids with regard to the reversal of acute renal allograft rejection (*56*, *127–129*). Between 60% and 76% of approximately 100 rejection episodes were reversed with methylprednisolone as compared with 56–72% with oral steroids (*56*, *127–129*). In 2 studies, oral doses were associated with a higher frequency of gastrointestinal bleeding, aseptic necrosis, diabetes (*127*), hypertension, fluid retention, and infection (*128*); however, this higher frequency of adverse effects may have been related more closely to the duration of therapy than to the total dose administered (*10*).

### *Dose Comparison Studies*

In 1974, Webel et al. (*130*) studied the effect of a single intravenous bolus of methylprednisolone on lymphocyte transformation stimulated by mitogens or allogeneic cells and found no significant difference in the magnitude of the response to the 4 doses studied: 80 mg, 250 mg, 500 mg, or 1 g. Similarly, Fan et al. (*131*) compared single intravenous methylprednisolone doses of 50 mg and 1 g, and 1-g doses administered on 3 consecutive days and found that each dose produced a similar suppression of lymphocyte responsiveness to a variety of mitogens. These early studies suggested that the corticosteroid doses in clinical use for the treatment of rejection in the mid-1970s may have been excessive. Although some investigators have suggested a therapeutic ceiling of 3 g of methylprednisolone (*56*), the dosing schedules used for the treatment of acute renal allograft rejection have been and continue to be chosen empirically.

When low-dose (3 mg/kg or 250 mg) and high-dose (30 mg/kg or 1 g) intravenous methylprednisolone therapies were compared in double-blind randomized studies, there were no significant differences in renal allograft rejection reversal, graft function, or occurrence of side effects (*10*, *11*). In one study, however, patients receiving high doses tended to have greater reductions in serum creatinine levels while patients receiving low doses tended to have fewer infections (*11*).

### *Antilymphocyte Antibodies versus Corticosteroids*

In a number of investigations in the late 1970s and early 1980s, the use of antilymphocyte globulins for first-line treatment of rejection resulted in higher graft survival than did steroids at 1–4 years after rejection (*132–135*). Difficulties in lot standardization of the antilymphocyte globulin preparations, however, were associated with variations in potencies and side effects from contaminating antibodies. These problems are among the concerns that led to the development in the mid-1980s of the mAb OKT3 (*136*).

When OKT3 therapy was compared with milligrams of intravenous methylprednisolone (for a maximum of 3 days) for the treatment of renal rejection in a randomized prospective trial (*137*) in 123 patients, OKT3 reversed significantly more rejection episodes than did steroids (94% vs. 75%, respectively). One-year graft survival was also significantly different (62% vs. 45%, respectively). In a study (*138*) comparing the use of OKT3 and high-dose steroids for first- and second-line treatment of renal allograft rejection, 82% of first-rejection episodes were reversed with OKT3, whereas 63%, significantly fewer, were reversed in patients treated with 1-g bolus doses of intravenous methylprednisolone for 3 days, with an increase in oral prednisone gradually tapered to 30 mg/day over a period of 10–14 days. No significant differences in rejection reversal were found between OKT3 or steroids in their use for rescue treatment. Three-month allograft survival was 74% in both groups. A retrospective review (*139*) of 314 consecutive kidney transplants in patients receiving either OKT3 or oral prednisone for treatment of rejection provided further evidence supporting the use of OKT3 for first-line therapy of rejection. Subgroup analysis showed significantly superior 2-year actuarial graft survival among 39 patients treated with first-line OKT3 (87%) compared with 38 patients receiving steroids (54%).

Despite these observations, many transplant centers continue to use high-dose steroid therapy as first-line treatment for acute allograft rejection, reserving antibody therapy for patients with steroid-resistant rejection episodes. This antirejection strategy can be rationalized on the basis of the proven efficacy and relative ease of administering high-dose steroids, but is probably equally motivated by the unproven assumption that the morbidity associated with high-dose steroid therapy is less than that associated with antilymphocyte antibody preparations. Clearly, additional studies are warranted to determine whether steroids should remain the agents of choice in the first-line treatment of allograft rejection.

## CONCLUSIONS AND CONSIDERATIONS FOR THE FUTURE

Historical trends in the use of glucocorticoids in renal transplantation have been characterized by lowering of doses

for both prophylactic immunosuppression and treatment of rejection. These trends have been motivated by the desire to minimize the complications of long-term steroid therapy and by the recognition that low doses of these agents may provide satisfactory immunosuppression, at least when combined with other immunosuppressive drugs. The availability of CsA has prompted an interest in completely eliminating steroid therapy after renal transplantation, either by steroid avoidance or by withdrawal of steroids at some time after transplantation.

Unfortunately, much of the published experience with steroid-free immunosuppression describes the results of uncontrolled trials. Moreover, published studies of steroid withdrawal vary widely with respect to patient selection criteria, the timing and rapidity of steroid withdrawal, definitions of failure and success, residual immunosuppression after elimination of steroids (CsA alone vs. CsA and AZA), and duration of follow-up. Because the benefits and risks of completely withdrawing steroids have not been fully evaluated, this immunosuppressive strategy should be regarded as experimental, pending the results of ongoing randomized prospective trials.

Because few clinical variables reliably predict the outcome of steroid reduction or withdrawal protocols, it would be reasonable to consider in vitro immunologic parameters as guides in the future. To date, investigators have touted the use of CD4 to CD8 ratios (*140*, *141*), cytotoxic T cell reactivity (*142*), the lymphocytic response to PHA (*143*), and the mixed lymphocyte reaction (*144*, *145*) as guides to eliminating steroids, but results are inconclusive and more controlled studies are needed. Whether such immunologic monitoring will reduce the incidence of rejection associated with steroid reduction remains to be proved. Clinical studies in which steroids have been eliminated empirically indicate that between 50% and 80% of transplant recipients can be maintained on steroid-free, CsA-based immunosuppression without the precipitation of acute rejection.

Additional long-term studies are required to assess the risk of chronic rejection in patients withdrawn from steroid therapy. Based on available data, at least 5 years of follow-up may be required to assess this risk satisfactorily. Finally, encouraging preliminary data indicate a need for additional randomized trials comparing high-dose steroids to antilymphocyte antibodies in the first-line treatment of allograft rejection. While the overall benefits and risks of steroid-free immunosuppression in CsA-treated transplant recipients require further scrutiny, studies considered in this review have undoubtedly laid the groundwork for the future immunosuppressive strategies in that drugs or drug combinations of the future likely will be judged, in part, on their ability to spare steroid therapy.

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