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# Pretreatment with cyclosporine and anti-interleukin 2 receptor antibody abrogates the anti-idiotype response in rat recipients of cardiac allografts

(transplantation immunobiology/ART-18 monoclonal antibody)

KAZUO TANAKA*†, NICHOLAS L. TILNEY*, KLAUS G. STUNKEL‡, WAYNE W. HANCOCK*§, TIBOR DIAMANTSTEIN¶, AND JERZY W. KUPIEC-WEGLINSKI*‖

*Surgical Research Laboratory, Harvard Medical School, Department of Surgery, Brigham and Women's Hospital, Boston, MA 02115; ‡Institute of Immunology and Oncology, BAYER AG, Wuppertal, Federal Republic of Germany; and ¶Institute of Immunology, Free University, West Berlin, Federal Republic of Germany

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ABSTRACT **A 10-day course with ART-18, a mouse monoclonal antibody (mAb) directed against the rat interleukin 2 receptor (IL-2R), prolongs the survival of (LEW × BN)$F_1$ cardiac allografts in LEW recipients to ≈3 weeks (acute rejection = 8 days, $P < 0.001$). We examined host responses against ART-18 idiotype (Id) and mouse immunoglobulin in recipients immunomodulated with ART-18 mAb. Treatment with ART-18 elicited high titers of anti-Id antibodies 14 days after transplantation. However, naive rats given ART-18 before transplantation showed strong anti-Id responses as early as day 4 after engraftment, coinciding with abrogation of the treatment effect (graft survival, ≈10 days). Preimmunization with irrelevant mouse IgG, which elicited high titers of anti-IgG, did not influence the efficacy of ART-18 upon graft survival (17 days). The use of cyclosporin A (CsA) in conjunction with ART-18 prior to transplantation suppressed the anti-Id response and led to dramatic graft prolongation (>58 days), with two of five grafts surviving indefinitely. This striking effect of CsA plus ART-18 pretreatment did not depend upon CsA *per se*, as grafts were rejected within 12 days in animals pretreated with CsA alone; in both groups CsA trough levels were comparable. Moreover, administration of CsA before transplantation in concert with control IgG (instead of ART-18) prompted rejection within 2–4 weeks. Thus, discrete interaction(s) between anti-IL-2R mAb and CsA prior to engraftment induces partial host unresponsiveness/tolerance to anti-IL-2R mAb treatment following transplantation and suppresses the neutralizing anti-Id responses, which results in long-term/permanent graft acceptance. This study provides a strategy to overcome the anti-Id response mounted by graft recipients that otherwise limits the efficacy of anti-IL-2R mAb treatment.**

A long-desired goal in transplantation has been the development of specific and selective means of suppression of the immune responses mounted by the host in response to the foreign tissue. Recent advances in hybridoma technology have raised hopes of achieving this goal. Indeed, monoclonal antibodies (mAbs) against major histocompatibility complex class II antigens, as well as against CD3, CD4, and CD25 surface antigenic markers, have been used as therapeutic agents following transplantation in experimental models (1–4) and in some clinical trials (5–7). However, the induction of anti-idiotype (anti-Id) and anti-Ig antibodies represents the major limiting feature of this treatment strategy (8), as they limit or prevent immunosuppression mediated by OKT3 in humans (9) or by anti-CD25 mAbs such as 33B3.1, anti-Tac, and M7/20 in humans (7), subhuman primates (10), and mice (11), respectively. ART-18 is a mouse IgG mAb that recognizes the p55 subunit of the rat high-affinity interleukin 2 receptor (IL-2R) complex *in vitro* (12), whereas *in vivo* it prevents acute rejection of cardiac, renal, and pancreatic islet allografts as well as small bowel and peripheral nerve transplants in rats (reviewed in ref. 13).

The present studies were designed to evaluate the role of and the interactions between anti-Id and anti-Ig responses in rat recipients of cardiac allografts treated with ART-18. The results demonstrate direct effects of host anti-Id antibodies upon graft survival in this model. However, cyclosporin A (CsA) given as an adjunct into the hosts conditioned with ART-18 *prior* to transplantation decreased titers of anti-Id antibodies and enhanced the efficacy of subsequent IL-2R targeted therapy, an observation of potential importance for clinical use.

## MATERIALS AND METHODS

**Animals and Grafting Technique.** Male inbred adult rats (Harlan–Sprague–Dawley) were used. Cardiac allografts from Lewis × Brown Norway $F_1$ hybrids ($RT1^{l/n}$, $LBNF_1$) were transplanted to the abdominal great vessels of LEW hosts using microvascular techniques. Ventricular contractions were assessed by palpation through the recipient flank. Rejection was defined as the day of cessation of myocardial contractions.

**ART-18, Production of Isotype Switch Variants, and F(ab)/F(ab')$_2$ Fragments.** Cloning, production, and characterization of ART-18 have been described (12). This IgG1 material defines an epitope that is identical to or overlapping with the functional IL-2 binding domain of the receptor as it inhibits IL-2 binding and IL-2-dependent T-cell growth *in vitro*. The sequential sublining technique has been used to select variant cells secreting proteins of IgG2a and IgG2b isotypes from the parental ART-18 hybridoma (14). Ascites fluid-containing IgG antibodies were purified by ammonium sulfate precipitation and ion-exchange chromatography. ART-18 IgG1 was incubated with 1% papain (Sigma) or with 25 μg of pepsin per ml (Sigma) to obtain F(ab) and F(ab')$_2$,

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Abbreviations: CsA, cyclosporin A; Id, idiotype; IL, interleukin; IL-2R, IL-2 receptor; mAb(s), monoclonal antibody(ies).

†Present address: Department of Surgery, National Higashi-Fukuoka Hospital, Fukuoka, Japan.
§Present address: Department of Nephrology, Prince Henry's Hospital, Melbourne, Australia.
‖To whom reprint requests should be addressed at: Surgical Research Laboratory, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115.

respectively (15). Residual Fc portion was removed by a DEAE-Sephacel column (Pharmacia).

**mAb Therapy.** ART-18 or its F(ab)/(F(ab')$_2$ fragments were administered i.v. to rat recipients of cardiac allografts at a dose of 300 μg/kg per day for 10 consecutive days after transplantation.

**Immunization Regimen.** Groups of naive LEW rats, the prospective recipients, were immunized prior to transplantation to induce high titers of anti-ART-18(Id)/IgG antibodies by i.v. challenge with 1 mg of ART-18 per kg (IgG1, IgG2a, or IgG2b), mouse IgG1 (mouse myeloma protein derived from MOPC-21, Cappel Laboratories), or mouse IgG2a/IgG2b (UPC-10/MOPC-195, Cappel Laboratories) on days −21, −14, −7, and −3. LBNF$_1$ cardiac allografts were then placed on day 0, followed by a 10-day ART-18 therapy.

CsA (Sandoz, East Hanover, NJ) was used as an adjunct in the immunizing protocol. CsA was dissolved in olive oil and administered i.m. to LEW rats at a dose of 15 mg/kg every 2–3 days (total, nine injections); the final injection was given 2 days before heart transplantation. CsA serum levels were determined by fluorescent polarization immunoassay using a TDx analyzer (Abbott).

**Serum Levels of ART-18 and Its Fragments.** Serum levels of ART-18 or its F(ab)/F(ab')$_2$ fragments were measured by ELISA as described (15). Briefly, 96-well enzyme immunoassay microplates (Costar) were coated overnight at 4°C with rat absorbed goat anti-mouse IgG (2 μg/ml, Sigma) in 0.05 M bicarbonate buffer (pH 8.2). Plates were blocked with phosphate-buffered saline (PBS) containing 3% non-fat dry milk, followed by addition of serially diluted rat serum samples. Captured mouse IgG was detected using a peroxidase-conjugated goat anti-mouse IgG (1 μg/ml, Sigma). After adding the substrate and stopping the reaction with 1 M $H_2SO_4$, the optical density (OD) of each well was measured at 488 nm by ELISA reader.

**Rat Antibodies to ART-18 or Mouse IgG.** Rat antibodies against ART-18 or mouse IgG were quantitated by the modified ELISA as described (10). EIA microplates (Costar) were coated with 20 μg of ART-18 or mouse IgG per ml in 1 M carbonate/bicarbonate buffer at 200 μl per well overnight at 4°C. The plates were washed with PBS/0.05% Tween 20 (Fisher) and after incubation with PBS containing 3% of non-fat dry milk for 1 hr at 4°C, serially diluted serum samples (50 μl per well) were added. After 1 hr at 4°C, the plates were incubated for 45 min at room temperature with peroxidase-conjugated mouse anti-rat κ chain (50 μl per well; Zymed Laboratories) and treated with 2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid) in 0.1 M sodium citrate (100 μl per well, pH 4.2) for 30 min at room temperature. The OD of each well was measured at 405 nm using the ELISA reader.

**Detection of Anti-Id Antibodies.** The presence of circulating antibodies to the Id of ART-18 was tested directly by the ability of serum to block the binding of ART-18 to its antigen (11). Briefly, biotinylated ART-18 or ART-65 (mAbs of distinct IL-2R epitope specificities, 1 mg/ml) was incubated with serum from ART-18-treated or naive rats (1:10 dilution) for 1 hr at 25°C and then added (50 μl) to ConA-stimulated spleen cells ($10^6$). The cells were incubated for 45 min on ice, washed, resuspended with streptavidin/phycoerythrin (2.5 μl; Becton Dickinson, Sunnyvale, CA) and their binding patterns with mAb were analyzed on a FACS1 Cell Analyzer (Becton Dickinson). Inhibition of binding of the biotinylated mAbs to stimulated spleen cells was measured by a decrease of fluorescence intensity. To verify that the blocking activity was due to anti-Id antibodies and not to soluble IL-2R or free ART-18, serum samples were incubated with protein A-Sepharose (Pharmacia).

**Statistical Analysis.** Experimental data were tested for normality by the Wilk–Shapiro test and were compared for significant differences by using Student's *t* test for two- and Newman–Keul test for multiple-sample comparisons. A $P$ value of $<0.05$ was considered significant.

## RESULTS

**Anti-Mouse Response in Rat Recipients of Cardiac Allografts Treated with ART-18.** Anti-mouse response in LEW recipients of LBNF$_1$ cardiac allografts conditioned with ART-18 of IgG1 isotype was first detected 6 days after transplantation (Table 1). Administration of F(ab) or F(ab')$_2$ fragment induced a response comparable to that of intact ART-18. Thus, the F(ab) portion of IL-2R mAb is responsible for the rat anti-mouse reactivity. Interestingly, the host response to F(ab) occurred despite rapid clearance of the fragments from circulation (serum levels <0.5 μg/ml) compared with animals given intact ART-18 or its F(ab')$_2$ (≈5 μg/ml and 3 μg/ml, respectively, Table 1). Similar results were recorded for purified mouse myeloma Fab/F(ab')$_2$ (data not shown).

**Effects of ART-18 on Cardiac Allograft Survival in Recipients Preimmunized with ART-18 or Mouse IgG.** A 10-day i.v. course following transplantation with ART-18 isotype switch variants abrogated acute rejection and prolonged cardiac allograft survival to 16–21 days (Table 2, groups A, F, and H vs. group K, $P < 0.001$); ART-18 IgG2a was somewhat less effective than IgG1 and IgG2b. When naive rats were given ART-18 IgG1, IgG2a, or IgG2b prior to transplantation, the therapeutic efficacy of subsequent ART-18 IgG1 or IgG2b treatment was abrogated (Table 2, groups C, D, E, and J), regardless of the isotype of the ART-18 used as an immunizing modality. In contrast, treatment of the prospective recipients with irrelevant mouse IgG1, IgG2a, or IgG2b did not affect at all the efficacy of ART-18 upon graft survival (Table 2, groups B, G, and I), even if the same isotype of IgG served for preimmunization and treatment after transplantation. These results are consistent with the view that anti-ART-18 antibodies, defined operatively as those that react with ART-18 regardless of isotype but not with isotype-matched control IgG, abrogated the effect of mAb treatment *in vivo*.

**Anti-ART-18 (Id)/IgG Responses in Recipients Pretreated with ART-18 or Mouse IgG.** Antibodies to the Id of the administered ART-18 IgG2b were first measured indirectly by comparing the relative ELISA titers to the administered mAb and to the irrelevant mouse IgG2b. An elevated anti-ART-18 response in graft recipients conditioned with ART-18 IgG2b was noted 14 days after transplantation (Table 3, column 3, group H). However, treatment with ART-18 IgG2b before the surgery prompted an early (day 4) and dramatic

Table 1. Anti-ART-18 mAb response in rat recipients of cardiac allografts treated with ART-18 and its F(ab)/F(ab')$_2$ fragments

| Treatment* | Serum level of ART-18 or its fragments,† μg/ml | Anti-ART-18 titer in serum‡ |
|---|---|---|
| ART-18 IgG1 | 4.8–5.6 | 4, 5, 5 |
| ART-18 F(ab) | <0.5 | 4, 4, 7 |
| ART-18 F(ab')$_2$ | 2.9–3.6 | 3, 4, 5 |

*Recipients of cardiac allografts were treated with intact ART-18 (IgG1) or its F(ab)/F(ab')$_2$ fragments (300 μg/kg per day i.v.) for 10 consecutive days after transplantation.

†Level of whole IgG or its fragments in the serum of treated recipients 6 days after transplantation as determined by ELISA. Standard curves were obtained by using known concentrations of intact ART-18 or its fragments.

‡Titers of anti-ART-18 were determined by ELISA. The samples of experimental sera were diluted 2-fold from 1:25 to 1:3200. Data are expressed as the end point of the greatest dilution of the sample serum that gave an OD higher than the mean + 4 SD of that in the control (normal) serum.

Table 2. Effects of preimmunization with mouse immunoglobulin or ART-18 on the survival of cardiac allografts in recipients treated with ART-18

| Group | Preimmunization* | Therapy† | Graft survival, days | MST,‡ days |
|---|---|---|---|---|
| A | — | ART-18 IgG1 | 20, 21, 21, 22 | 21.0 ± 0.8 |
| B | Mouse IgG1 | ART-18 IgG1 | 16, 20, 20, 23 | 19.8 ± 2.9 |
| C | ART-18 IgG1 | ART-18 IgG1 | 9 (×3), 10 | 9.3 ± 0.5 |
| D | ART-18 IgG2a | ART-18 IgG1 | 9 (×4), 10 | 9.2 ± 0.4 |
| E | ART-18 IgG2b | ART-18 IgG1 | 12, 12, 13 | 12.3 ± 0.6 |
| F | — | ART-18 IgG2a | 15, 16, 16, 17 | 16.0 ± 0.8 |
| G | Mouse IgG2a | ART-18 IgG2a | 13, 13, 16, 18 | 15.0 ± 2.4 |
| H | — | ART-18 IgG2b | 16, 21, 22, 23 | 20.5 ± 3.1 |
| I | Mouse IgG2b | ART-18 IgG2b | 11, 16, 20, 22 | 17.3 ± 4.9 |
| J | ART-18 IgG2b | ART-18 IgG2b | 9 (×3), 10, 11 | 9.6 ± 0.9 |
| K | — | —§ | 7, 8 (×4) | 7.8 ± 0.4 |

The statistical significance between experimental groups is as follows: $P < 0.001$ when group A vs. group C, D, or E is compared or when group H vs. group J is compared.

*LEW recipients were preimmunized with ART-18 or mouse IgG on days −21, −14, −7, and −3 (1 μg/kg i.v.); $LBNF_1$ cardiac allografts were transplanted on day 0.

†Allograft recipients were treated with ART-18 daily (day 0–9, 300 μg/kg i.v.).

‡Mean graft survival time ± SD.

§No prolongation of cardiac allograft survival was recorded in rats treated with mouse IgG1, IgG2a, or IgG2b materials or with irrelevant mouse anti-human TSH mAb (13).

rise in anti-ART-18 levels (Table 3, column 3, group J); this high reactivity correlated with abrogation of the therapeutic effectiveness of ART-18 administered after transplantation (Table 2, group J). In contrast, the high anti-IgG2b response in the animals pretreated with mouse IgG2b (Table 3, column 4, group I) had no detrimental effect upon ART-18 IgG2b-mediated prolongation of graft survival (Table 2, group I). Thus, high anti-ART-18, but not anti-IgG, titers abrogate the therapeutic effects of IL-2R-targeted therapy following transplantation.

To demonstrate directly the presence of circulating anti-Id antibodies, ConA-activated spleen cells were stained with biotinylated ART-18 or biotinylated ART-18 that has been preincubated with serum from normal or ART-18-pretreated and/or -treated hosts. The naive serum did not affect the binding of ART-18 to IL-2R expressed by activated splenocytes (>95% binding). In contrast, serum from hosts pretreated/treated with ART-18 uniformly inhibited ART-18 binding (45–65%). This effect was ART-18 specific, as the test serum did not affect the binding of biotinylated ART-65, the mAb of distinct IL-2R epitope specificity. Absorption of serum with a protein A-Sepharose column removed all blocking activity. Thus, the blocking activity following ART-18 treatment is primarily due to the protein A binding anti-Id antibodies and not to the circulating free ART-18 or soluble IL-2R.

Table 3. Effects of ART-18/IgG pretreatment on anti-Id/IgG responses in allograft recipients treated with ART-18

| Group* | Time,† days | Titer‡ Anti-ART-18 | Titer‡ Anti-IgG |
|---|---|---|---|
| H (21) | 4 | 1, 1, 2, 3 | 2, 2, 3, 4 |
| | 7 | 1, 2, 2, 3 | 3, 3, 4, 4 |
| | 14 | 3 (×3), 4 | |
| I (17) | 4 | 1, 1, 3 | 3, 3, 6 |
| | 7 | 1, 1, 2, 3 | 4, 5, 5, 6 |
| J (10) | 4 | 6, 7, 7 | 3, 3, 4 |
| | 7 | 5, 6, 7, 7, 8+ | 4, 5, 7 (×3) |

*The detailed description of the individual experimental groups is shown in Table 2. The mean graft survival time (days) in the respective animal groups is given in parentheses.

†Days after heart transplantation.

‡Anti-ART-18/IgG titers were measured by ELISA. Sera were diluted 2-fold from 1:25 to 1:3200. Data are expressed as the end point of the greatest dilution that gave an OD higher than the mean + 4 SD of that in the normal serum. If the highest dilution of serum (1:3200) remained positive, the sample titer was designated as "8+".

**Effects of CsA Plus ART-18 Pretreatment on Cardiac Allograft Survival in ART-18-Treated Hosts.** Adjunctive use of CsA prior to transplantation erased sensitization to ART-18 given pre- and postoperatively and strikingly prolonged allograft survival to >58 days ($P < 0.001$); in addition, two of five grafts survived indefinitely (Table 4, group L). Such a marked graft prolongation resulted from indirect effects of CsA upon the host immune responses against ART-18 itself. (*i*) Pretreatment with CsA alone or in conjunction with mouse IgG (instead of ART-18) did not add to the effect exerted by ART-18 after transplantation (graft survival, 20–24 days; Table 4, groups M and N). (*ii*) The relatively low CsA trough levels in hosts pretreated with CsA plus ART-18 (50–200 ng/ml; Fig. 1) were comparable to those in recipients pretreated with CsA alone, suggesting that mAb did not affect CsA clearance. (*iii*) Only marginal graft prolongation was noted when the latter regimen on its own was employed (Table 4, group O). Thus, combined pretreatment with CsA and IL-2R mAb enhances the posttransplant mAb therapeutic effect.

**Anti-Id/IgG Responses in Allograft Recipients Pretreated with CsA Plus ART-18.** CsA plus ART-18 pretreatment diminished serum titers of anti-Id and anti-IgG antibodies in rats given ART-18 after grafting (Table 5, group L vs. group J). The decrease of anti-Id responses ($P < 0.001$) resulting from interaction between CsA and ART-18 was critical for prolongation of graft survival >58 days in this experimental group. The following observations in Table 5 support this notion. (*i*) Although pretreatment with CsA and control mouse IgG, instead of ART-18 (group M), diminished anti-IgG titers, the anti-Id titers remained elevated ($P < 0.01$ compared with group L), coinciding with graft survival of only ≈24 days. (*ii*) Pretreatment with CsA alone followed by ART-18 after grafting (group N), which produced the effect as if ART-18 alone had been given (graft survival, ≈20 days), did not decrease anti-Id responses ($P < 0.001$ compared with group L). (*iii*) The elevated anti-Id titers on day 14 in one rat of group L were followed by the graft loss 5 days later. Thus, pretreatment with ART-18 and CsA decreased anti-Id antibody levels; these correlated with actual graft survival.

In contrast, the posttransplant CsA therapy (15 mg/kg per day for 7 days), which prolongs graft survival to >100 days

Table 4. Effects of pretreatment with CsA and ART-18/IgG on the survival of cardiac allografts in recipients treated with ART-18

| Group | Preimmunization | Therapy | Graft survival, days | MST,* days |
|---|---|---|---|---|
| L | ART-18 IgG2b + CsA | ART-18 IgG2b | 19, 31, 42, >100, >100 | >58.4 ± 38.8 |
| M | Mouse IgG2b + CsA | ART-18 IgG2b | 21, 21, 27, 27 | 24.0 ± 3.5 |
| N | CsA | ART-18 IgG2b | 18, 20 (×3), 21 | 19.8 ± 1.1 |
| O | CsA | — | 12 (×4), 16 | 12.8 ± 1.8 |

For details, see Table 2. CsA was given prior to transplantation (15 mg/kg i.m.) on days −21, −19, −16, −14, −12, −9, −7, −5, and −2.
*Mean graft survival time ± SD.

on its own (16), had less pronounced effect upon anti-Id titers in rats conditioned simultaneously with ART-18 (data not shown). Thus, treatment with CsA plus IL-2R mAb before transplantation decreases host sensitization to mAb; the diminished anti-Id response ultimately potentiates the beneficial effect of IL-2R-targeted therapy after grafting.

## DISCUSSION

The ability of anti-IL-2R mAbs to combat graft rejection has been shown in various animal models and in clinical settings (4, 7, 10, 13, 17–19). However, as most data on host anti-Id/Ig responses derive from humans (7, 18) and monkeys (10), which limits the effectiveness of these materials, more basic information is needed to understand the complex interactions between mAb and the host immune system itself. In an attempt to dissect some aspects of Id/Ig network, we used a panel of anti-IL-2R mAbs in rat recipients of cardiac allografts.

The sensitization regimen consisting of four injections with ART-18 to the prospective hosts before transplantation provoked strong operational anti-Id responses, regardless of the isotype of mAb employed. Anti-Id antibodies have been identified indirectly *in vivo* by their ability to abrogate the efficacy of posttransplant ART-18 therapy and directly *in vitro* through inhibition of a specific ART-18 binding to IL-2R upon Con A-activated blasts. In contrast, the host humoral response to the constant domain of the mouse IgG had no effect upon the beneficial outcome of ART-18 treatment. Similarly, in mice treated with anti-murine IL-2R mAb M7/20 (IgM) and in monkeys conditioned with mouse anti-human IL-2R mAb anti-Tac (IgG2a), the neutralizing anti-Id antibodies blocked binding of mAb to its targets *in vitro* and abrogated the *in vivo* suppressive effects upon delayed-type hypersensitivity and renal allograft survival, respectively (10, 11). Comparable clinical data have been recorded for 33B3.1, a rat anti-human IL-2R mAb (IgG2a) (7). Thus, as the anti-Id response represents the major limiting factor of IL-2R mAb treatment, concomitant/sequential use of mAbs against distinct Id may be feasible. Indeed, rejection of renal transplants in patients given anti-Tac can be reversed by a subsequent course of OKT3 (17). The murine responses against anti-CD3 mAb were suppressed after pretreatment with anti-CD4 mAb (20), whereas immunosuppression could be maintained for prolonged periods following consecutive use of mAbs against distinct Id of the CD4 molecule (21).

Approximately 50% of patients receiving OKT3 develop an oligoclonal B-cell response directed predominantly (>80%) against the Id of the mAb (9, 22). Its down-regulation by azathioprine and steroids (23) or cyclophosphamide (24) provides the rationale for repeated courses of OKT3. Although addition of CsA to OKT3 had no effect upon anti-Id titers in some transplant patients (25), it decreased anti-OKT3 responses in others (26). However, this treatment may be associated with the risk of generating too profound suppression. In rats, a posttransplant regimen of subtherapeutic doses of CsA synergizing with ART-18 often produces permanent graft acceptance (13), despite highly elevated anti-Id titers. The putative mechanism of such interaction between the two agents is speculative and not directly related to the present work.

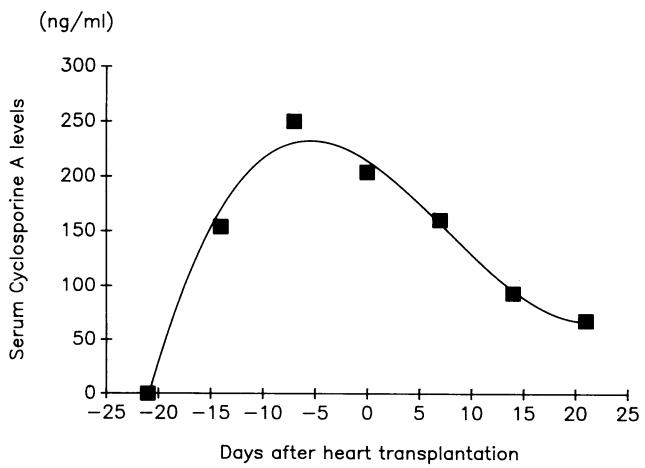


FIG. 1. CsA levels in the serum of animals treated with CsA (15 mg/kg i.m. between days −21 and −2, every 3 days; total of nine injections) and ART-18 (group L in Table 4). ■, Means of four or five experimental samples. Means ± SD on days −14, −7, 0, 7, 14, and 21 were 154.0 ± 30.9, 249.8 ± 25.7, 203.8 ± 41.0, 160.2 ± 44.8, 93.2 ± 27.7, and 67.8 ± 27.7 ng/ml, respectively. As the CsA serum level on day 0 in group M in Table 4 (CsA given alone without ART-18 pretreatment) was 197.3 ± 101.0 ng/ml, simultaneous administration of ART-18 did not affect the CsA serum level.

Table 5. Effects of CsA plus ART-18/IgG pretreatment on anti-Id/IgG responses in allograft recipients treated with ART-18

| Group* | Time,† days | Titer‡ Anti-Id | Titer‡ Anti-IgG |
|---|---|---|---|
| L (>58) | 7 | 1, 2, 3, 3, 4 | 2, 2, 3, 3, 4 |
| | 14 | 2, 2, 3, 4, 8+ | 2, 2, 3, 4, 5 |
| | 21 | 2, 3, 4, 4 | |
| | 28 | 1, 1, 3, 4 | |
| M (24) | 7 | 1, 1, 2, 2 | 1, 1, 2, 2 |
| | 14 | 3, 4 (×3) | 1, 1, 2, 4 |
| | 21§ | 4, 5, 5, 6, 7, 7 | 1, 1, 2, 3 (×3) |
| N (20) | 7 | 2, 2, 3, 3 | 2, 2, 3, 4 |
| | 14 | 3, 4 (×3) | 3, 3, 4, 4 |
| | 21¶ | 6, 6, 7, 8, 8 | 3, 3, 4 (×3) |

*The detailed description of the individual experimental groups is shown in Tables 2 and 4. CsA (15 mg/kg) was administered i.m. into the prospective graft recipients on days −21, −19, −16, −14, −12, −9, −7, −5, and −2. The mean graft survival time (days) in the respective animal groups is given in parentheses.
†Days after heart transplantation.
‡Anti-Id/IgG titers were measured by ELISA (see Table 3 for details).
§Measurements at day 21 and the time of graft rejection; $P < 0.01$ compared with anti-Id titers at day 21 in group L.
¶Measurements at day 21 or the time of graft rejection; $P < 0.001$ compared with anti-Id titers at day 21 in group L.

We now provide evidence for a strategy to overcome the anti-Id response by manipulating the host anti-Id network before grafting. Thus, the ability of CsA given in concert with ART-18 (but not with irrelevant immunoglobulin) before transplantation to "tolerize" the animal and abrogate its anti-Id response against subsequent posttransplant ART-18 therapy, with resultant long-term graft acceptance, is of particular interest. A course of CsA combined with antigenic protein challenge may influence directly the posttransplant host anti-mAb humoral responses by modulating B-cell-mediated immunity, with the resultant Id/anti-Id images in the recipient being disturbed (27). CsA depresses production of IL-2, which consequently induces many secondary events, including inhibition of IL-2R expression and IL-1 and interferon $\gamma$ production (28). It is conceivable that IL-2 represents one of the humoral factors involved in differentiation of "antigen-specific" B-cell lineage (29). We have recently shown that functionally potent antigen-specific T-suppressor cells may develop in an IL-2-independent fashion (30). Thus, CsA spares T-suppressor cells involved in T- and B-cell immunity, which may contribute, at least in part, to the biological effects observed in the present studies. Alternatively, as CsA depresses elaboration of IL-4 (31), the actual T-cell response to foreign protein may well be directly diminished by CsA pretreatment, even proximal to its effects on IL-4 production and the B-cell response.

In conclusion, we have demonstrated that host anti-Id responses limit the efficacy of IL-2R-targeted therapy but that, importantly, this anti-Id response can be reduced significantly by administration of CsA and mAb *prior* to transplantation. This regimen may induce partial unresponsiveness/tolerance to subsequent anti-IL-2R mAb treatment, which results in long-term/permanent graft acceptance, an observation of considerable relevance to the clinical application of IL-2R-targeted therapy.

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