A High-Capacity Quantitative Mouse Model of Drug-Mediated Immunosuppression Based on Rejection of an Allogeneic Subcutaneous Tumor

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ABSTRACT

We describe a high-capacity in vivo assay to measure drug-mediated transplant immunosuppression using a mouse model of Sa1 tumor rejection. Sa1 grew poorly and was rejected by 14 days in immunocompetent allogeneic recipient mice. In nude (nu/nu) mice, Sa1 grew more rapidly and was not rejected, confirming the T cell dependence of this response. In immunocompetent animals, administration of immunosuppressive agents resulted in increased tumor growth relative to vehicle-treated animals. Treatment with immunosuppressive drugs such as cyclosporin A (CsA), 40-O-(2-hydroxyethyl)-rapamycin (SDZ RAD), or 2-amino-2-[2-(4-octylphenyl)ethyl]-1,2-propanediol hydrochloride (FTY720) produced dose-dependent inhibition of tumor rejection. By contrast, the drugs did not affect Sa1 tumor growth in nu/nu mice, which is consistent with their predicted indirect effect on tumor size by suppressing immunity, rather than by directly stimulating Sa1 growth. Drug potency, which is usually not described for immunosuppressive agents, was calculated from the linear relationship between drug dose and tumor volume. The potency of CsA was inversely related to the stringency of the histocompatibility barrier. Another advantage of this assay is that the endpoint is an objective size measurement over a short time period, compared with transplant models where the endpoint may not be reached for many weeks and may be more subjective. In addition, this model can measure the potency of combination drug treatments and compare new immunosuppressive drug regimens. For example, the administration of SDZ RAD or FTY720 with CsA resulted in a more than additive increase in potency, compared with the sum of the drugs as single agents.

Discovery of new immunosuppressive drugs remains a challenge today despite the existence of in vitro and in vivo models. Current methods have significant limitations. In vitro models do not mimic the complexity of the immune response or measure the pharmacodynamic properties of drugs. Models of vascularized solid organ transplantation in rodents are limited by technical difficulty. An experienced scientist can perform up to five vascularized grafts per day, an insufficient number for extensive drug screening. The skin graft assay is limited by the poor ability to detect efficacy of several clinically relevant drugs, including rapamycin and cyclosporin A (CsA) (Eng et al., 1991).

Furthermore, there is no animal model in which the endpoint linearly correlates with the degree of immunosuppression. In most models of allogeneic rejection, the endpoint is median survival time. Graft survival of a solid organ or skin graft may not correlate linearly with drug potency because less immunosuppression may be needed later in the response than in the early response. Also, median survival times must be calculated because some grafts may survive for extended times, thus skewing the average. In these models, rejection does not occur with a fully suppressed immune response. Thus, there is no endpoint for 100% immunosuppression; so, a dose of drug that produces 50% immunosuppression (or a dose resulting in a 50% effect; ED50) cannot be determined.

To overcome these difficulties, we have used a model of acute rejection of an allogeneic s.c. tumor. Rejection of solid organ grafts is a T cell-dependent process. Rejection of most allogeneic vascularized solid tumor grafts is also T cell-dependent (Smith et al., 1988). The model described here is based upon rejection of Sa1, a chemically induced spindle-cell strain A fibrosarcoma line originally derived from an A/J mouse (Mitchison and Dube, 1955). Sa1 is a rapidly growing vascularized tumor. Sa1 expresses MHC class I but not MHC class II (Smith and Fitch, 1989) and thus may be considered analogous to the parenchymal cells of a solid organ graft. As

ABBREVIATIONS: CsA, cyclosporin A; ED50, dose resulting in a 50% effect; MHC, major histocompatibility complex; SDZ RAD, 40-O-(2-hydroxyethyl)-rapamycin; FTY720, 2-amino-2-[2-(4-octylphenyl)ethyl]-1,2-propanediol hydrochloride; nu/nu, nude mice; DMSO, dimethyl sulfoxide; AUC, area under the curve; ED30, dose resulting in a 30% effect.
with most models of vascularized solid organ grafts, alloantibodies do not cause rejection of Sa1. The generation of anti-Sa1 antibodies by the recipient results in a delay in the rejection ("enhancement"); Gorer and Kaliss, 1959), a process also observed under some conditions with solid vascularized organ grafts (Morris, 1980).

Rejection of allogeneic tumors in mice has been used as a model of acute allograft rejection since the 1950s (Gorer and Kaliss, 1959). These studies often focused on the immunological mechanisms of rejection. For example, some studies used monoclonal antibodies to determine the role of CD4 and CD8 T cells in rejection (see, e.g., Nakayama and Uenaka, 1985).

Models were based on the rejection of an ascites tumor (see, e.g., Rakhamilevich et al., 1993), a subcutaneous tumor (see, e.g., Smith and Fitch, 1989), or a tumor injected into the footpad of the recipient (see, e.g., Schmits et al., 1996). In another example, the "Winn tumor assay" involved mixing allogeneic tumor cells with immunocompetent T cells at various ratios, inoculating irradiated recipient mice (in the footpad, in the peritoneal cavity, or s.c.), and assessing growth of the allogeneic tumor (Winn, 1961; Loveland and McKenzie, 1982; Cole and Ostrand-Rosenberg, 1991).

More recent studies have assessed immunosuppressive effects of gene therapy approaches by transflecting the tumor with the gene of interest. Examples include viral interleukin-10 (Suzuki et al., 1995), CD95 (Arai et al., 1997), and soluble tumor necrosis factor receptor (Sabatine et al., 1998). Other studies have assessed rejection of an allogeneic tumor in mice deficient in specific immune mechanisms using genetically engineered (knockout) mice. For example, mice deficient in integrin LFA-1 are unable to reject allogeneic tumors from the footpad (Schmits et al., 1996), and perforin-deficient mice eliminate an allogeneic intraperitoneal tumor less efficiently (Kagi et al., 1994).

In none of the previous reports described was an effort made to measure drug-mediated immunosuppression. Clinical solid organ transplantation is only feasible because of immunosuppressive drugs; thus, models in which drug immunosuppression can be quantified are of significant utility. Here, we confirm that rejection of Sa1 is T cell-dependent and that immunosuppression can be measured by the extent of Sa1 growth in drug-treated allogeneic recipient mice. We calculate an ED$_{50}$ for CsA and show that the potency of CsA depends upon the stringency of the histocompatibility barrier between the tumor donor and the recipient. Finally, we show that immunosuppression can be measured by the extent of Sa1 growth in drug-treated allogeneic recipient mice. We calculate an ED$_{50}$ for CsA and show that the potency of CsA depends upon the stringency of the histocompatibility barrier between the tumor donor and the recipient. Finally, we show that immunosuppression can be measured by the extent of Sa1 growth in drug-treated allogeneic recipient mice.

Materials and Methods

Cell Culture. Sa1 cells derived from A/J mice (H-2k$^d$A$^k$K$^d$D$^d$) were obtained from Professor Robert North (Trudeau Institute, Saranac Lake, NY). To prevent contamination of Sa1 with passenger leukocytes, the tumor cells were propagated in vitro and not as in vivo ascites. Cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD) with 10% fetal bovine serum and 1 mM t-glutamine. To minimize variation between experiments, cells were frozen at $8 \times 10^6$ cells/vial, and for each experiment a vial was thawed and the cells cultured 2 days prior to use. One vial provided sufficient numbers of cells to inoculate 50 mice.

Mice. A/J, C57BL/6, C57BL/10, B10.BR, B10.A, and nude (nu/nu) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The MHC class I haplotype of each strain used as recipients is shown in Table 1. C57BL/6 and C57BL/10 are closely related strains sharing MHC and most non-MHC antigens. Also listed in Table 1 are the matching of non-MHC antigens (i.e., background antigen), and the overall stringency of the mismatch of the recipient with Sa1. Since Sa1 does not express MHC class II, differences in MHC class II genotype between Sa1 (A/J) and the recipient are probably not relevant. B10.A, B10.BR, or C57BL/10 mice share the same background (non-MHC antigens), but differ from each other in MHC class I antigens (Table 1). Thus, B10.A differs from Sa1 (A/J) at non-MHC antigens only (i.e., "minor antigens") and is the least stringent mismatch. B10.BR differs at minor antigens and the H-2D antigens, and thus is an intermediate mismatch. C57BL/10 and C57BL/6 differ from Sa1 at minor antigens and at both H-2D and H-2K.

All facilities have been fully accredited by the Association for Assessment and Accreditation of Laboratory Animals. All studies were done in accordance with applicable state and federal regulation. Furthermore, all studies carried out according to procedures reviewed and approved by the Institutional Animal Care and Use Committee.

Growth of Allogeneic Tumors In Vivo. A vial of Sa1 cells previously frozen as described was thawed 2 days before use and grown at 37°C in 10% CO$_2$ as described in the section on Cell Culture. Sa1 cells were harvested from culture flasks using phosphate-buffered saline containing 1 mM EDTA and rinsed three times in Hanks' balanced salt solution supplemented with 10 mM HEPES buffer. The right thoracic flank of the mouse was shaved and the tails of the mice marked so that individual mice could be monitored over time. Sa1 cells ($2 \times 10^6$ cells/mouse in 100 &mu;L) were s.c. injected on the right thoracic flank of the recipient.

Drug Administration. Cyclosporin A, SDZ RAD, and FTY720 were obtained from Novartis Pharmaceuticals (Basel, Switzerland). CsA stock solutions were prepared in five parts absolute ethanol to one part Tween-80 and stored at $-20^\circ$C. Dosing solutions were made daily by diluting the CsA stock solution in high-performance liquid chromatography grade water such that the final concentration of ethanol was 5% and Tween-80 was 1%. CsA was s.c. administered in a volume of 8 &mu;L/kg (0.2 &mu;L/kg mouse). FTY720 was dissolved in DMSO and stored at $-20^\circ$C as a stock solution. Dosing solutions were prepared daily by diluting the stock solution in 0.5% carboxymethyl cellulose to a final concentration of 10% DMSO/90% carboxymethyl cellulose. FTY720 was administered by oral gavage in a volume of 8 &mu;L/kg (0.2 &mu;L/kg mouse). SDZ RAD was chemically derived from rapamycin and used as previously described (Schuler et al., 1997; Schuurman et al., 1997). SDZ RAD was dissolved in a microemulsion preconcentrate optimized specifically for RAD. This material was aliquoted into vials and stored at $-20^\circ$C as a stock solution. Dosing solutions were prepared daily by diluting a vial to the appropriate concentration using sterile saline. RAD was administered by oral gavage in a volume of 8 &mu;L/kg (0.2 &mu;L/kg mouse). CsA, FTY720, and SDZ RAD were administered daily beginning 16 h prior to tumor challenge.

Monitoring of Tumor Rejection. The length and width of the tumor were measured using digital calipers. Tumor growth was
monitored for individual mice beginning at day 5, and continued until 15 days after tumor inoculation. Experiments were not extended beyond 15 days because in immunosuppressed animals the tumors became necrotic.

Data Analysis. The ability of drugs to prevent tumor rejection was determined by comparing one of two endpoints, the volume of the tumor at day 15 or the area under the tumor growth curve (AUC). These endpoints are described in detail below. If a given data point was 2 S.D. from the mean, it was not included in the data analysis. This usually included 1 to 3 mice/100 mice. Volume of the tumor (mm\(^2\)) on days 5, 9, or 15 was calculated by the formula volume = L(W\(^2\)/2). AUC for a graph of the “tumor volume versus day” was estimated by the trapezoidal rule: AUC = \(\frac{1}{2}(D_n + 1 - D_n)(V_n + 1 + V_{n+1})\), where \(D_n\) = the day of measurement, \(n\) = the nth measurement, and \(V\) = tumor volume. The average AUC was determined by averaging AUC for each individual mouse within a group. Statistical significance in comparing AUC or day 15 tumor volume between groups for each experiment was determined by one-way ANOVA or a Mann-Whitney Rank Sum test.

ED\(_{50}\) Determination. ED\(_{50}\) is defined as the concentration of a drug needed to result in 30% of the AUC of Sa1 grown in the nude mouse. ED\(_{50}\) is defined as the concentration of a drug needed to result in 50% of the AUC of Sa1 grown in the nude mouse. ED\(_{50}\) was calculated when the ED\(_{50}\) was not achieved.

Results

Sa1 Rejection Is T Cell-Dependent. Sa1 initially grows in allogeneic C57BL/6 mice (“most stringent mismatch”; see Materials and Methods) resulting in a small tumor, peaking in size by day 11 with a volume of about 200 mm\(^3\). The tumor is completely rejected and not detectable by palpitation by day 15 (Fig. 1). Sa1 is not rejected in the syngeneic A/J mouse (Fig. 1). Thus, in the absence of either MHC or non-MHC (“minor”) histocompatibility antigen differences, the tumor is not rejected, suggesting a lack of significant tumor associated antigens.

Sa1 is also not rejected in C57BL/6 mice treated with CsA (Fig. 1), suggesting that rejection is T cell-dependent. This conclusion is further supported by the lack of Sa1 rejection in nude mice. As shown in Fig. 2 allogeneic nu/nu mice on a C57BL/6 background, which lack T cells, do not reject Sa1.

CsA does not affect Sa1 growth in nu/nu mice on a C57BL/6 background (Fig. 2), or growth in beige/nude/xid mice or nu/nu mice on an outbred background (data not shown). Thus, CsA appears to act indirectly on the recipient T cells to prevent Sa1 rejection, but does not directly effect Sa1 growth. Despite recent evidence that CsA can affect tumorigenicity in vitro and in vivo (Hojo et al., 1999), we do not detect similar effects under the conditions of this model.

As shown in Fig. 3, CsA delays or prevents rejection of the Sa1 tumor in B10.BR recipient mice (“intermediate stringency”) in a dose-dependent manner. The maximal difference in tumor growth is measured at day 15; experiments were stopped at day 15 to prevent the tumors from becoming necrotic.

CsA Potency Depends on the Histocompatibility Barrier. The ability of CsA to prevent rejection in B10.A, B10.BR, or C57BL/10 mice was compared with determine the impact of histocompatibility barriers on CsA efficacy. B10.A, B10.BR, and C57BL/10 mice share the same background (non-MHC antigens), but differ from each other in class I MHC antigens (see Materials and Methods, Table 1). Thus, B10.A differs from Sa1 (A/J) at non-MHC antigens only (i.e., “minor antigens”) and is the least stringent mismatch, B10.BR differs at minor antigens and the H-2D antigens and thus is an intermediate stringency mismatch, and C57BL/10 differs at minor antigens and at both H-2D and H-2K antigens and is the most stringent mismatch.

As shown in Fig. 4, A and B, a suboptimal dose of CsA (10 mg/kg) significantly inhibits Sa1 rejection in B10.A (least stringent mismatch) and B10.BR (intermediate mismatch) mice, but has no effect on rejection in C57BL/10 (most stringent mismatch) mice. Significant inhibition of Sa1 rejection is seen in all three strains at 20 or 30 mg/kg CsA. Data in Fig. 4B shows AUC as an endpoint (see Materials and Methods). AUC provides a quantitative endpoint that is based upon all of the data collected and thus has less variation than other endpoints (data not shown). In summary, more immunosuppression is needed to overcome a greater histocompatibility barrier.

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**Fig. 1.** Sa1 is rejected in allogeneic immunocompetent recipients, but not in syngeneic or in CsA-treated mice. CsA was administered daily at 25 mg/kg s.c. Values represent the average tumor volume (n = 10 mice per group). Error bars (S.D.) are shown only for the day 15 tumor volume. Statistical analysis was performed for day 15 tumor volume only (\(^*\)*p < 0.05 versus untreated C57BL/6). The differences between C57BL/6 with CsA are not statistically different than the values for Sa1 in A/J mice.

**Fig. 2.** Sa1 is not rejected in allogeneic nu/nu recipients and CsA does not effect Sa1 growth in nu/nu recipients. CsA was administered daily at 25 mg/kg s.c. Values represent the average tumor volume (n = 10 mice/group). Error bars (S.D.) are shown only for day 15 values.
Effect of Delaying Drug Treatment. CsA treatment (30 mg/kg s.c.) of B10.BR and C57BL/10 mice challenged with Sa1 tumor was begun on day 1, day 4, day 7, or day 10. As shown in Fig. 5, CsA is less effective in preventing Sa1 rejection if administration is delayed. A significant effect on Sa1 rejection is still obtained in B10.BR mice when immunosuppression is delayed for 4 or 7 days, but no effect is seen when dosing is delayed 10 days. In data not shown, high-dose CsA treatment (30 mg/kg) can only be delayed by 4 days and still partly suppress Sa1 rejection in fully allogeneic C57BL/10 recipient mice (most stringent mismatch). Thus the greater the degree of histocompatibility mismatch, the more difficult it is to suppress an ongoing immune response. These results may reflect differences in the strength of the response.

Effect of Other Immunosuppressive Drugs on Sa1 Rejection. FTY720 and RAD are new drugs currently in clinical trials that act by different mechanisms than cyclo-

Fig. 3. Dose-dependent inhibition of Sa1 rejection by CsA. B10.BR mice (intermediate mismatch) were treated with solvent, 10, 20, or 30 mg/kg of CsA beginning the day prior to Sa1 inoculation. Values represent the average tumor volume (n = 15 mice/group). Error bars (S.D.) and statistical significance are shown only for the day 15 volume. **p < 0.05 for day 15 volume versus solvent-treated mice. In addition, the day 15 volume for mice treated with CsA at 10 mg/kg s.c. was significantly different than that for mice treated with 30 mg/kg. The differences between the 10 mg/kg and 20 mg/kg or between 20 mg/kg and 30 mg/kg did not reach statistical significance.

Fig. 4. Potency of CsA is dependent on the stringency of the histocompatibility barrier. A, Sa1 volume in recipient mice of different strains treated with CsA (10 mg/kg). Data points represent the average of 14 mice for the C57BL/10 group, 15 mice for the B10.BR group, and 10 mice for the B10.A group. Error bars (S.D.) and statistical significance are shown only for the day 15 volume. B, Sa1 AUC versus CsA dose in three strains of mice. Columns represent the average AUC (tumor volume versus day) from day 0 to 15 for each group ± S.D. **p < 0.05 using one-way ANOVA analysis of ranks.
sporin A. Both FTY720 and SDZ RAD dose-dependently inhibit Sa1 rejection in B10.A mice (Fig. 6), B10.BR mice (data not shown), and in C57BL/6 mice (data not shown). As shown in Fig. 6, in B10.A recipients (least stringent mismatch), FTY720 statistically significantly inhibits Sa1 rejection at 0.3 mg/kg and 1 mg/kg, and SDZ RAD inhibits rejection at 10 mg/kg and at 30 mg/kg. As with cyclosporin A, SDZ RAD and FTY720 are also less effective in preventing rejection in B10.BR mice or C57BL/6 mice (data not shown). Thus, this model is suitable for measuring immunosuppressive drugs that act by a variety of mechanisms.

**Combination of New Therapies with CsA.** In clinical organ transplantation, CsA-based combination therapy with other immunosuppressive drugs is used to maximize the therapeutic benefit and minimize the side effects of individual drugs. Therefore, efficacy of combinations of different immunosuppressive drugs at suboptimal doses was determined using C57BL/6 (Fig. 7A; most stringent mismatch) mice as recipients or B10.A mice as recipients (Fig. 7B; least stringent mismatch). In C57BL/6 mice, neither CsA at 5 mg/kg, nor SDZ RAD at 1 mg/kg nor FTY720 at 0.1 mg/kg results in a significant increase in Sa1 growth. However, the combinations of either SDZ RAD with CsA or FTY720 with CsA at the above doses result in a more than additive effect on Sa1 growth. A similar result is seen in the B10.A mouse. In the B10.A recipients, the combination of CsA (5 mg/kg) and SDZ RAD (3 mg/kg) results in an effect that is more than additive. The ideal method to study drug interactions is by use of an isobologram (Berenbaum, 1989). The studies presented here were not designed to generate an isobologram, or to assess the pharmacokinetic interactions of the drugs. These studies are currently being planned. Here, we show that this model is suitable for testing for additive effects with combinations of drugs.

**Quantification of Drug Potency.** Defining a precise measure of drug potency is a challenge in immunology. However, here we show that using this model, it is possible to define an ED\(_{50}\). We define the ED\(_{50}\) as the dose of a drug that
produces a tumor that is 50% of the tumor size in the absence of an immune response.

To determine the best experimental approach to define tumor size in the absence of an immune response, Sa1 was grown in syngeneic mice and in nude mice. The growth of Sa1 is less rapid in the syngeneic A/J mice (Fig. 2) than in the nu/nu mice on the B10 background (Fig. 2) or nu/nu mice on the outbred background (data not shown). Reduced growth of Sa1 in the syngeneic A/J recipient may reflect differences in the vascularization of the tumor, some tumor-associated antigens that delay growth but do not cause rejection, or other recipient factors. By contrast, equivalent Sa1 growth is seen in nu/nu mice on the outbred background as in nu/nu mice on a C57BL/6 background. Also, equivalent Sa1 growth is seen on nu/nu mice on the outbred background as in beige/nude/xid mice on a C57BL/6 background. Because beige/nude/xid mice lack NK cells and certain B cells, this suggests that NK cells do not play a critical role in this model. Based on this data, 100% immunosuppression is arbitrarily defined as the growth of Sa1 in nu/nu outbred mice.

Defining the size of the tumor in nu/nu outbred mice as 100% immunosuppression, the dose of cyclosporin A necessary to reach a 50% effect was determined. As shown in Fig. 8A, AUC and the day 15 tumor volume in B10.BR (intermediate stringency) increases linearly with the dose of CsA. However, the effect of CsA does not reach 50%, although an ED₅₀ value is reached. The ED₅₀ and ED₃₀ values for CsA in B10.BR recipients, calculated using both the day 15 tumor volume (Fig. 8B) and AUC (not shown), is summarized in Table 2. It is not surprising that CsA does not reach a 50% effect. CsA is known to have poor efficacy in mouse models (Eng et al., 1991; Yuh and Morris, 1991). FTY720 and SDZ RAD also do not reach a 50% effect, although antibodies to CD18, CD4, CD8, or CD3 do (data not shown). Anti-CD18 monoclonal antibody, anti-CD8 monoclonal antibody, or anti-CD3 monoclonal antibody result in complete inhibition of Sa1 rejection, and enable Sa1 to reach similar size in drug-treated animals as in nude animals. The ability of these agents to completely prevent rejection in this model further validates the use of ED₅₀ as experimentally defined in this model as a mechanism to compare immunosuppressive drugs.

**Discussion**

We have developed a model of acute rejection of an allogeneic graft that enables the measurement of the efficacy of a drug to elicit immunosuppression. The model is of high capacity; an experienced scientist can perform 100 transplants in a day. The model is based on the growth and rejection of a subcutaneous allogeneic tumor in immunocompetent mice. We show that the model is T cell-dependent and that drugs that inhibit T cells, including CsA, SDZ RAD, and FTY720, are active in this model.

In this model, the potency of CsA varies indirectly with the stringency of the histocompatibility barrier, suggesting that CsA is acting by the predicted mechanism. Efficacy of other therapies such as anti-CD18 monoclonal antibody or anti-CD4 monoclonal antibody also varied with the histocompatibility barrier in this model (data not shown), suggesting that this is a general phenomenon. Efficacy of anti-CD4 antibodies has been shown to vary with the barrier of the mismatch in both rat (Qi et al., 1997) and murine models (Hamano et al., 1999) of cardiac allograft rejection.

Advantages of the assay include the following. First, the assay effectively converts the endpoint of time to an endpoint of size. Thus, the endpoint is reached in 14 to 21 days, which is substantially less than weeks to months for vascularized heart grafts or skin grafts. Second, by using size as an endpoint, true ED₅₀ values can be calculated for immunosuppressive drugs. The effect of these drugs on the endpoint of the assay is highly linear, and this enables comparisons of potency between drugs. Third, a normal distribution in tumor size is expected so that the mean values can be compared instead of the median values. Fourth, because a competent scientist can perform 100 tumor grafts per day, the system is
ideally suited for high-capacity drug screening, drug profiling, and the testing of novel drug combinations.

Some parameters of rejection of an allogeneic tumor may differ from those for rejection of a vascularized solid organ. We have found that MHC class II is not detected by fluorescence activated cell scanning on Sa1 in vitro, even after 48-h treatment with interferon-γ (data not shown). In contrast, allogeneic organ grafts probably contain donor endothelium and donor passenger leukocytes that express MHC class II. Second, there may be a more important role for CD8 T cells, compared with CD4 T cells in this model. Smith et al. (1998) demonstrated that Sa1 can be rejected by MHC class I disparate recipients by CD8+ T cells, independent of CD4+ T cell help. This premise is also supported by the studies of Lamouse-Smith et al. (1993), showing that MHC-1 (CD8)-deficient mice are unable to reject Sa1. The greater role for CD8 T cells in acute rejection of the Sa1 tumor differs from rejection of vascularized organ grafts and skin grafts, where CD4 T cells play a more critical role (for examples, see Pearson et al., 1992; Wise et al., 1999). Other differences between this model and a model of solid organ transplantation include the lack of donor endothelium and the large alloantigen burden compared with many solid organ transplant models, and the ability of the tumor to regenerate more rapidly than a solid organ graft. However, the doses of CsA and other immunosuppressive drugs that are effective in the model correlate well with those published in the literature for other murine models of T cell-dependent pathology (Lems et al., 1980; Yuh and Morris, 1991). Thus, based on the data presented in this paper, we conclude that this model is pharmacologically predictive.

We did not test cytotoxic drugs in this model. Cytotoxic agents that inhibit Sa1 cell proliferation might affect tumor growth in addition to inhibiting the immune system; thus, these compounds may not prevent Sa1 tumor rejection. The effect of a drug on Sa1 tumor growth, independent of immune effects, can be assessed by the effect of the drug on Sa1 growth in nude mice (see Fig. 3 showing the lack of effect of CsA on Sa1 growth in nude mice). Although SDZ RAD and CsA have been reported to have antineoplastic activity against some tumors (Hojo et al., 1999; Majewski et al., 2000), neither RAD, CsA, nor FTY720 inhibited the growth of the Sa1 tumor in nu/nu mice (Fig. 2 and data not shown). SDZ RAD has been shown to inhibit growth of Epstein-Barr virus-transformed B cells (Majewski et al., 2000), but no other studies have been reported. Rapamycin, a macrolide related to RAD, has been shown to have antitumor activity in some types of tumors (Dourois and Suffness, 1981); however, the doses used against nonhematopoietic cells were much higher than those described here (250–400 mg/kg i.p.). Also, in the literature, some tumors were insensitive to rapamycin, and sensitivity may reflect the role of autocrine growth factors in tumor growth.

New immunosuppressive drugs currently in clinical trials include SDZ RAD and PFTY720. SDZ RAD binds to the cyclophilin protein FKBP12. FKBR2/SDZ RAD acts to inhibit the signal from the interleukin-2 receptor, a process at a later stage in T cell activation than CsA. FTY720 is a novel immunosuppressant that acts to modulate lymphocyte homing to peripheral lymph nodes and Peyser’s patches. FTY720 causes immunosuppression by sequestering immunocompetent T cells and reducing the circulating peripheral T cell population (Chiba et al., 1998).

FTY720 is not active in the in vitro models of immunosuppression (Brinkmann et al., 2000), highlighting the importance of high-capacity animal models for discovering and characterizing new drugs. Furthermore, potency in vivo is influenced by the pharmacokinetic properties of drugs, and this cannot be predicted by in vitro studies. For example, SDZ RAD is more potent than CsA in vitro and in vivo if administered by the same route of administration (Schuler et al., 1997; Schuurman et al., 1997). However, oral exposure of both SDZ RAD and cyclosporine A is limiting in the mouse. Thus, we have found that following subcutaneous administration of CsA and oral administration of SDZ RAD, the two drugs are equally immunosuppressive, a result not predicted by the in vitro potency of the compounds.

To achieve a maximum therapeutic benefit, two or more immunosuppressants are often used in combination. A preclinical model capable of comparing the potency of different combinations of drugs is of significant value. Here, we show that both SDZ RAD or FTY720 show an effect in combination with CsA that may be more than additive, consistent with previously published reports (Chiba et al., 1996; Schuurman et al., 1997, Troncoso et al., 1999). Because of the high capacity of this model, extensive studies to assess the degree of synergy between these drugs are possible.

In summary, we have shown that a murine model of allogeneic tumor rejection first described in the 1950s can be adapted for high-capacity studies that precisely measure the degree of drug-mediated immunosuppression. Because of its simplicity, this model can be used to screen for novel drugs, profile existing drugs, or to test combinations of drugs.

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