Effects of CD28 blockade on subsets of naïve T cells in cats

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Objective—To determine whether human CTLA4-Ig (huCTLA4-Ig) inhibits costimulation-dependent lymphocyte proliferation in vitro, compare the effects of (hu)CTLA4-Ig with cyclosporine and steroids on CD4+ and CD8+ T-cell lymphocyte proliferation, and determine whether memory T-cell function remains intact in the presence of (hu)CTLA4-Ig.

Animals—29 cats.

Procedure—Peripheral blood mononuclear cells (PBMCs) were stimulated with concanavalin A (costimulation-dependent mitogen) or phorbol 12-myristate 13-acetate and ionomycin (costimulation independent mitogen) alone or in the presence of (hu)CTLA4-Ig, cyclosporine, or dexamethasone; effects of these treatments on lymphocyte proliferation were assessed by incorporation of thymidine labeled with tritium or flow cytometry. Antigen-specific proliferation was determined by stimulating PBMCs from 2 healthy cats seropositive for Toxoplasma gondii with soluble Toxoplasma antigen alone or in the presence of (hu)CTLA4-Ig or cyclosporine.

Results—(hu)CTLA4-Ig inhibited costimulation-dependent lymphocyte proliferation in vitro but had no effect on costimulation-independent lymphocyte proliferation. Compared with mitogen alone, (hu)CTLA4-Ig caused a significant decrease in responder frequency and proliferative capacity of CD4+ T cells; however, the effect on CD8+ T cells was not significant. Cyclosporine alone or with dexamethasone had a significantly greater suppressive effect on responder frequency and proliferative capacity of CD4+ and CD8+ T cells, compared with (hu)CTLA4-Ig. Compared with cyclosporine, (hu)CTLA4-Ig appeared to have a sparing effect on antigen-specific proliferation of memory CD4+ and CD8+ T cells.

Conclusions and Clinical Relevance—(hu)CTLA4-Ig selectively inhibited costimulation-dependent proliferation of lymphocytes in vitro and had a sparing effect on antigen-specific proliferation of memory T cells. The specificity of its mechanism of action suggests that (hu)CTLA4-Ig may prevent allograft rejection but leave memory responses to previously encountered antigens intact. (Am J Vet Res 2005;66:483-492)
associated with chronic rejection. In addition, results of previous studies have suggested that memory T cells, which are less dependent on B7-1/CD28 interaction for activation, will be less affected. Thus, theoretically, (hu)CTLA4-Ig therapy may selectively inhibit naive T-cell activation and subsequent graft rejection, leaving memory T-cell responses intact to maintain immunity to previously encountered pathogens such as \( T \) gondii.

This study investigated the potential of (hu)CTLA4-Ig as a selective immunosuppressive agent for use in cats. The purposes of the study reported here were to determine whether (hu)CTLA4-Ig inhibits costimulation-dependent feline lymphocyte proliferation in vitro, compare the effects of (hu)CTLA4-Ig with the current immunosuppressive therapy of cyclosporine and corticosteroids on CD4+ and CD8+ T-cell lymphocyte proliferation by use of flow cytometric analysis, and determine whether memory T-cell function remains intact in the presence of (hu)CTLA4-Ig.

**Materials and Methods**

**Animals**—Twenty-nine healthy male domestic shorthair cats were used in the study. Ages of cats ranged from 1 to 3 years.

**Sample collection**—Blood was obtained by jugular venipuncture from conscious cats. Samples (2 to 3 mL) were collected in EDTA, and peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. A modification of a previously described technique was performed. Briefly, PBMCs were isolated by centrifugation over ficoll (density, 1.077) for 20 minutes at 250 \( \times \) g at 22°C. The mononuclear cell layer was washed twice in Dulbecco’s PBS solution and centrifuged for 10 minutes at 184 \( \times \) g. The pellet was resuspended in 800 \( \mu \)L of DPBSS.

**PBMC stimulation assay**—Stock solutions of the costimulation-dependent mitogen concanavalin A (ConA; 0.2 mg/mL), the cosuppression independent mitogens phorbol 12-myristate 13-acetate (PMA; 0.1 mg/mL) and ionomycin (1 mg/mL) and the immunoglobulin fusion protein (hu)CTLA4-Ig (1 mg/mL) were used at a concentration of 10 \( \mu \)g/mL. Cyclosporine (50 \( \mu \)g/mL) was used at a concentration of 0.05 \( \mu \)g/mL, and dexamethasone (4 \( \mu \)g/mL) was used at a concentration of 0.04 \( \mu \)g/mL (1 \( \times \) \( 10^7 \)M).

Peripheral blood mononuclear cells from each cat were plated in triplicate at a concentration of 1 \( \times \) \( 10^7 \) cells/mL; 100 \( \mu \)L of cells were placed into each well. Cells were stimulated with ConA (10 \( \mu \)g/mL) or PMA and ionomycin (10 \( \mu \)g/mL), alone or in the presence of (hu)CTLA4-Ig (10 \( \mu \)g/mL). Cells were incubated at 37°C in 5% \( CO_2 \) for 72 hours. Cells were then harvested, and \( \text{H} \)-thymidine incorporation was determined by use of a liquid scintillation counter. The stimulation index was calculated.

**Flow cytometry**—Peripheral blood mononuclear cells were labeled with carboxyfluorescein succinimidyl-ester (CFSE; 0.8 mM), as previously described. Cells from each cat were plated in triplicate at a concentration of 2 \( \times \) \( 10^7 \) cells/mL. Cells were stimulated with ConA (10 \( \mu \)g/mL) alone or ConA in the presence of (hu)CTLA4-Ig (10 \( \mu \)g/mL), cyclosporine (0.05 \( \mu \)g/mL), dexamethasone (0.04 \( \mu \)g/mL), or a combination of cyclosporine (0.05 \( \mu \)g/mL) and dexamethasone (0.04 \( \mu \)g/mL). Cells were labeled with mouse anti-feline CD4-PE (0.1 mg, 2 \( \mu \)L/tube) or mouse anti-feline CD8-PE (0.1 mg, 2 \( \mu \)L/tube). Briefly, cells were washed with fluorescence-activated cell sorting (FACS) buffer (1 g of bovine serum albumin into 1 L of DPBSS and 0.1 g of 0.01% sodium nitrite [NaNO₃]) and then incubated in FACS block (5 \( \mu \)L rat immunoglobulin, 1 mL of FACS buffer, 5 \( \mu \)L of mouse serum, and 50 \( \mu \)L of Fc receptor block) for 30 minutes at 4°C. Cells were labeled with anti-CD4+ or anti-CD8+ for 20 minutes at 4°C. Cells were washed once and analyzed by use of a flow cytometer. The responder frequency (percentage of cells proliferating in culture) and the proliferative capacity (mean number of daughter cells produced/proliferating cell) were determined as previously described for CD4+ and CD8+ T cells.

**Blood** (2 to 3 mL) was obtained via jugular venipuncture from 2 clinically normal cats seronegative for \( T \) gondii and 2 healthy cats seropositive for \( T \) gondii. Peripheral blood mononuclear cells were isolated, labeled with CFSE, and stimulated with soluble Toxoplasma antigen (STAG; 25 \( \mu \)g/mL) either alone or in the presence of (hu)CTLA4-Ig (50 \( \mu \)L of a 10 \( \mu \)g/mL solution) or cyclosporine (50 \( \mu \)L of a 10 \( \mu \)g/mL solution). The STAG was prepared from vitro–cultured tachyzoites of \( T \) gondii, as previously described. Cells were incubated for 96 hours, surface stained for CD4+ and CD8+, and analyzed by flow cytometry.

**Antigen-specific proliferation** was similarly evaluated by use of PBMCs isolated from a healthy adult cat that had been vaccinated with a feline viral rhinotrachitis-calcivirus-panleukopenia (FVRCP) vaccine at 8, 10, and 12 weeks of age. Peripheral blood mononuclear cells were isolated and labeled, as previously described. Labeled cells were stimulated with the FVRCP vaccine alone or in the presence of (hu)CTLA4-Ig (50 \( \mu \)L of a 10 \( \mu \)g/mL solution) or cyclosporine (50 \( \mu \)L of a 10 \( \mu \)g/mL solution). Optimal antigen concentration for lymphocyte proliferation was determined by titration experiments. Briefly, feline PBMCs were stimulated with the FVRCP vaccine undiluted or diluted with RPMI medium to dilutions of 1:10, 1:100, and 1:1,000. The RPMI medium (250 mL) was supplemented with 20% fetal calf serum (50 mL), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). On the basis of results of titration experiments, an optimal dilution of the FVRCP vaccine was determined to be 1:100.

**Statistical analyses**—All variables (stimulation index, responder frequency, and proliferative capacity) were considered to be continuous variables and were not normally distributed as determined by visual inspection of graphical displays of the data and the Shapiro-Wilk test. Therefore, median was used to describe these variables. The Wilcoxon signed rank test was used to evaluate the effect of (hu)CTLA4-Ig on lymphocyte proliferation in the presence of ConA (costimulation-dependant mitogen) or PMA and ionomycin (costimulation independent mitogens). The Wilcoxon rank sum test was used to compare the effects of each drug or drug combination on CD4+ and CD8+ T-cell lymphocyte proliferation. Additionally, a comparison was made between the effect of each drug on both CD4+ and CD8+ T cells to determine if there was a greater effect on 1 population of cells. Statistical significance was set at \( P < 0.05 \).

**Results**

Lymphocytes from 29 cats were evaluated in 6 separate experiments. By use of \( \text{H} \)-thymidine incorporation, the addition of (hu)CTLA4-Ig to lymphocytes stimulated with the costimulation-dependent mitogen ConA resulted in a significant decrease in the stimulation index, compared with ConA alone (\( P < 0.001 \)). In the presence of ConA alone, the median stimulation...
index for all 29 cats was 29 (range, 1.7 to 291.1). With the addition of (hu)CTLA4-Ig, the median stimulation index was 15 (range, 0.1 to 138.2). In contrast, (hu)CTLA4-Ig had no effect on PMA- and ionomycin-induced (costimulation-independent) proliferation, compared with PMA and ionomycin alone. In the presence of PMA and ionomycin alone, the median stimulation index was 39 (range, 1.3 to 145.2). No significant change was seen with the addition of (hu)CTLA4-Ig (P = 0.36; Figure 1). These results indicate that (hu)CTLA4-Ig does inhibit costimulation-dependent feline lymphocyte proliferation in vitro.

Use of CFSE, in contrast to 3H-thymidine, permits the differentiation of CD4+ and CD8+ T-cell responses. Presently, literature suggests that CD4+ T cells are more dependent on costimulation for activation and proliferation than CD8+ T cells. Therefore, to determine whether (hu)CTLA4-Ig preferentially suppresses CD4+ T cells in cats, the effects of (hu)CTLA4-Ig on feline CD4+ and CD8+ T cell proliferation were determined by flow cytometry by use of CFSE. In the presence of ConA, (hu)CTLA4-Ig caused a significant decrease of the responder frequency (P = 0.003) and proliferative capacity (P = 0.027) of CD4+ T cells, compared with mitogen alone. In contrast, (hu)CTLA4-Ig did not cause a significant decrease in the responder frequency (P = 0.221) or proliferative capacity (P = 0.256) of CD8+ T cells, compared with mitogen alone (Figure 2). These results indicate that, similar to other models, feline CD4+ T cells are more dependent on costimulation for activation and proliferation than feline CD8+ T cells.

In a previous study using 3H-thymidine incorporation, cyclosporine caused a noncytotoxic decrease in feline whole-blood lymphocyte proliferation. In the study reported here, FACS analysis was used to determine whether conventional immunosuppressive therapy, including cyclosporine and dexamethasone, causes a differential effect on proliferation of specific populations of T cells. In the presence of ConA, cyclosporine caused a significant decrease in the responder frequency of CD4+ (P < 0.001) and CD8+ (P < 0.001) T cells, compared with mitogen alone. Although dexamethasone, in the presence of ConA, had no significant effect on the responder frequency of CD4+ (P = 0.336) and CD8+ (P = 0.256) T cells, compared with ConA alone, there was an increase in the responder frequency of both subsets when dexamethasone was added to ConA-treated cultures. When dexamethasone was combined with cyclosporine, the effect on the responder frequency of CD4+ (P < 0.001) and CD8+ (P < 0.001) T cells was synergistic (Figure 3). Cyclosporine alone (CD4+,
had a significantly greater effect on the responder frequency of CD4+ and CD8+ T-cell subsets, compared with (hu)CTLA4-Ig. Similarly, cyclosporine caused a significant decrease in the proliferative capacity of both CD4+ (P = 0.001) and CD8+ (P < 0.001) T cells, compared with mitogen alone. Dexamethasone alone had no significant effect (CD4+, P = 1.000; CD8+, P = 0.460), and although not significant, dexamethasone caused an increase in the proliferative capacity of CD8+ T cells, compared with mitogen alone. Cyclosporine alone (CD4+, P = 0.001; CD8+, P < 0.001) and in combination with dexamethasone (CD4+, P = 0.001; CD8+, P = 0.001) had a significantly greater effect than (hu)CTLA4-Ig on the proliferative capacity. Compared with (hu)CTLA4-Ig, the greater effect of cyclosporine alone and in combination with dexamethasone on the responder frequency and proliferative capacity of feline lymphocytes suggested that, because of the more potent effect on both CD4+ and CD8+ T cells, current immunosuppressive therapy used for feline renal transplant recipients may result in more global immunosuppression.

To determine whether (hu)CTLA4-Ig selectively spares memory T-cell proliferation, memory cell responses to *T. gondii* were examined in 2 cats seropositive for *T. gondii* (seropositive cat 1 and seropositive cat 2) with or without (hu)CTLA4-Ig. Two cats seronegative for *T. gondii* were used as controls. Peripheral blood mononuclear cells from each cat were stimulated with STAG alone or with (hu)CTLA4-Ig or cyclosporine. The results from both seronegative cats were similar; therefore, only results for 1 cat will be described. After 96 hours of culture with STAG, approximately 11.7% of CD4+ and 8.0% of CD8+ T cells had proliferated, compared with 2.2% of CD4+ and 1.6% of CD8+ T cells that proliferated with medium alone. Antigen-specific proliferation of CD4+ or CD8+ T cells did not occur in the presence of (hu)CTLA4-Ig or cyclosporine (Figure 4). In the presence of (hu)CTLA4-Ig, approximately 2.7% of CD4+ and 1.1% of CD8+ T cells responded to the antigen. In the presence of cyclosporine, approximately 2.2% of CD4+ and 1.4% of CD8+ T cells responded to the antigen.

In seropositive cat 1, approximately 36.4% of CD4+ and 34.9% of CD8+ T cells had proliferated in response to STAG, indicating an antigen-specific memory cell response. In the presence of cyclosporine, antigen-specific proliferation of both T-cell subsets returned to baseline values; only 11.5% of CD4+ and 11.3% of CD8+ T cells proliferated. In the presence of (hu)CTLA4-Ig, approximately 11.2% of CD4+ and 17.1% of CD8+ T cells were able to respond to the antigen, compared with 11.9% of CD4+ and 12.1% of CD8+ T cells that were able to respond to the antigen with medium alone (Figure 5). In seropositive cat 1, an antigen-specific memory cell response was identified for CD8+, but not for CD4+ T cells, in the presence of (hu)CTLA4-Ig. In seropositive cat 2, both CD4+ and CD8+ antigen-specific memory cell responses were identified in the presence of (hu)CTLA4-Ig.
Figure 4—Proliferation profiles of CD4+ (panel A) and CD8+ (panel B) T cells from a cat seronegative for Toxoplasma gondii in the presence of medium alone, soluble Toxoplasma antigen (STAG), STAG plus (hu)CTLA4-Ig, STAG plus cyclosporine (Cyclo). Approximately 11.7% of CD4+ and 8.01% of CD8+ T cells had proliferated in response to STAG. Antigen-specific proliferation of CD4+ and CD8+ T cells did not occur in the presence of (hu)CTLA4-Ig or cyclosporine. See Figure 2 for key.
Figure 5—Proliferation profiles of CD4+ (panel A) and CD8+ (panel B) T cells from a cat seropositive for *T. gondii* in the presence of medium alone, STAG, STAG plus (hu)CTLA4-Ig, or STAG plus cyclosporine. Approximately 36.4% of CD4+ and 34.9% of CD8+ T cells had proliferated in response to STAG indicating an antigen-specific memory cell response. In the presence of cyclosporine, antigen-specific proliferation of both T cell subsets returned to baseline values. In the presence of (hu)CTLA4-Ig, an antigen-specific memory cell response was identified for CD8+ but not CD4+ T cells. See Figure 4 for key.
Figure 6—Proliferation profiles of CD4+ (panel A) and CD8+ (panel B) T cells from a healthy adult cat that had been vaccinated at 8, 10, and 12 weeks of age with a feline viral rhinotracheitis-calicivirus-panleukopenia (FVRCP) vaccine. Cells were exposed to medium alone, vaccine (Vacc), vaccine plus (hu)CTLA4-Ig, or vaccine plus cyclosporine. After 96 hours of culture with the FVRCP vaccine, approximately 13.4% of CD4+ T cells had proliferated, indicating an antigen-specific memory cell response. In the presence of cyclosporine, antigen-specific proliferation of CD4+ T cells was suppressed to baseline values (6.5%). Cyclosporine completely inhibited proliferation in response to the antigen. An antigen-specific memory cell response could be detected for CD4+ T cells (11.4%) in the presence of (hu)CTLA4-Ig. See Figure 4 for key.
The effect of (hu)CTLA4-Ig on memory cell responses was further examined by use of recall responses to a commercial antigen used to vaccinate cats. Memory cell responses were examined by use of PBMCs from a healthy adult cat that had been vaccinated at 8, 10, and 12 weeks of age with the FVRCP vaccine. Peripheral blood mononuclear cells were stimulated with FVRCP (1:100 dilution) alone or with (hu)CTLA4-Ig or cyclosporine. After 96 hours of culture with the FVRCP vaccine, approximately 13.4% of CD4+ and 6.9% of CD8+ T cells had proliferated, indicating an antigen-specific memory cell response. In the presence of cyclosporine, antigen-specific proliferation of CD4+ T cells was suppressed to baseline values (6.5%) and to less than baseline values for CD8+ T cells (1.4%). Cyclosporine completely inhibited proliferation in response to the antigen. Although the addition of (hu)CTLA4-Ig to these cultures inhibited proliferation, an antigen-specific memory cell response could be detected for CD4+ T cells. Thus, in the presence of (hu)CTLA4-Ig, approximately 11.4% of CD4+ and 3.3% of CD8+ T cells were able to respond to the antigen (Figure 6). Compared with cyclosporine, (hu)CTLA4-Ig appeared to have a sparing effect on antigen-specific proliferation of memory CD4+ and CD8+ T cells.

Discussion

During the past decade, advances in immunobiology have permitted a better understanding of the intercellular interactions between T lymphocytes and the APC. The 2-signal model for T-cell activation (the first signal provided by the antigen and the second signal by other antigen nonspecific interactions) suggests novel approaches to therapeutic immune regulation. As naive T cells are more dependent on costimulatory signals for activation and proliferation, compared with memory T cells, inhibitors of costimulation may selectively prevent naive T-cell responses and leave memory cell function intact.

Results of in vitro studies with human leukocytes indicate that (hu)CTLA4-Ig is an effective antagonist of the B7/CD28 costimulatory pathway. The CTLA4 (CD152), which is structurally similar to CD28, is expressed only on activated T cells and has a 20- to 200-fold higher binding affinity for the B7-1 and B7-2 molecules, compared with CD28. In small animal and nonhuman primate models of transplantation, (hu)CTLA4-Ig has been used successfully to prolong allograft survival and prevent acute rejection and development of graft arteriosclerosis associated with chronic rejection. Additionally, short-course administration of (hu)CTLA4-Ig has been successful in inducing T-cell anergy, thus avoiding long-term treatment with the drug. Results of the study reported here indicated that (hu)CTLA4-Ig inhibits costimulation-dependent proliferation of feline lymphocytes and suggested that this drug may be an effective immunosuppressive drug in cats.

Although the histopathologic features of renal allograft rejection have been described in cats, information regarding populations of T lymphocytes involved in graft rejection has been extrapolated from the human literature. In humans, both CD4+ and CD8+ T cells are involved in acute graft rejection. Activated T cells direct immune responses by the production of cytokines, including interleukin-2 (IL-2) and interferon-γ, which in turn induce the activation and growth of other immune effector cells. During allograft rejection, CD4+ T cells produce large amounts of IL-2, which subsequently stimulates the conversion of CD8+ precursor T cells to CD8+ cytotoxic T cells, which are involved in graft rejection. The expression of various cytokines depends primarily on cell division. Limiting or inhibiting T-cell division and cytokine production is beneficial in slowing down or inhibiting the rejection process. The TCR and costimulatory signals of T-cell activation are involved in determining the responder frequency and proliferative capacity of an immune response. The first signal (TCR engagement) is important during the induction phase because it controls the number of T cells that will divide. The costimulatory signal recruits additional T cells into the proliferating pool and permits the responding T cells to undergo multiple cell divisions. With this in mind, by use of CFSE labeling, we evaluated the responder frequency and proliferative capacity of feline lymphocytes in the presence of (hu)CTLA4-Ig. The addition of (hu)CTLA4-Ig caused a significant decrease in the responder frequency and proliferative capacity of CD4+ T cells, compared with ConA alone. In contrast, although (hu)CTLA4-Ig caused a decrease in the responder frequency and proliferative capacity of CD8+ T cells, compared with mitogen alone, the results were not significant. In humans, CD4+ T cells are more costimulation dependent than CD8+ T cells. A possible explanation for this may be that, in humans, CD28 glycoprotein is expressed on all CD4+ T cells but only 50% of CD8+ T cells. The type and percentage of feline lymphocytes that express the CD28 glycoprotein are unknown; however, this likely varies between species because 100% of murine T cells express the CD28 glycoprotein. The suppressive effect of (hu)CTLA4-Ig on feline lymphocyte proliferation was greater on CD4+ than CD8+ T cells, similar to previous findings in humans. The ability of (hu)CTLA4-Ig to have a significant inhibitory effect on both the responder frequency and proliferative capacity of feline lymphocytes, particularly CD4+ T cells, is likely to result in decreased production of cytokines involved in mediating rejection. Therefore, (hu)CTLA4-Ig may be efficacious in preventing graft rejection in the feline transplant patient.

Presently, successful renal transplantation in cats has required the continuous administration of cyclosporine, a calcineurin phosphatase inhibitor, in combination with glucocorticoids for immunosuppression. Both drugs act by preventing the T-cell–mediated release of activated cytokines, particularly IL-2. Although successful in preventing allograft rejection, these drugs cause indiscriminate suppression of T cells; as a result, patients taking them are at risk for reactivation of latent infections, neoplasia, and other adverse effects not associated with their immunosuppressive actions. Additionally, although the effects of cyclosporine and glucocorticoids on specific popula-
tions of T cells in human patients are known, these data are not yet available for cats. In the study reported here, cyclosporine had a similar suppressive effect on both CD4+ and CD8+ T cells and, when combined with dexamethasone, the effect on both populations of cells was synergistic. Cyclosporine alone and in combination with dexamethasone had a significantly greater effect than (hu)CTLA4-Ig with regards to the responder frequency and proliferative capacity of both CD4+ and CD8+ T cells. The dose of cyclosporine was chosen on the basis of results of a previous in vitro study that determined a dose range of cyclosporine (0.01 to 0.1 µg/mL) that caused suppression of feline lymphocyte proliferation.

The dose chosen for the study reported here was 0.05 µg/mL. How this in vitro dose corresponds to an in vivo model is not known; therefore, although the effect of cyclosporine was greater than (hu)CTLA4-Ig, the dose of 0.05 µg/mL may have been excessive. Additionally, the doses of (hu)CTLA4-Ig and dexamethasone used in our study were extrapolated from human studies. How these doses will correspond to an in vivo model is not known. Interestingly, dexamethasone alone did not suppress lymphocyte proliferation, and in fact, although not significant, an increase in CD4+ and CD8+ lymphocyte proliferation was detected in the presence of dexamethasone, compared with the mitogen alone. Although glucocorticoids are known to inhibit mitogen-induced proliferation of T cells, results of several studies in humans indicate the opposite effect.

In our study, memory T-cell function in the presence of (hu)CTLA4-Ig was determined for feline lymphocytes. Because cats are a common host for T gondii and reactivation of latent infections has resulted in death of the feline renal transplant patient, one of the purposes of our study was to determine whether (hu)CTLA4-Ig would spare memory cells that could respond to a T gondii antigen. In cats seronegative and seropositive for T gondii, the presence of STAG in culture stimulated lymphocyte proliferation of CD4+ and CD8+ T cells. A cumulative effect of proliferation during the 96-hour incubation period likely contributed to the proliferative response in all cats. Seropositive cat 1 was described because this cat had the strongest proliferative response to STAG. This seropositive cat spontaneously had an active T gondii infection, which had manifested itself by causing neurologic signs. The cat was clinically normal when the blood was obtained for the study reported here. The strong proliferative response in this cat supports an antigen-specific memory cell response. In this cat, the addition of cyclosporine either returned to baseline or completely abolished proliferation of both T-cell subsets. Although in the presence of (hu)CTLA4-Ig proliferation was decreased, an antigen-specific T-cell response could be detected for CD8+ but not CD4+ T cells. This may support the point that CD4+ T cells are more costimulation dependent and thus are more affected by the immunosuppressive effects of (hu)CTLA4-Ig. In seropositive cat 2, however, both CD4+ and CD8+ antigen-specific memory cell responses were identified.

Compared with cyclosporine, the results indicated that (hu)CTLA4-Ig appeared to have a sparing effect on antigen-specific proliferation of CD4+ and CD8+ memory cells. Thus, (hu)CTLA4-Ig may prevent immune responses to novel antigens, such as grafts, and leave memory responses to previously encountered antigens and latent infections intact.

Current immunosuppressive therapy available for the feline renal transplant recipient has shortcomings resulting in an increase in morbidity and mortality rates and a decrease in life expectancy of the graft and patient. Immunosuppressive strategies targeted at blocking T-cell costimulation are novel approaches to immunoregulation. In the study reported here, (hu)CTLA4-Ig was successful in selectively inhibiting costimulation-dependent lymphocyte proliferation in an in vitro feline lymphocyte model. In addition to encouraging in vitro and in vivo studies in humans and other animal models, results of this study have important implications for clinical transplantation for the feline renal transplant recipient.

References