

Successful Cyclosporine Treatment for Atopic Dermatitis in a Rhesus Macaque (*Macaca mulatta*)

Shira Ovadia, DVM,^{1,*} Steven R. Wilson, VMD,² and Caroline J. Zeiss, BVSc, PhD³

A juvenile (1 year old) female rhesus macaque (*Macaca mulatta*) developed a chronic active skin condition characterized by pruritus, erythema, alopecia, scaling, exfoliation, and lichenification. Lesions were limited to the ventrum, specifically rostral mandible and neck, axilla and inguinal regions, distal extremities, and interdigital regions. Differential diagnoses included infection, dietary deficiency, metabolic abnormality, endocrinopathy, and immunological injury. Diagnostic tests included complete hemogram, serum chemistry, skin scrapes for ectoparasite detection, hair plucks for dermatophyte culture, and a serum-based hypersensitivity panel. All results were within normal limits. Dermal biopsies revealed lesions consistent with active allergic dermatitis, and a diagnosis of atopic dermatitis was made. Oral cyclosporine (5 mg/kg daily) rapidly eliminated clinical evidence of dermatitis. Histologically, lesions resolved after 12 months of treatment. Atopic dermatitis is an inflammatory skin condition for which there are neither pathognomonic clinical or diagnostic features nor a single successful therapy. Basic criteria such as pruritus, lichenification, a chronic course, and history of allergies strongly support the diagnosis. One successful therapeutic agent is a macrolide calcineurin inhibitor, cyclosporine. It represents a safer class of immunomodulatory drugs than corticosteroids and provides targeted alteration of lymphocyte function. To our knowledge this case represents the first reported successful treatment of atopic dermatitis in a nonhuman primate utilizing cyclosporine.

Atopic dermatitis (AD) is a chronic relapsing, highly pruritic, inflammatory skin disease with increasing prevalence in human beings and canines. AD is extremely devastating during infancy. It negatively affects the quality of life because of intense pruritus causing insomnia and secondary bacterial and fungal dermatitis. Immune mechanisms underlying asthma and AD have great similarities; however the exact etiology for the condition is still not fully understood (14). In this case report we describe the clinical manifestations, diagnostic work up, and successful management of AD in a rhesus macaque with emphasis on the targeted immunomodulatory therapy using a T-cell inhibitor, cyclosporine (CYS). In light of limited available information on naturally occurring atopic disease in captive nonhuman primates, this report provides useful clinical overview about the condition for clinicians and investigators.

Materials and Methods

Case report. A juvenile female rhesus macaque (*Macaca mulatta*) was born at Yale University nonhuman primate colony by a natural delivery in July 2001. Upon weaning (7 months), the macaque was pair-housed with an age-matched Yale-born female rhesus macaque in an indoor animal facility accredited by the Association for the Advancement and Assessment of Laboratory Animal Care International and managed in accordance with the

United States Public Health Service Policy for the Humane Care and Use of Laboratory Animals. Specifically, routine husbandry practices consisted of 12:12-h light:dark cycles; controlled temperature, humidity, and ventilation (18 to 29°C, 30 to 70%, and 10 to 15 air changes/h, respectively), commercial primate chow (Certified Primate Diet #5048 LabDiet, PMI Nutrition International, St. Louis, Mo.), and daily supplementation with a variety of fruits and vegetables. The animal had social contact with its cage mate and audio-visual and olfactory contact with about 14 macaques housed in the same room. In addition, a series of toys and treats was rotated as part of the environmental enrichment program. Daily sanitation consisted of hosing the waste pan with warm water. The macaque had no direct contact with chemicals or detergents. The macaque was seronegative for cercopithecine herpesvirus 1, simian immunodeficiency virus, simian T-cell leukemia virus 1, simian retrovirus (BioReliance Corporation, Rockville, Md.) and tested negative for *Mycobacterium tuberculosis* by an intradermal test (Colorado Serum Company, Denver, Colo.) and by a cell-mediated immunity assay (Primagam, Biocor Animal Health Inc., Omaha, Nebr.). At weaning, the animal was vaccinated for measles (Attenuvax, Merck & Co. Inc., West Point, Pa.). The macaque was trained for a behavioral cognitive task and had a single brain magnetic resonance imaging (MRI). The Institutional Animal Care and Use Committee approved the experimental use of this animal. Relevant previous medical history consisted of an abscess with cellulitis in the left arm at the age of 9 months treated successfully with parenteral sulfadiazine-trimethoprim for 14 days without adverse effects.

At the age of 12 months the animal developed chronic active dermal lesions. Clinical signs observed were pruritus, erythema,

Received: 10/5/04. Revision requested: 11/8/04. Accepted: 11/22/04.

¹The National Institute of Biotechnology of the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel 84105; ²Section of Comparative Medicine, ³Department of Ophthalmology, School of Medicine, Yale University, New Haven, Connecticut 06520-8016.

*Corresponding author.

alopecia, scaling, excoriation, and lichenification of the skin. Affected areas involved the ventrum, especially the rostral mandible and neck, axilla and inguinal regions, interdigital areas, and distal extremities. Diagnostic tests performed included a complete blood count, serum chemistry profile (Antech Diagnostics, Lake Success, N.Y.), skin scrapings for ectoparasite detection, hair plucks for dermatophyte culture (Microbiology Laboratory, Section of Comparative Medicine, Yale University), and serum hypersensitivity panel (IBT Reference Laboratory, Lenexa, Kans.). The panel determined general serum immunoglobulins E (IgE), histamine levels, and serum IgE for selected allergens. Testing for potential allergens included latex, American cockroach, sulfamethoxazole, whey, apple, banana, cabbage, carrot, corn, cucumber, grape, orange, pear, sweet potato, white potato, zucchini, oat, wheat, green bean, soybean, beef, chicken, pork, turkey, peanut, chocolate (cacao), Brewer's yeast, and gluten. Results for all these tests were within normal limits or negative.

Subsequently, full-thickness skin punch biopsies 2 to 3 mm in diameter were collected from affected as well as unaffected areas. Lesions were consistent with active allergic dermatitis. On histological examination there was marked epidermal hyperplasia accompanied by superficial dermal perivascular inflammatory infiltration and dermal edema. Infiltrates consisted primarily of lymphocytes and plasma cells with occasional eosinophils (Fig. 1). Initial treatments with soothing bath and vitamins A and D ointment were ineffective as well as impractical, because husbandry practices and occupational health and safety concerns precluded daily application (3). The animal continuously exhibited the described clinical signs, and at the age of 16 months, therapy with 2.5 mg/kg daily CYS (Sandimmune, Novartis Pharmaceutical Corporation, East Hanover, N.J.) intramuscularly was initiated. The initial CYS dose over a 17-day period proved ineffective, whereas a twofold increase in dose to 5 mg/kg daily CYS intramuscularly eliminated lesions and pruritus within 2 weeks. After a 5-month period of chronic administration of CYS, the dose was reduced to 2.5 mg/kg daily followed by immediate recurrence of the clinical signs. Subsequently, the animal was maintained on oral CYS at dose of 5 mg/kg daily, with resolution of dermal inflammation as determined by clinical appearance and repeat biopsies (Fig. 2 and 3). The clinical progress was accompanied with a marked improvement in performing cognitive tasks. Monitoring for renal dysfunction by routine (biannual) serum chemistry test revealed no noticeable elevation in serum creatinine, which is the accepted marker for renal toxicity. The diagnosis of AD was made on the basis of clinical, laboratory and histology findings as well as the response to therapy and challenge. Once the animal reached puberty (34 months of age, after 18 months of therapy) the daily CYS dose was gradually reduced by 10% every month. At the same time, the initial CYS formulation (Sandimmune) was replaced with a formulation of CYS with increased bioavailability (Neoral, Novartis Pharmaceutical Corporation, East Hanover, N.J.). The therapeutic plan was to reduce the CYS dose gradually to the minimum effective dose or, in case of complete remission, discontinue the administration of CYS.

Discussion

AD is known to affect humans and canines of all ages, with increased incidence at younger ages (1, 4, 7, 11-13, 24, 25). Its etiology is unknown, but current information suggests that it is an inherited defect of a bone marrow-derived cell, resulting in

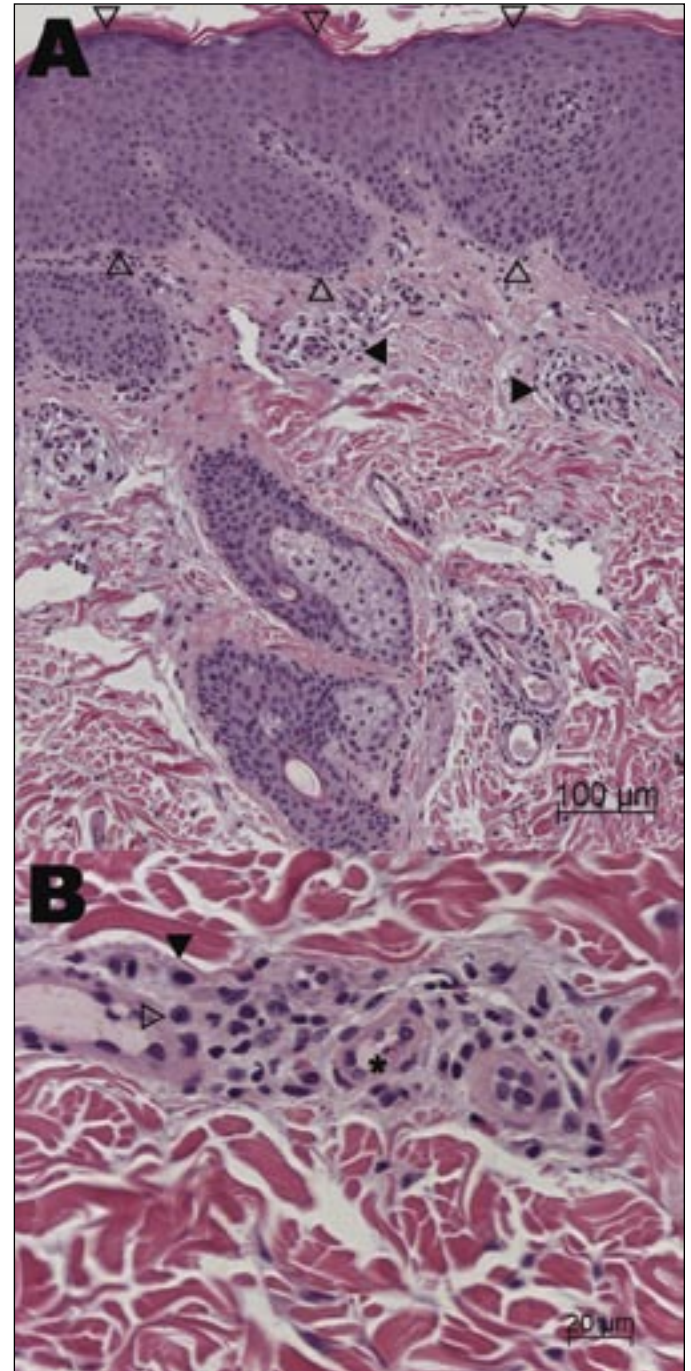


Figure 1. Haired skin, rhesus macaque, pretreatment. Digital micrographs of punch biopsy stained with hematoxylin and eosin. A) Marked epidermal hyperplasia (open arrows) accompanied by superficial dermal perivascular inflammatory infiltrate (arrows) and dermal edema. B) Infiltrates consist primarily of lymphocytes (open arrow) and plasma cells (arrow) with occasional eosinophils (asterisk). Lesions are consistent with active allergic dermatitis. Magnification, $\times 110$ (A); $\times 500$ (B).

a variety of cutaneous and generalized immune abnormalities. Reported immune abnormalities in human patients with AD include altered T lymphocyte function, hyperstimulated Langerhans cells, eosinophilia, and increased production of mast cells and prostaglandin E₂ (PGE₂). Elevation in the serum IgE level is reported commonly in humans but rarely seen in canines (25).

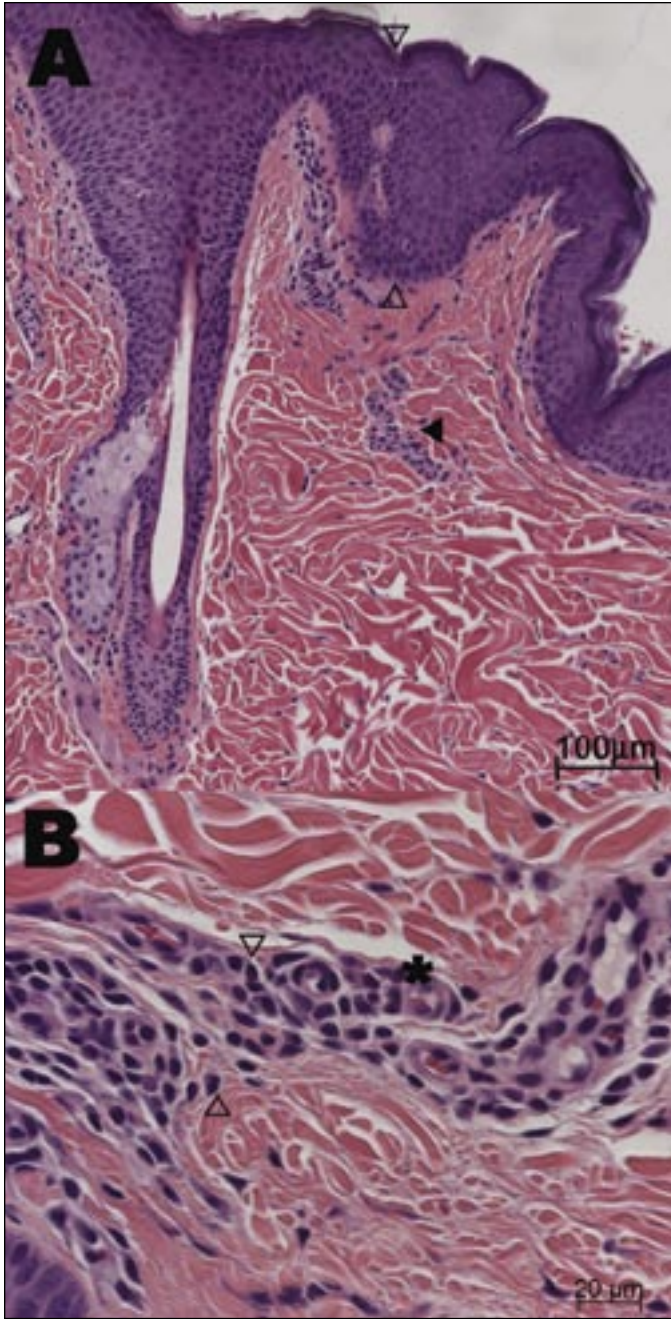


Figure 2. Haired skin, rhesus macaque, 4 months after initiation of treatment. Digital micrographs of punch biopsy stained with hematoxylin and eosin. (A) Moderate epidermal hyperplasia (open arrows) and superficial perivascular inflammation (arrows). (B) The inflammatory infiltrate consisted predominantly of lymphocytes (open arrows) with occasional mast cells (not shown) and eosinophils (asterisk). The lack of edema and decreased number of inflammatory cells compared with the pretreatment histology (Fig. 1A, B) are indicative of remission. This superficial perivascular dermatitis is indicative of a chronic allergic process. Magnification, $\times 110$ (A); $\times 500$ (B).

Identification of CD4⁺ T lymphocytes as the predominant cell type in AD-lesional skin supports the concept of a T cell-mediated disorder. Thus, therapeutic drugs, such as CYS, that affect T-cell activation and cytokine production, have the potential to be effective in ameliorating this condition (1, 14). The current

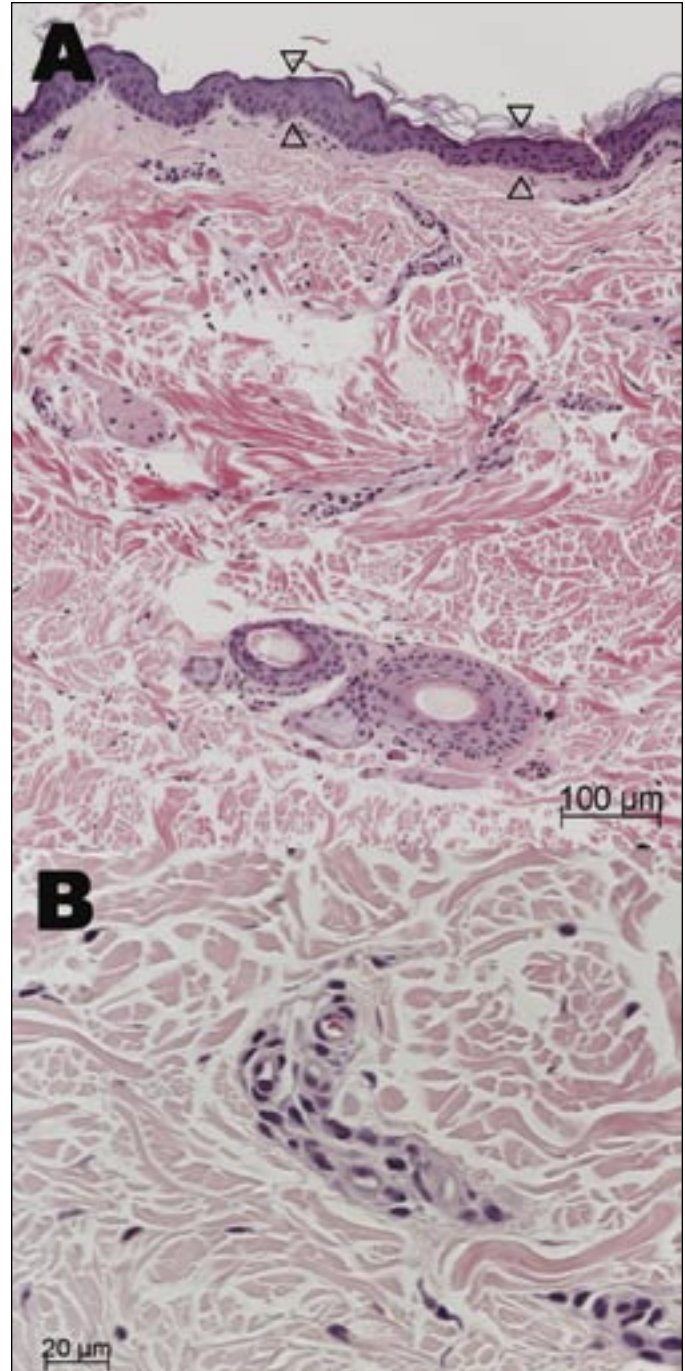


Figure 3. Haired skin, rhesus macaque, 12 months after initiation of treatment. Digital micrographs of punch biopsy stained with hematoxylin and eosin. No evidence of active inflammation is present. The dermis and epidermis (open arrows) are of normal thickness and cellularity. Magnification, $\times 110$ (A); $\times 500$ (B).

accepted model proposes a predominant type 2 (Th2) cytokine milieu in the initial acute phase of AD and a mixed Th1, Th2 pattern in chronic lesions (1, 8, 14, 21, 24, 29).

The macaque's serum allergen hypersensitivity panel was normal. Serum histamine and specific and nonspecific IgE levels were low. In addition the proportional and absolute number of eosinophils was within normal limits in all hemograms. Eo-

Table 1. Clinical features of atopic dermatitis (AD) described in humans (10) in comparison with our clinical findings in the rhesus macaque

Clinical features in humans ^a	AD features observed in rhesus macaque
Basic features: at least three must be present for diagnosis of AD	
1. Pruritus	Pruritus
2. Typical morphology and distribution: Flexural lichenification or linearity in adults Facial and extensor involvement in infants and children	Lichenification Facial involvement
3. Chronic or chronically relapsing dermatitis	Chronicity
4. Personal or family history of atopy (asthma, allergic rhinitis, atopic dermatitis)	Not determined
Minor features: at least three must be present for diagnosis of AD	
1. Xerosis (dry skin)	
2. Ichthiosis or palmar hyperlinearity or keratosis pilaris	
3. Immediate (type I) skin test reactivity	
4. Elevated serum IgE	
5. Early age of onset	Onset at 1 year
6. Tendency toward cutaneous infections (<i>Staphylococcus aureus</i> and herpesvirus) or impaired cell-mediated immunity	
7. Tendency toward nonspecific hand or foot dermatitis	Interdigital dermatitis
8. Nipple eczema	
9. Cheilitis (lip desquamation)	
10. Recurrent conjunctivitis	
11. Dennie-Morgan infraorbital fold (?)	
12. Keratoconus	
13. Anterior subcapsular cataracts	
14. Orbital darkening (?)	
15. Facial pallor or facial erythema (?)	Anterior neck involvement
16. Pityriasis alba	
17. Anterior neck folds	
18. Itch when sweating (?)	
19. Intolerance to wool and lipid solvents (?)	Not detected
20. Perifollicular accentuation	
21. Food intolerance	
22. Course influenced by environmental or emotional factors	
23. White demographism or delayed blanch (?)	

^aClinical features in humans that are not relevant to rhesus macaques are marked with a question mark.

sinophils were occasionally present in dermal biopsies obtained during the pretreated active phase as well as 4 months after initiation of therapy but not in samples taken after 12 months of treatment (Fig. 1 through 3). The finding of lymphocytes, plasma cells, and eosinophils within lesions supports the diagnosis of AD. In addition there was no histological evidence of an infectious cause of dermatitis in this animal.

There is no objective laboratory marker or test for AD, because of the complicated pathogenesis and unknown etiology of this condition. Clinical diagnostic guidelines for AD have been developed for humans and canines. Among the basic clinical features of AD are pruritus, lichenification, a chronic or chronic relapsing course, and history of atopy. This history may involve other individual atopies, e.g., allergic respiratory disease or history of allergic disease in pedigree or breed predilection. In humans, the presence of at least three of the major features and three minor features are necessary for a diagnosis of AD (7, 10, 25). The commonly used diagnostic criteria for AD in humans are listed in Table 1. In our animal, the basic criteria were met, excluding history of atopy, which could not be confirmed. The animal also exhibited some of the minor features seen in humans and canines: early age of onset and facial, ventral, distal extremity, and interdigital pattern of involvement. AD in young adult rhesus macaques has been described in conjunction with experimentally induced IgE-mediated asthma. These animals responded well to immunosuppressive corticosteroid therapy (22). However, the specific clinical features characteristic of AD in rhesus macaques had not yet been defined.

Diagnostic tests to confirm AD are unreliable, suffering from inadequate specificity and sensitivity. The differential diagnoses

for the condition are infectious causes (bacterial, viral, parasitic, fungal), dietary deficiencies, metabolic or developmental (cornification) disorders, and immunological injuries such as contact, dietary, and inhalant hypersensitivity. Allergy tests may provide additional evidence and provide potential therapeutic strategies, like allergen avoidance and allergen-specific immunotherapy (7, 9). This macaque was IgE-seronegative for all specific allergens tested including latex and sulfonamides, which have been reported to cause hypersensitivity reactions in macaques and other species including humans (15, 26). Absence of specific causative allergens precluded the opportunity to pursue allergen-specific therapy. Moreover, husbandry practices and occupational health and safety concerns prohibited the use of many common modes of therapy in humans and canines, such as topical application of antiinflammatory agents (3, 6, 18).

Once a diagnosis of AD had been made, systemic treatment was initiated with CYS, a macrolide calcineurin inhibitor with potent immunosuppressive properties. Its primary mode of action is to suppress cell-mediated reactions seen in allograft rejection, delayed hypersensitivity, experimental allergic encephalitis, Freund's adjuvant arthritis and graft-versus-host disease. To a lesser extent, it is capable of suppressing humoral immunity. The exact mechanism of pharmacological action of CYS is unknown, but it appears to exert its immunomodulatory effect through specific and reversible inhibition of immunocompetent lymphocytes in the G₀ or G₁ phase of the cell cycle. T lymphocytes are preferentially inhibited through formation of a complex with cyclophilin. CYS interferes with calcium-dependant T-cell signal transduction pathways, thus preventing activation of specific transcription factors involved in cytokine gene expression (5).

The T helper (Th0, Th1, Th2) cell is the primary target, and T suppressor activity may be inhibited as well as lymphokine production, such as interleukin-2 and transforming growth factor (1, 14, 16, 23). Currently, systemic CYS is used clinically to prevent allograft rejection and treat rheumatoid arthritis, psoriasis, and AD (1, 17). In the presented case, a therapeutic dose was determined by using published human and canine literature and the drug datasheet (1, 2, 12, 17-20, 28-30). The response to CYS therapy and relapse of AD when challenged by a 50% reduction in dose confirmed the initial diagnosis. Treatment with CYS was selected over more traditional approaches involving systemic administration of corticosteroids. Because of the potential adverse local and systemic effects of corticosteroids seen especially in juveniles, long-term use of corticosteroids is an inferior alternative and is generally not recommended (27). Among those adverse effects are increased risk of infection, hypertension, psychic derangement, growth and developmental retardation, Cushingoid features, and peptic ulcers. Other T-cell inhibitors such as azathioprine, methotrexate, and mycophenolate mofetil (1) were not pursued due to the therapeutic response and tolerance observed with CYS administration. The chronic use of CYS mandates close monitoring of blood pressure and renal function in order to avoid long-term toxic effects of CYS. Therapeutic doses should be maintained at the minimum required to prevent the recurrence of dermatitis (1, 11, 12). The therapeutic plan in this case consisted of regular health monitoring and gradual reduction of the CYS dose. The therapeutic goal was to discontinue its administration, because AD in human beings and canines, in many cases, is ameliorated or resolves at puberty (1, 27).

In conclusion, CYS, a T-lymphocyte modulator, has been shown to effectively treat AD in a juvenile female rhesus macaque with no apparent adverse effects. To our knowledge, this report represents the first described case of AD in a nonhuman primate with clinical and histological evidence of remission after 12 months of daily administration of injectable or oral CYS.

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