

Rostrum

With the increased use of "immunosuppressive" agents in severe asthma and the increasing use of cyclosporine in other conditions of possible immune pathogenesis, the following provocative article should interest many of our readers.

Is there a role for cyclosporine in asthma?

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Airway inflammation is a characteristic feature of asthma. After contact with a specific allergen on the surface of bronchial airway mast cells, IgE-dependent degranulation induces the early phase bronchial response, consisting of smooth muscle contraction and increased vascular permeability. The inflammatory LPR that begins 4 to 8 hours after the early phase is characterized by inflammatory cellular infiltrates in the bronchial mucosa and submucosa. The cells present in the bronchial airway wall and lumen, including mast cells, basophils, eosinophils, neutrophils, platelets, T- and B-lymphocytes, macrophages, and epithelial cells, are all involved in the pathogenesis of the inflammation in asthma. Although none of the cells can be identified as the primary effector cell, lymphocytes appear to have a critical role because of their immunoregulatory functions.

Systemic glucocorticoids remain the cornerstone of the treatment of severe asthma in patients refractory to bronchodilator therapy. Their long-term use systemically, however, is associated with substantial and often irreversible adverse side effects. The favorable experience with nonsteroidal anti-inflammatory therapy of dermatologic and rheumatologic diseases has prompted studies of drugs in the treatment of severe asthma. Anti-inflammatory agents studied for their steroid-sparing capability include methotrexate, gold, nedocromil, colchicine, dapsone, hydroxychloroquine, and azathioprine, among other drugs. Only methotrexate, gold, and nedocromil have proven to

Abbreviations used

CsA:	Cyclosporine
IL:	Interleukin
IL-2R:	Interleukin-2 receptor
PG:	Prostaglandin
FO:	Fish oil
LT:	Leukotriene
mRNA:	Messenger ribonucleic acid
PAF:	Platelet-activating factor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
INF:	Interferon
TNF:	Tumor necrosis factor
LPR:	Late-phase reaction

have some effectiveness in randomized, double-blind, placebo-controlled studies.¹⁻⁴

CsA, as discussed below, affects inflammatory cells and their mediators. Its effects appear to exceed those effects induced by other anti-inflammatory agents and suggest its probable usefulness in the management of severe steroid-dependent asthma. Mechanisms of the action of CsA have been reviewed with that objective in mind.

CsA

CsA is a cyclic undecapeptide metabolite extracted from the fungus, *Tolypocladium inflatum*. CsA, available as Sandimmune (Sandoz Pharmaceuticals, East Hanover, N.J.), is used to prevent and treat organ allograft rejection and a variety of inflammatory and autoimmune diseases. The actions of CsA on cells and their mediators involved in bronchial airway inflammation indicate the rationale of its use in the management of bronchial asthma.

Mechanisms of action of CsA

The precise mechanism of action of CsA is not defined, but studies with lymphocytes and thymocytes have revealed

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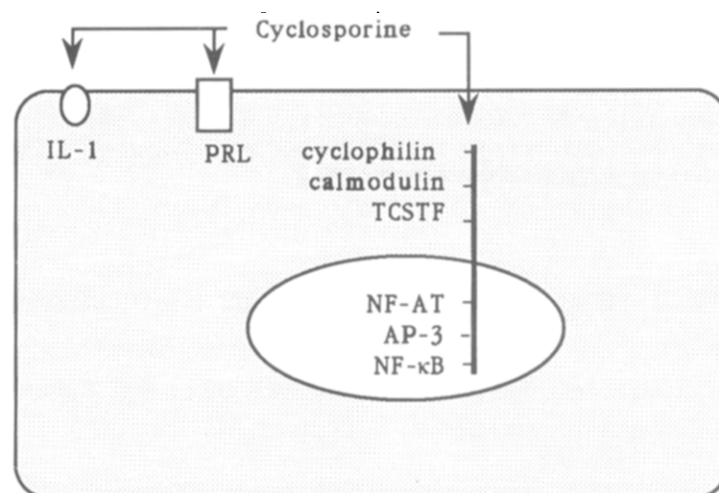


FIG. 1. CsA blocks IL-1 and prolactin (*PRL*) receptors and also blocks cytosolic (cyclophilin, calmodulin, and T cell-specific transcription factor [*TCSTFs*]) and nuclear (nuclear factor of activated T cells [*NF-AT*], activator protein 3 [*AP-3*], and nuclear factor- κ B [*NF-κB*]) lymphocytic proteins after diffusing through cell membrane.

that (Fig. 1) (1) CsA diffuses readily and completely into the cell, although there is no specific CsA membrane receptor.⁵ Of the cytoplasmic proteins that bind to CsA with varying affinity, the best characterized is cyclophilin, which is an immunophilin with protein kinase activity that may catalyze gene activation of T cells.^{6,7} CsA could block this activation by directly inhibiting the protein kinase activity of cyclophilin. Cyclophilin has identical enzymatic activity to peptidyl prolyl *cis-trans* isomerase, the latter of which is essential for refolding of proteins.^{8,9} Calmodulin, another cytosolic protein that activates exocytosis, binds CsA with less affinity than cyclophilin and regulates other kinases, cyclic nucleotides, and phospholipase A₂.¹⁰ T cell-specific transcription factors are additional cytoplasmic CsA-binding proteins. (2) CsA binds to nuclear regulatory proteins, such as nuclear factor of activated T cells, activator protein 3, and, to a lesser extent, nuclear factor- κ B. These regulatory proteins are important in the transcriptional activation of the genes for IL-1, IL-2, IL-6, TNF- α and β , IFN- β , and for the IL-2R.^{11,12} CsA also inhibits the synthesis of mRNAs for other lymphokine genes, such as IL-3 and IFN- γ .¹³⁻¹⁵ Other cytokines, such as IL-4, IL-5, colony-stimulating factor, and GM-CSF are also decreased by CsA.¹⁵⁻¹⁸ (3) The receptors for IL-1 and prolactin can be blocked by CsA.^{19,20} Therefore, CsA inhibits the synthesis of a whole cascade of lymphokines and has various sites of action for its anti-inflammatory properties.

Pharmacokinetics of CsA

CsA is available for oral, topical, intralesional, and intravenous use but is not administered intramuscularly because of unpredictable absorption by this route.²¹ Approximately 40% of an oral dose is absorbed; of that dose, 30% is bioavailable.²² CsA is metabolized by the hepatic cytochrome P-450 system and is eliminated primarily via biliary excretion with a significant enterohepatic circulation.²³

Drugs that are also nephrotoxic, for example, amphotericin B (Lymphomed, Inc., Rosemont, Ill.) and the aminoglycosides, among others, should be avoided.²³⁻²⁵ CsA, >7.5 mg/kg/day, is considered a high dose; 5 to 7.5 mg/kg/day, a medium dose; and <5 mg/kg/day, a low dose.²⁴

CsA has a variable absorption and elimination rate and a narrow concentration range for safe and effective therapy.²⁶ Optimal doses to achieve maximal anti-inflammatory and minimal side effects require a maintenance level in whole blood of between 80 and 150 ng/ml that can be determined by high-performance liquid chromatography.²⁷⁻²⁹

Side effects of CsA

Acute nephrotoxicity, the most common and serious side effect of CsA, can occur rapidly, is dose-related, and is reversible. Chronic CsA-related nephrotoxicity is irreversible but is infrequent with lower-dose regimens.³⁰ Other side effects include hypertension, mild hepatotoxicity (transient hyperbilirubinemia and transient elevated transaminases), gastrointestinal disturbances (anorexia, nausea, and vomiting), neurologic problems (muscular weakness, tremor, paresthesias, and seizures), hypertrichosis, gingival hyperplasia, and normochromic anemia.^{31,32} The risk of infection and neoplasms is also low.^{23,32}

Nephrotoxicity can be avoided by (1) initially administering CsA at a low dose not to exceed 5 mg/kg/day, (2) maintaining serum creatinine at no more than 150% of the baseline level, (3) increasing the dose gradually, as tolerated, (4) maintaining CsA levels <150 ng/ml in blood (high-performance liquid chromatography), (5) not administering CsA to individuals with preexisting kidney disease, and (6) obtaining a renal biopsy specimen after long-term therapy.²⁷ The concurrent use of omega-3 (n = 3) fatty acid-rich FO has been proposed to prevent the nephrotoxicity.³³ CsA selectively increases intrarenal vasoconstrictor

thromboxane A₂ synthesis and reduces synthesis of the vasodilator PGE₁ and PGE₂. FO maintains balanced production of renal vasodilating and vasoconstricting prostanoids. Studies with FO, 6 gm/day, with low-dose CsA in 13 patients with psoriasis and in a group of transplant recipients, confirmed that FO attenuates CsA-associated renal dysfunction.^{34, 35}

Clinical uses of CsA as an anti-inflammatory drug

CsA has been well tolerated at low dose and had significant anti-inflammatory effects in randomized, double-blind, placebo-controlled trials in rheumatoid arthritis, psoriasis, atopic dermatitis, oral lichen planus, nephrotic syndrome, and primary biliary cirrhosis.^{27, 28, 36-39} A therapeutic response was also observed in open, uncontrolled studies of systemic lupus erythematosus, Sjögren's syndrome, dermatomyositis, myasthenia gravis, vernal keratoconjunctivitis, uveitis, Behçet's syndrome, and ulcerative colitis.⁴⁰⁻⁴⁷

ACTIONS OF CsA ON CELLS AND MEDIATORS INVOLVED IN THE INFLAMMATION OF ASTHMA

Mast cells and basophils

CsA inhibited, *in vitro*, in a dose-dependent fashion, the release of histamine and PGD₂ from human lung mast cells induced by anti-IgE.⁴⁸ At lower concentrations, 3×10^{-2} µg/ml, CsA inhibited IgE-mediated and non-IgE-mediated release of LTC₄ by human lung mast cells.⁴⁹ In addition, CsA, *in vitro*, induced a dose-dependent inhibition of IgE receptor-mediated serotonin release from rat basophilic leukemia cells as well as histamine and LTC₄ release from human basophils.^{50, 51} Thus, CsA, at low concentrations, is an effective inhibitor of the release of the proinflammatory and vasoactive mediators from mast cells and basophils.

These laboratory observations have been confirmed in clinical studies of 21 children with severe vernal keratoconjunctivitis who had been refractory to treatment with topical systemic glucocorticoids and cromolyn sodium. Treatment with 2% CsA eye drops inhibited local mast cell degranulation and mediator release with considerable clinical improvement.⁴⁴

Lymphocytes

CsA most likely affects lymphocytes by suppressing lymphokine production. This likelihood has been observed both *in vivo* and *in vitro*.⁵² CsA suppressed the production of IL-2, IL-4, IL-5, and other lymphokine mRNA transcripts. Two hours of preincubation with CsA inhibited the concanavalin A-, lectin-, and phorbol myristate acetate-induced synthesis of IL-4 mRNA in a T cell line.^{16, 53} CsA inhibited, in a dose-dependent manner, the early events of IgE isotype selection induced by IL-4-stimulated human B-lymphocytes from atopic individuals but did not inhibit the ongoing and IL-4-independent IgE synthesis by the same cells.⁵⁴ The spontaneous and the IL-4-induced IgE

synthesis by human mononuclear cells also can be suppressed by CsA, 1 µg/ml.⁵⁵ The primary mechanism for the enhanced IgE production is through the IL-4-mediated increase in numbers of IgE secretory B-lymphocytes. CsA not only inhibits IgE production but also decreases the numbers of IgE-secreting B cells.

A clinical study that supports an immunomodulatory effect of CsA, 3 mg/kg/day, for 15 days on lymphocytes, was performed in seven adult patients with severe psoriasis. The results demonstrated a significant decrease in the total number of T cells with a more pronounced reduction of CD4 cells. Clinical improvement occurred concomitantly, suggesting that the therapeutic effects of CsA on psoriasis were due to inhibition of CD4 cells.⁵⁶

CsA, 25 mg/kg/day for 5 days, prevented the endotoxin-induced impairment of β₂-adrenergic-receptor lymphocytes in guinea pigs.⁵⁷

Eosinophils

Blood, tissue, and bone marrow eosinophilia are suppressed by CsA in a time and dose-dependent fashion. Cyclophosphamide-induced eosinophilia in rats was not inhibited on days 14 and 21 by CsA, 10 or 25 mg/kg/day for 21 days, but bone marrow eosinophilia was depressed significantly on day 21.⁵⁸ The lymphoid tissue and liver eosinophil infiltrates were more effectively reduced on days 14 and 21 by the higher dose.

Accessory cells

The effect of CsA, 1 µg/ml, on the antigen-presentation capacity has been studied in two human monocyte subpopulations. The monocyte subpopulation that is most effective in presenting antigens was relatively more sensitive to CsA than the subpopulation that induces immunosuppression.⁵⁹

Macrophages have receptors specific for CsA,⁶⁰ and 1 µg/ml interferes with the monocyte/macrophage function of antigen presentation to T-lymphocytes. CsA inhibits this initial step of the specific immune response by interference with the presentation of processed antigen by accessory cells to specific T cells and by reduced production of IL-1 by monocytes and macrophages. Before T cell recognition, antigen is processed by accessory cells. Subsequently, the processed antigen is complexed with the major histocompatibility complex and displayed on the extracellular membrane surface. Specific T cell responses cannot occur without the recognition of processed antigen in the context of major histocompatibility.⁶¹

The *in vitro* synthesis and release of IL-1 by macrophages and dendritic cells are decreased by CsA at concentrations of 30 µg/ml.⁶² CsA also inhibits the induction of macrophage membrane IL-1 expression.⁶³ Thus, both the initial antigen recognition and subsequent T cell-accessory cell interaction are inhibited by CsA. The effects of CsA, at low concentrations, are specific for the inductive interaction of accessory cells and T-lymphocytes. Other accessory-cell effector functions of the immune response, such as antigen uptake, are normal.

Production of superoxide anions is a mechanism by which

mononuclear cells eliminate invading microbes or tumor cells and contributes to the inflammatory response. CsA, 1 $\mu\text{g}/\text{ml}$, inhibits the respiratory burst and generation of superoxide in mouse peritoneal macrophages, as demonstrated by a decrease in phorbol myristate acetate-stimulated superoxide and H_2O_2 production.⁶⁴

Neutrophils

In a study of seven patients with psoriasis, the migration of neutrophils through the dermal capillaries was inhibited by CsA, 3 mg/kg/day, administered for 3 to 4 weeks.⁶⁵ The decline in neutrophil chemotaxis coincided with significant clearing of the psoriasis.

PAF

The administration in human subjects of a single dose of CsA, 5 mg/kg/day, at the time of intradermal injection of antigen, completely suppresses the cutaneous IgE-dependent LPR, antigen activation of mast cells, or direct PAF-induced inflammation.⁶⁶ PAF-induced bronchoconstriction was unaffected in guinea pigs administered a single dose of CsA, 25 mg/kg, but was markedly inhibited when it was administered with a dose of the PAF antagonist, BN 52021, which by itself was insufficient to cause bronchodilatation.⁶⁷ However, pretreatment with CsA alone for 2 days inhibited PAF-induced bronchoconstriction threefold, decreasing it from 60% in control-treated to 20% in the CsA-treated guinea pigs ($p < 0.01$).

DISCUSSION

Mast cells and basophils, which release proinflammatory mediators, are important in the pathogenesis of asthma. These mediators stimulate mucus secretion, are chemotactic for inflammatory cells, increase microvascular permeability and airway edema, and cause smooth muscle contraction. These mediators include histamine, LTs, PGs, chemotactic factors for lymphocytes, monocytes, neutrophils and eosinophils, IL-3, IL-4, IL-5, IL-6, and GM-CSF.^{68, 69} IL-3, IL-5, and GM-CSF recruit basophils and IL-5 enhances degranulation of basophils and histamine release.⁷⁰ CsA may prevent the activation and release of mediators by mast cells and basophils and result in the reduction of the inflammatory component of asthma.

Histologic studies of bronchi, obtained at autopsy of subjects with asthma, reveal that lymphocytes play an important role in asthmatic bronchial inflammation. CsA inhibits the synthesis of many lymphokines that may contribute to inflammation of the airways. CsA, like corticosteroids, inhibits IL-2 and IL-3 synthesis and the expression of IL-2R on lymphocytes. Although the role of IL-2 in asthma has not been established, IL-2 would increase the levels of several cy-

tokines by activating T-lymphocytes, and IL-2 augments IL-4-induced synthesis of IgE.⁵⁵ IL-2 production by T cells from children with asthma, but not from control subjects, has been observed after stimulation of lymphocytes with *Dermatophagoides farinae* antigens.^{71, 72} The serum concentrations of IL-2R and INF- γ were elevated in patients with severe acute asthma compared with that of all control groups.⁷³ Concentrations decreased as the patients clinically improved during the first 3 days of therapy. Improvement in airway obstruction correlated with the decrease in the serum concentrations of soluble IL-2R and CD4 cells expressing IL-2R. CsA reduces formation of mRNA of IL-3, IL-4, IL-5, and GM-CSF. IL-3 is essential for differentiation and growth of mucosal mast cells from bone marrow precursors and causes histamine release from mast cells and basophils. Atopic patients have a higher proportion of circulating T cells capable of producing IL-4 than nonatopic subjects.⁷⁴ IL-4 is an important lymphokine regulating IgE production and mast cell differentiation.⁷⁵ IL-4 and IL-5 are potent stimulants of IgE secretion and synthesis and induce expression of the IgE low-affinity receptors ($\text{Fc}\epsilon\text{RII}$) on B cells.^{76, 77} IL-3, IL-5, and GM-CSF have pronounced effects on eosinophils, including enhancement of adherence and activation of the hypodense phenotype.⁷⁸ IL-5 can preferentially augment IgA secretion by B cells that may be important to the initiation of inflammation at mucosal surfaces.⁷³ Another potential benefit of CsA in bronchial asthma can be its protective action on β_2 -adrenergic receptors, since their blockade in respiratory airways promotes bronchial hyperresponsiveness and production of IgE.⁵⁷

Eosinophils are important in the pathophysiology of asthma, a disease some authors have referred to as chronic eosinophilic bronchitis. These cells infiltrate the lungs, and when they are degranulated, produce functional and lytic damage to the airway and alveolar epithelial cells through the synthesis and release of bronchoconstrictor, chemotactic, and vasoactive factors, such as LTs, PAF, and cationic proteins. CsA inhibited eosinophilia at doses of 10 mg/kg/day after 21 days of treatment.⁵⁸ This dose is twice the anti-inflammatory dose, and additional research is needed to determine if lesser dosages of CsA can inhibit eosinophilia in allergic human diseases. CsA also inhibits the production of several factors capable of stimulating eosinophil growth and migration, including GM-CSF, IL-3, and IL-5.

Macrophages constitute the majority of cells recovered by bronchial alveolar lavage of both normal

subjects and individuals with asthma.⁷⁹ Activated macrophages secrete potent chemotactic substances, such as LTs, PAF, IL-1, IL-6, IL-8, TNF, platelet-derived growth factor, and GM-CSF.⁸⁰ Alveolar macrophages can recruit monocytes, eosinophils, lymphocytes, and neutrophils into the lungs, all of which are involved in the pathogenesis of asthma. The production of monocyte chemotactic factors and the macrophage response to lymphokines are blocked by CsA.⁶³ CsA also decreases the production of superoxide and H₂O₂, substances that damage the airways.

Evidence of the role of neutrophils in human asthma is based mainly on (1) *in vitro* studies with peripheral blood cells, (2) histologic studies of the bronchial infiltration observed at the beginning of the LPRs, and (3) a study of human bronchial biopsy specimens with immunofluorescence techniques, which demonstrated an association between the epithelial deposition of neutrophil elastase and bronchial epithelial damage.⁸¹ The neutrophil chemotactic activity that appears in serum during the early and late asthmatic reactions after provocative challenge by allergen or exercise also suggests an important role of neutrophils in asthma.⁸² Bronchial alveolar lavage fluids obtained during the LPR after antigen challenge of a sensitized individual contain increased numbers of neutrophils. The presence of neutrophil chemotactic activity in serum of the patients with LPRs correlates with the appearance of neutrophils in the circulation.⁸³ Neutrophils accumulate in many tissues involved in acute IgE-dependent allergic responses. Bronchial damage results from the release of the neutrophil enzyme, nicotinamide adenine dinucleotide (reduced) oxidase, as well as superoxide radicals and proteolytic enzymes.⁸² The production and release of LTB₄ and PAF, potent chemotactic factors for neutrophils, are blocked by CsA, and neutrophil emigration is thus inhibited.

CsA blocks production by basophils, alveolar macrophages, platelets, neutrophils, and eosinophils of the chemoattractants LTB₄ and PAF. The latter is a potent bronchoconstrictor, induces microvascular leakage, and is chemotactic for eosinophils. It regulates bronchopulmonary reactivity by recruiting platelets, neutrophils, and eosinophils into the lung.^{84, 85} The presence of PAF receptors on T cells suggests that T cells are also a target of this mediator. PAF also acts synergistically with IFN- γ and TNF to increase the production of IL-1 and additional PAF.^{86, 87} The lungs of guinea pigs, treated with PAF for 2 weeks, revealed congestion, bronchial smooth muscle contraction, smooth muscle hyperplasia, meta-

plasia of the mucus glands, and eosinophil and mast cell infiltration.^{88*} PAF itself may lead to airway edema, plasma extravasation, inflammatory cell infiltration, bronchoconstriction, and smooth muscle hyperplasia. CsA, at low dose, inhibits the cutaneous IgE-dependent LPR, which is also PAF dependent. Higher doses of CsA have inhibitory effects on PAF-induced bronchoconstriction.

CONCLUSIONS

CsA does more than inhibit production of cytokines; it interferes with at least two series of biochemical events in human inflammatory cells: (1) those occurring at the pretranslational mRNA level of lymphocytes and (2) those controlling the release of chemical mediators from inflammatory cells involved in the pathogenesis of asthma. These potentially beneficial effects in asthma include inhibition of (1) mast cell exocytosis and release of histamine, serotonin, LTC₄, PAF, PGD₂, and chemotactic peptides, (2) basophil release of histamine and LTC₄, (3) lymphocyte synthesis of IL-2, IL-3, IL-4, IL-5, GM-CSF, IFN- γ , and the IL-4-induced synthesis of human IgE, (4) eosinophilia, (5) macrophage synthesis of IL-1, TNF, superoxide, and H₂O₂, (6) chemotaxis of neutrophils, (7) cutaneous LPR and bronchoconstriction induced by PAF, and (8) the prevention of β_2 -adrenergic-receptor impairment on lymphocytes and probably other cells.

It is possible that CsA affects a critical Ca⁺⁺-dependent step in cell activation resulting in inhibition of both gene transcription and granule exocytosis in proinflammatory cells involved in asthma. Since CsA is currently used in humans and has high efficacy as an anti-inflammatory drug with safe doses tenfold less than that used for immunosuppression, CsA should be evaluated, both systemically and by inhalation, for the therapy of chronic asthma, especially of the steroid-dependent type.

*Studies demonstrating CsA glucocorticoid-sparing properties have been published since this article was prepared. They include:

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