Suppression of Inflammation by Cyclosporin A Is Mediated via a T Lymphocyte-Independent Process

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An athymic mutant rat strain was used to examine the hypothesis that modification of diseases with an inflammatory component by cyclosporin A (CsA) results from the suppression of nonspecific inflammatory mechanisms, rather than T-lymphocyte function, as is commonly inferred. Confirmation that the animal host was grossly depleted of T cells was obtained from anatomic and morphologic examination and functional tests of T-lymphocyte responsiveness. The experimental approach was to determine the effect of CsA on the course of experimentally induced infection with *Escherichia coli*, and extracellular pathogen. Host protection against this microorganism is dependent on an effective nonspecific inflammatory response. CsA administration prior to bacterial challenge resulted in a highly significant increase in bacterial numbers in the kidneys of both euthymic and athymic hosts. The data have provided a direct demonstration that modulation of the nonspecific inflammatory response by CsA can occur via a T lymphocyte-independent process.

KEY WORDS: cyclosporin A; inflammation; athymic host; experimental pyelonephritis.

INTRODUCTION

Cyclosporin A (CsA) is widely used to prevent allograph rejection (1). More recently, the agent has been employed in the management of disorders with an inflammatory component, including Crohn's disease, rheumatoid arthritis, uveitis, and psoriasis (2–5). The ability of CsA to control organ graft rejection has been attributed to an effect on lymphokine synthesis by T-helper lymphocytes and an extensive body of literature has accumulated detailing the effects of the agent on lymphocyte biology (6). As a result, it is generally believed that the T lymphocyte is the specific target for CsA and this precept has been commonly used to explain the effects of CsA on inflammatory mechanisms. Our own studies of the effect of CsA on experimentally induced infection have suggested that, in addition to an effect on T lymphocytes, CsA might also modify the nonspecific inflammatory response. One observation of particular relevance showed that CsA exacerbated acute pyelonephritis, experimentally induced with *Escherichia coli* (7). Host resistance to extracellular pathogens, such as *E. coli*, is not adversely affected by modulation of T-lymphocyte function, indicating that CsA affects defense mechanisms other than T cell-mediated immunity. The availability of an athymic rat strain, which lacks T cells and hence the accepted target for CsA, provided an opportunity to examine the hypothesis that CsA might modify inflammatory processes via a T lymphocyte-independent process.

MATERIALS AND METHODS

Inbred female Dark Agouti (DA) rats, 3–4 months of age, were used as the euthymic strain. In experiments involving athymic animals, female "nude" progeny from the rnu^{nz}/rnu^{nz} mutant (8), backcrossed onto a DA background, were used (kindly provided by Professor B. F. Heslop, University of Otago).

Cyclosporin A (Sandimmun, Sandoz, Basle, Switzerland) was administered by intramuscular injection into the thigh muscle of the rat. The agent was administered at a dose of 50 mg/kg every second day (9), starting 7 days before challenge of the kidney and continuing for the duration of the experiment. The carrier oil in which CsA is suspended was administered to control animals.

Pyelonephritis was induced as described previously (10). To estimate renal bacterial numbers, the animals were sacrificed, and the kidneys removed aseptically and homogenized in 9 ml of saline. Columbia agar pour plates were made from serial 10-fold dilutions of the homogenate to obtain the bacterial count per kidney.

Peripheral blood was collected from the ventral tail vein into EDTA anticoagulant. Samples were processed in a Model S Coulter counter (Coulter Electronics Ltd., Dunstable, Beds., England) and the total leukocyte count was determined. A blood film was examined for the differential leukocyte count. T lymphocytes were identified using the rat T-lymphocyte peripheral thymocyte surface antigen system (Pta) described previously (11,12). B lymphocytes were identified as cells bearing surface immunoglobulin. Goat anti-rat $F(ab)_2$ was prepared by immunizing a goat with $F(ab)_2$ fragments produced by pepsin digestion of IgG and the antiserum produced purified by passage through an immunoadsorbent column of rat γ-globulin (Pentax Incorporated, Kankakee, IL) bound to Sepharose 4B gel (Pharmacia Fine Chemicals, Uppsala, Sweden) activated by cyanogen bromide. The eluted antisera was then labeled with fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, MO). Wet preparations of spleen cells were examined for peripheral fluorescence using a Leitz Standard 14 microscope fitted with a 100-W halogen lamp and incident light fluorescence. The popliteal lymph node weight method for determining the graft-versus-host activity of rat lymphoid cells was carried out as described by Ford *et al.* (13), using the F_1 progeny of a cross between the DA strain and an inbred white Wistar strain. A similar protocol was used to determine the host-versus-graft response of euthymic and rnu^{nz} rats (14). Details of the method for *in vitro* analysis of the mitogenic response to T lymphocyte-specific mitogens have been published previously (15).

Statistical analysis was carried out using the Wilcoxon sum of ranks test for nonparametric data.

RESULTS AND DISCUSSION

Anatomic, histopathologic, and functional tests con-

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firmed the original findings that the mutant strain was grossly depleted of T lymphocytes (8). At autopsy, the most noteworthy feature of the gross anatomy of the athymic host was the age-dependent changes in the mediastinum. Under 10 weeks of age, there was a clear absence of any tissue resembling the thymus, but in older animals, lobules of light brown fatty tissue were observed that appeared grossly similar to the thymus of euthymic animals. Histologically, the lymphoid system of the mutant was similar to those found in the Rowett athymic mutant (16). Lymph nodes from nude animals showed a consistent reduction in the number of small lymphocytes found in the paracortical region compared with lymph nodes from the wild-type strain. A reduced density of small lymphocytes in the interfollicular zones of Peyers' patches from mutant animals was also found. Arterioles in splenic tissue from the mutant strain showed a marked general reduction in the number of small lymphocytes forming the periarticular cuffs, and in some cases, arteriolar cuffing was totally absent.

Total leukocyte numbers in the athymic strain were decreased in comparison to the wild type, which was accounted for by a decrease in the absolute numbers of lymphocytes. A differential leukocyte count also showed a reduction in the number of lymphocytes in the nude strain and a corresponding increase in the numbers of neutrophils (Table I). T-cell differentiation antigens on splenic lymphocytes could be demonstrated on 35% of splenic lymphocytes from euthymic hosts but were absent from splenocytes from most mutant animals (Table II). Functional analysis of the T-cell status of the athymic strain was carried out by quantifying and comparing with euthymic subjects. The results of the mitogenic responsiveness of splenic lymphocytes, the graft-versus-host reaction, and the host-versus-graft response confirm the absence of T cell-mediated immune capability in the athymic strain predicted by the morphologic evaluation (Table III).

Histologic evidence of renal infection appeared as early as 6–12 hr after challenge, and by 48 hr, foci of neutrophils were commonly seen in the cortical and medullary interstitium. The foci developed into wedge-shaped inflammatory lesions extending from the inner medulla to the cortex. Renal bacterial numbers reached a peak of 10^6 viable microorganisms per gram of tissue 24–48 hr after challenge and then slowly declined over a period of months. The absence of T lymphocytes did not affect bacterial numbers in the kidney 7 days after the induction of pyelonephritis (Fig. 1, euthymic *vs* athymic controls). However, when animals were treated with CsA according to the protocol outlined in Fig. 1 and then challenged, a highly significant and analogous increase in bacterial numbers occurred in both treated groups compared with their untreated controls (Fig. 1, euthymic CsA Rx *vs* athymic CsA Rx, $P < 0.01$).

Table I. Peripheral Blood Leukocyte Components of the Wild-Type and rnu^{nz} Strains

Cell type	Wild type[a]	rnu^{nza}
Leukocytes ($\times 10^9$/L)	15.6 ± 2.7	11.3 ± 1.7*
Lymphocytes	13.3 ± 2.1 (86%)	6.6 ± 0.9* (60%)
Neutrophils (PMN)	1.8 ± 0.9 (12%)	4.1 ± 1.5* (37%)
Monocytes	0.4 ± 0.3 (2%)	0.4 ± 0.3 (3%)

[a] Mean ± SD. $n = 19$ in both cases.
* Values significantly different from each other at $P < 0.001$.

Table II. Morphological Characterization of Splenic Cells in Wild-Type and rnu^{nz} Strains

Cell type	Wild type[a]	rnu^{nza}
Lymphocytes (%)		
T cells (Pta A.1 +)	35 ± 7	3 ± 4[b]*
B cells (SIg +)	45 ± 2	68 ± 6*
"Null cells"	8 ± 6	13 ± 6**
Phagocytic cells (%)		
Monocytes	7 ± 2	7 ± 2
Polymorphs	5 ± 1	9 ± 2*

[a] Mean of 13 animals.
[b] Results from individual animals: 0, 0, 0, 0, 0, 0, 0, 0, 1, 3, 6, 11, 12.
* Values significantly different from each other at $P < 0.001$.
** Values significantly different from each other at $P < 0.05$.

Cyclosporin A is known to modify inflammation in a number of diseases with an inflammatory component. In the present experiments CsA was administered to athymic rats to determine whether the agent affected cellular components of the immune and nonspecific inflammatory systems other than T lymphocytes. An extracellular pathogen (*E. coli*) was chosen as the challenging microorganism because of its ability to establish a local infection which could be exacerbated by modulation of the inflammatory response (17) but not affected by changes in T cell-mediated immunity (18). Bacterial numbers at the infection site were used to indicate the status of the cellular host defense mechanism. These experiments provided evidence for an effect of CsA on non-T-lymphocyte-associated inflammatory mechanisms, by demonstrating an increase in the number of bacteria in the kidneys of CsA-treated, athymic animals. Although the study would have benefited from an evaluation of the effect of CsA on inflammatory mechanisms in the athymic host, this was not possible on account of the difficulties in obtaining sufficient animals to provide comparable groups.

Evaluation of the state of the T-lymphocyte compartment of the athymic mutant was an important part of the experimental protocol. Laboratory evidence, based on anatomic, morphologic, and functional tests, validated the selection of this host and confirmed that the strain was severely depleted of T lymphocytes and had a grossly depressed T cell-mediated immune response. In such a host the most likely explanation for the observed results is an effect on a cellular defense process other than T cell-mediated immunity. However, it could be argued that CsA affected a small, but critical population of residual T lymphocytes. This seems unlikely in the absence of evidence of a role for T lymphocytes in the early stages of the host response to infection with extracellular pathogens, even in an immunologically intact host (18).

Studies of the cellular basis of host defenses to *E. coli* renal infection point to the nonspecific inflammatory response, in particular the neutrophil, as being critical in de-

Table III. Functional T-Cell Status in the Athymic Mutant Rat

	Euthymic host	Athymic mutant
Mitogenic responsiveness	95,149 ± 21,575[a]	923 ± 0
Graft-versus-host response	25.3 ± 5.0 mg[b]	1.2 ± 0.2 mg
Host-versus-graft response	24.1 ± 2.6 mg[b]	1.1 ± 0.2 mg

[a] Disintegrations per minute/10^6 lymphocytes (n = 10).
[b] Increase in popliteal lymph node weight over baseline (mean weight, 3.2 mg) (n = 8).

termining the outcome of an infectious challenge with *E. coli* and, therefore, most likely to be affected by CsA (17,19). Supportive evidence for an effect of CsA on the early phase of the inflammatory response is emerging and this has been associated with quantitative changes in host defense status. Neutrophils from CsA-treated animals are qualitatively normal and show the expected level of metabolic, phagocytic, and bactericidal activity. However, the number of neutrophils infiltrating sterile, subcutaneously implanted sponges are significantly reduced, despite a two- to threefold increase in circulating leukocyte numbers (20). The evidence from this study suggests that CsA affects neutrophil adherence to the vascular endothelial lining or diapedesis, thus inhibiting the ability of neutrophils to traverse the endothelium.

The data demonstrate that modulation of the inflammatory response by CsA can be a T cell-independent process. Arguments involving the recognized effects of CsA on T-lymphocyte biology have been used to explain the favorable outcome of CsA administration on a number of clinical disorders. As has been mentioned already, these arguments have been influenced by the fact that the literature has focused on the ability of CsA to modulate the function of a subgroup of lymphocytes. While these cells are obviously a key target for CsA and explain the effectiveness of the agent in limiting organ graft rejection, evidence is mounting that CsA might also be an important agent in the modulation of neutrophil infiltration in acute inflammatory responses (21).

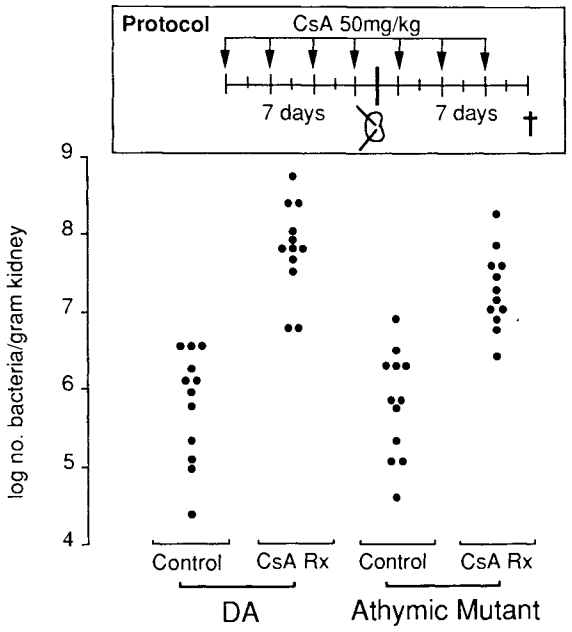

Fig. 1. Comparative effects of CsA on the bacteriologic status of kidneys from athymic and euthymic hosts, 7 days after the experimental induction of *E. coli* renal infection. n = 12 in each group; $P < 0.01$, control *vs* CsA Rx.

In the event that further experimentation proves the spectrum of CsA to extend beyond the T lymphocyte, current views on the mechanism by which CsA affects the inflammatory response will need to be reviewed. Possible effects such as an alteration to gene transcription of nonlymphocyte mediators of the inflammatory response also need to be investigated and could open up a new dimension to the interpretation of the pharmacologic and therapeutic activity of the agent.

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