Langerhans Cell Sensitivity to In Vitro Versus In Vivo Loading with Cyclosporine A

To the Editor:

Two recent manuscripts published in the Journal [1,2] confirm that Langerhans cell–dependent antigen presentation is sensitive to in vitro loading with cyclosporine A [3,4]. The authors speculate that one mechanism of CsA therapy in vivo may be inhibition of epidermal Langerhans cell antigen presentation. However, it is unlikely that the concentrations of cyclosporine A attained by the authors are achieved in vivo during psoriasis therapy. During daily oral cyclosporine therapy, epidermal keratomes taken at either the time of trough or peak blood levels generally reflect the peak blood level, indicating the ability of the epidermis to concentrate cyclosporine A approximately tenfold relative to trough blood levels (Table I) [5,6]. Upon in vitro culture in the presence of cyclosporine A, epidermal cells demonstrate even more extreme concentrating effects. Serum-free conditions particularly intensify intracellular concentrations, likely because all the cyclosporine is free (not serum protein-bound) and because of cyclosporine’s hydrophobicity. Most likely, this is because 10% serum or serum-free media have, at most, 1/10 (in the case of 10% serum) of in vivo plasma’s drug-binding capacity, leaving the highly lipophilic cyclosporine A mostly in a free state readily available for binding to cell membranes. Thus, the addition of 1 μg/ml of cyclosporine A to epidermal cells in serum-free culture resulted in an intracellular concentration of 9.4 ng/μg DNA, a value more than eightfold higher than the 1.1 ng/μg DNA achieved with a very high (not recommended) oral dose of 14 mg/kg/d [6,7], and 15 times higher than the 0.6 ng/μg DNA achieved with a lower, but still effective, oral dose of 3 mg/kg/d (Table I) [5,6]. These data make it unlikely that the concentrations for pre-loading Langerhans cells in vitro by Demidem et al (10 μg/ml, serum free) [1], Dupuy et al (1.2 μg/ml, serum present) [2], and Furue et al (5 μg/ml, serum present) [3] that were used to inhibit Langerhans cell APC activity are actually achievable in vivo. Furthermore, CsA concentrations in skin clearly exceed those required in vitro to inhibit the epidermal cell–lymphocyte reaction (0.25 μg/ml [4]; 0.012 ng/ml [2]), but are below the above APC inhibition concentrations. One might thus conclude that CsA-inhibition of Langerhans cell–T-cell responses in vivo would primarily be at the T-cell level.

We have previously published studies that directly address whether cyclosporine A therapy alters the level of in vivo epidermal APC activity by utilizing biopsies of clinically normal-looking skin from patients with psoriasis that were undergoing therapy with oral cyclosporine A [7]. Under these in vivo conditions, even following extensive in vitro manipulation including trypsinization and washing, Langerhans cells within the epidermal cell preparation were indeed markedly inhibited in their ability to activate T cells. We did not find release of cyclosporine A into the media by epidermal cells of individuals treated with cyclosporine A; nor were epidermal cell supernatants from treated patients inhibitory for epidermal cell–lymphocyte reactions [4], consistent with the findings of Furue et al [3] and Dupuy et al [2] that cyclosporine A-loaded Langerhans cells or keratinocytes fail to inhibit T-cell responses to untreated accessory cells. Thus, despite caveats regarding cyclosporine concentrations in the in vitro pre-loading studies, in vivo treatment with CsA can indeed result in inhibition of Langerhans cell–dependent T-cell activation, at the stimulator cell level.

The authors also speculate that Langerhans cell antigen-presentation activity in lesional psoriasis might be sensitive to cyclosporine A and thereby result in the observed improvement of psoriasis to cyclosporine A therapy [1,2]. However, the elevated antigen-presenting activity in psoriatic lesions appears due to a non-Langerhans antigen-presenting subpopulation [8,9], whose ability to present alloantigen was resistant to the levels of cyclosporine A achievable in psoriatic lesions, but whose activity was closely correlated to the level of activity of the lesions [4]. Thus, although cyclosporine A therapy did actually reduce Langerhans cells antigen-presenting activity in clinically normal appearing skin, there was a relative resistance of lesional psoriatic antigen-presenting non-Langerhans cells. These data suggested that cyclosporine A was more likely acting in lesional epidermis by inhibiting lymphokine or cytokine production, which was critical for continued APC infiltration, because lymphokine/cytokine-dependent expression of HLA-DR and ICAM-1 on keratinocytes, and ICAM overexpression on endothelial cells, disappears quite rapidly during therapy [4,10,11]. However, the sensitivity of dermal Langerhans cells and related perivascular dendritic antigen-presenting cells in the dermis remains to be determined, and these cells could well represent a relevant target of cyclosporine A in psoriasis.

Table I. Comparisons of Cell-Associated CsA Achieved by Various In Vivo and In Vitro Dosing Levels

<table>
<thead>
<tr>
<th>Cyclosporine Tissue Levels¹</th>
<th>Oral Dose in Vivo (mg/kg/d)</th>
<th>ng/μg DNA</th>
<th>μg/ml eq²</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0 (involved)</td>
<td>0.6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5.0 (involved)</td>
<td>1.0</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>7.5 (involved)</td>
<td>1.0</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>14.0 (involved)</td>
<td>1.1</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>14.0 (uninvolved)</td>
<td>0.6</td>
<td>1.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cyclosporine Cell-Associated Levels³</th>
<th>Dose in Vitro (μg/ml)</th>
<th>ng/μg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 (serum free)</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>10.0 (serum free)</td>
<td>56.1</td>
<td></td>
</tr>
<tr>
<td>1.0 (serum present)</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>10.0 (serum present)</td>
<td>20.5</td>
<td></td>
</tr>
</tbody>
</table>

¹ Keratome biopsies after 7 d of therapy analyzed by HPLC.
² Expressed as μg/ml equivalents for purposes of comparison to blood and solution levels; based on concentration in tissue (wt/wt) assuming epidermal tissue is 80% water.
³ Values obtained from Ellis et al [5].
⁴ Values obtained from Ellis et al [7] and Fisher et al [6].
⁵ Cultured keratinocytes incubated for 48 h, extracted and analyzed by HPLC.
⁶ Values obtained from Fisher et al [6].

Kevin D. Cooper
Ole Baadsgaard
Elizabeth Duell
Gary Fisher
Charles N. Ellis
John J. Voorhees
Department of Dermatology
University of Michigan Medical Center
Ann Arbor, Michigan

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REFERENCES


REPLY

The capacity of the immunosuppressive drug cyclosporine (CsA) to inhibit and resolve the cutaneous manifestations of psoriasis vulgaris has highlighted the important role that the immune system plays in the pathogenesis of this disease. But the mechanism(s) by which CsA achieves its therapeutic effect has (have) been unclear. Undoubtedly, CsA has a direct inhibitory effect on T lymphocytes, and it is this effect that has dominated the thinking of investigators interested in this disease. But CsA can also modify the functional properties of other cells that participate in cutaneous psoriasis: keratinocytes, Langerhans cells, dermal microvascular endothelium, and infiltrating blood-borne inflammatory cells. Determining the mode(s) of action of CsA in this disease is important, not only for understanding the immunopathogenic processes involved, but (hopefully) as a means to developing better therapeutic approaches.

Recent papers, including our own [1–3], have described the ability of CsA to inhibit the antigen-processing and accessory functions of epidermal Langerhans cells in vitro. In these experiments, putative antigen-presenting cells have been harvested from epidermis, treated with CsA, and then used as antigenic stimulators for T cells. Murine and human EAPC, including those obtained from involved and uninvolved psoriatic skin [4], were shown to be robbed of accessory and antigen-presenting function by CsA. Based on these findings, the possibility was advanced that CsA may have a therapeutic effect in psoriasis because it has a direct action on epidermal antigen-presenting cells. Cooper et al, in their Letter to the Editor, have questioned the appropriateness of this proposal. Studies in their laboratory have attempted to describe biochemically the content and distribution of CsA in the epidermis of patients treated with this agent, and they interpret their results to mean that the amount of CsA found within epidermis of treated patients is insufficient to have an effect on antigen-presenting cells, but sufficient to inhibit T-cell activation.

It is curious that this argument is raised by Cooper and his colleagues because their own experiments with antigen-presenting cells harvested from skin of psoriasis treated with CsA provide the best evidence that CsA acts directly on epidermal antigen-presenting cells. They have reported [6] that epidermal antigen-presenting cells harvested from uninvolved psoriatic skin are grossly deficient in their ability to activate T cells. Because uninvolved psoriatic skin is not heavily infiltrated with T cells it is difficult to understand how CsA inhibition of epidermal antigen-presenting cell function could be secondary to an effect of CsA on T cells. Instead, the simplest interpretation of this result is that CsA has a direct inhibitory effect on epidermal antigen-presenting cells independent of any potential effect on T cells. These studies by Cooper et al are complimentary to the in vitro studies mentioned above, and strongly support the contention that CsA inhibits epidermal antigen-presenting cells directly. The fact that the results of biochemical analysis of skin following CsA therapy fails to support this conclusion merely emphasizes that a biochemical approach is unlikely to resolve the controversy. It is easy to imagine that extremely small amounts of CsA, beyond the biochemical detection methods employed, are retained at critical sites within the epidermis where a direct effect on Langerhans cells is possible. For example, CsA may reside within the membranes or cytoplasm of epidermal Langerhans cells in amounts sufficient to suppress their functional activity, but insufficient to be detected by the biochemical methods used.

In their letter Cooper et al claim that elevated antigen-presenting cell activity in psoriatic lesions appears to be due to a non-Langerhans antigen-presenting subpopulation. The evidence in support of this statement [5] relies heavily on lack of CD1 expression by epidermal HLA-DR+ bone marrow–derived non–T cells in psoriatic skin. We feel that this reliance is ill-placed because Langerhans cells lose CD1 expression promptly after being placed in culture. We have proposed [6,7] that CD1– cells in psoriatic epidermis are the functional equivalents of cultured Langerhans cells, and our FACS analysis of psoriatic epidermal cells supports this proposal [4].

We agree with Cooper et al in the concluding statement of their letter that “the sensitivity (to CsA) of dermal Langerhans cells and related perivascular dendritic antigen-presenting cells remains to be determined, and these cells could well represent a relevant target of cyclosporine A in psoriasis.” We would merely reiterate that epidermal Langerhans cells are at least as relevant a target of CsA action, based on our own work [1,4], the work of Dupuy et al [2], the work of Furue and Katz [3], and the work of Cooper et al [5].

Aicha Demiden
Susan F. Grammer
J. Wayne Streilein
Department of Microbiology and Immunology
J. Richard Taylor
Department of Dermatology and Cutaneous Surgery
University of Miami School of Medicine
Miami, Florida

REFERENCES

