

Cyclosporine restores cytokine imbalance in childhood atopic dermatitis

Dianne E. Campbell, MBBS, FRACP, and Andrew S. Kemp, MBBS, FRACP, PhD
Melbourne, Australia

Childhood atopic dermatitis is associated with dysregulation of T-cell function, characterized by an imbalance in cytokine production in which T_{H2} (IL-4) responses predominate over T_{H1} (predominantly interferon- γ [INF- γ]) responses.¹ Marked improvement of atopic dermatitis has been reported after treatment with cyclosporine,² and although in vitro studies show that cyclosporine inhibits transcription of messenger RNA for both INF- γ and IL-4,³ its in vivo mechanism of action in atopic dermatitis is unclear.

We describe two highly atopic male children aged 6 years (case 1) and 12 years (case 2) whose atopic dermatitis was resistant to conventional therapy, including oral and pulse parenteral steroids. Serum IgE levels were extremely elevated at 27,000 IU/L and 41,600 IU/L, respectively (normal range, 0 to 200 IU/L). Administration of cyclosporine was started at 5 mg/kg daily, with significant reduction in extent and erythema of skin lesions within the first month of therapy in both cases. Concurrent topical therapy with emollients and non-fluorinated corticosteroids was unchanged over this period. Peak cyclosporine serum levels ranged from 370 to 756 μ g/ml, indicating adequate drug absorption and compliance with therapy. Cyclosporine dosage was reduced to 3 mg/kg in the third month of therapy in case 2, with some increase in erythema of affected skin.

METHODS

Peripheral blood mononuclear cells (PBMCs; 1×10^6 /ml) were obtained before and at 1, 2, and 3 months after onset of therapy by Ficoll (Pharmacia) density centrifugation of heparinized blood. In vitro production of INF- γ and IL-4 was examined after culture of PBMCs with staphylococcal enterotoxin B (SEB; Sigma Chemical Co., St Louis, Mo.) in serum-free media for 48 hours (AIM-V, Gibco). SEB was used because staphylococcal superantigens are considered to play a role in the cutaneous inflammation of atopic dermatitis, and we have previously shown SEB to be a potent stimulant for INF- γ production.⁴ Cytokine production was measured by sandwich ELISA of culture supernatants (stored at -20° C for <1 month) with anti-IL-4 and INF- γ monoclonal antibodies and recombi-

Abbreviations used

INF- γ :	Interferon- γ
PBMCs:	Peripheral blood mononuclear cells
SEB:	Staphylococcal enterotoxin B

nant human IL-4 and INF- γ (Pharmingen) as standard. Limits of detection for INF- γ and IL-4 were 10 pg/ml and 2 pg/ml, respectively.

RESULTS AND DISCUSSION

Before therapy, both patients showed low levels of INF- γ and high levels of IL-4 compared with normal children (Fig. 1). These cytokine profiles were in keeping with the children's highly atopic state and were similar to our previous reports of cytokine production by PBMCs obtained from children with moderate to severe atopic dermatitis after SEB and phytohemagglutinin stimulation.⁴ After onset of therapy, PBMCs from both subjects showed a marked increase in INF- γ production and a reduction in IL-4 production. The effect of the cyclosporine was to return in vitro production of INF- γ to levels in the range of those of nonatopic children and to cause a sustained reduction in IL-4 production in vitro. A similar effect was observed in case 1 in response to phytohemagglutinin: INF- γ and IL-4 were 771 pg/ml and 155 pg/ml (before treatment), 3637 pg/ml and 17 pg/ml (at 1 month), and 1776 pg/ml and 18 pg/ml (at 2 months), respectively. No pretreatment phytohemagglutinin stimulation was performed in case 2 because of small pretreatment blood sample. Eosinophil counts at onset of therapy and at 2 months into therapy were $3.3/3.02 \times 10^9$ /L (case 1) and $1.7/1.2 \times 10^9$ /L (case 2), respectively. No consistent pattern of change was observed in serum IgE, with the level in case 1 increasing to 48,856 IU/L and the level in case 2 decreasing to 32,569 IU/L after at least 1 month of cyclosporine therapy.

These findings indicate that cyclosporine may act in atopic dermatitis by promoting T_{H1} responses and/or by inhibiting T_{H2} responses. The enhancement of in vitro INF- γ production is somewhat surprising, given that cyclosporine has been shown in vitro to decrease transcription of INF- γ mRNA. This effect could be due to a differential effect of cyclosporine on particular T-cell functions or T-cell subtypes, as has been described in vitro in human⁵ and murine models.⁶ In these studies

From the Department of Immunology, Royal Children's Hospital, Melbourne.

Dianne E. Campbell was supported by an NHMRC postgraduate fellowship.

Reprint requests: D. E. Campbell, MBBS, FRACP, Department of Immunology, Royal Children's Hospital, Melbourne, Parkville, VIC 3052, Australia.

J Allergy Clin Immunol 1997;99:857-9.

Copyright © 1997 by Mosby-Year Book, Inc.

0091-6749/97 \$5.00 + 0 1/54/81428

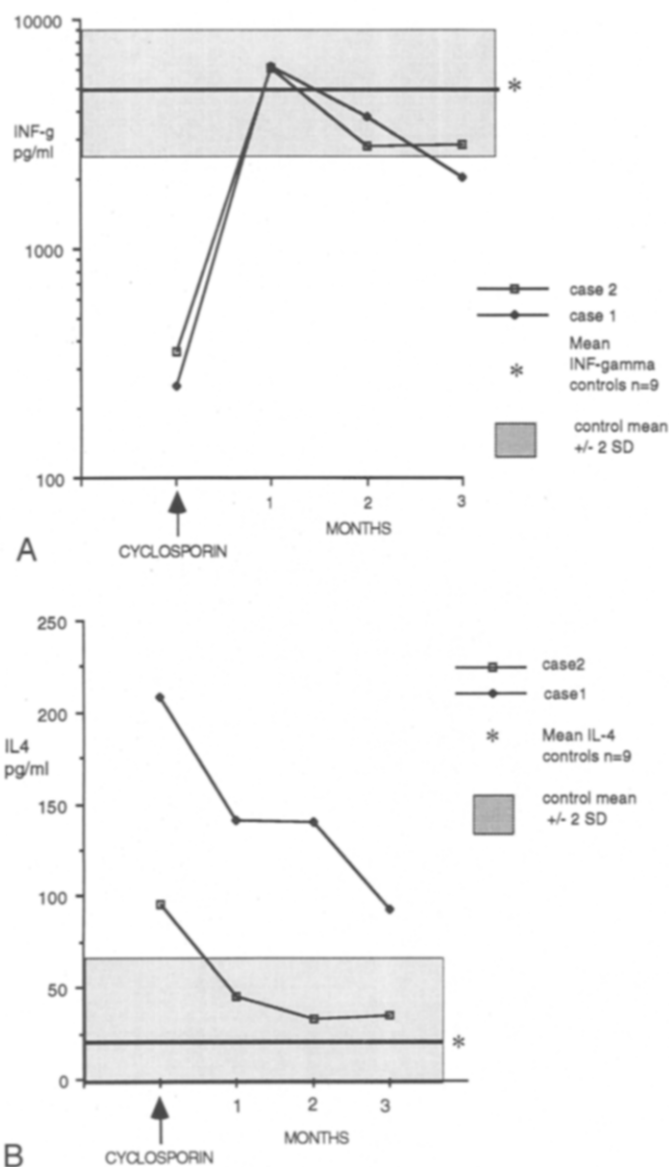


FIG. 1. IFN- γ (A) and IL-4 (B) production from 1×10^6 PBMCs from case 1 (male child, age 6 years) and case 2 (male child, age 12 years) after 48-hour culture with $1 \mu\text{g/ml}$ SEB. Cyclosporine dosage was reduced to 3 mg/kg in case 2 in the third month of therapy. All samples were analyzed in duplicate. *Bold line* represents mean of IFN- γ and IL-4 production from PBMCs (obtained from nine normal children), which were cultured under the same conditions. *Shaded area* represents 2 standard deviations from control mean.

cyclosporine has been shown to favor the differentiation of memory T cells⁶ and/or to not block IFN- γ production in the presence of effective T-cell costimulation.⁵ Alternatively, because IFN- γ and IL-4 are known to have reciprocal inhibitory effects, cyclosporine may act by decreasing the transcription of mRNA for IL-4, resulting in a secondary rise in IFN- γ . In this instance the inhibitory effect on IFN- γ produced by cyclosporine would be counteracted by removal of the inhibitory effects of IL-4. We believe this mechanism is less likely because decreased production of IFN- γ appears to be a fundamental defect in atopy, which precedes the devel-

opment of atopic dermatitis, and can be demonstrated in cord blood of infants who subsequently experience atopy,⁷ whereas IL-4 production has not been detected in supernatants from stimulated cord blood mononuclear cells. The effect of cyclosporine on in vitro cytokine production appears to be sustained over a 3-month treatment period; however, we do not yet know whether such alterations would be maintained after cessation of therapy. We propose that the unexpected elevation in IFN- γ production observed after cyclosporine treatment in patients with atopic dermatitis suggests a mechanism for its beneficial effect in this disorder.

REFERENCES

1. Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clin Exp Immunol* 1993;92:120-4.
2. Van Joost T, Heule F, Korstanje M, van den Boek MJ, Stenveld HJ, van Vloten WA, et al. Cyclosporin in atopic dermatitis: a multicentre placebo-controlled study. *Br J Dermatol* 1994;130:634-40.
3. Andersson J, Nagy S, Groth CG, Andersson U. Effects of FK506 and cyclosporin A on cytokine production studied in vitro at a single cell level. *Immunology* 1992;75:136-42.
4. Campbell DE, Kemp AS. Proliferation and production of INF- γ and IL4 in response to *Staphylococcus aureus* and staphylococcal superantigen in childhood atopic dermatitis. *Clin Exp Immunol* 1997;107:392-5.
5. Kubin M, Kamoun M, Trinchieri G. Interleukin 12 synergizes with B7/CD28 interaction in inducing efficient proliferation and cytokine production of human T cells. *J Exp Med* 1994;180:211-22.
6. Bemer V, Motta I, Perret R, Truffa-Bachi P. Interleukin-2 down modulates memory T helper lymphocyte development during antigenic stimulation in vitro. *Eur J Immunol* 1995;25:3394-401.
7. Tang MLK, Kemp AS, Thornburn J, Hill DJ. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994;344:983-5.