

Standardized extracts from Chinese herbs induce IL-10 production in human monocyte-derived dendritic cells and alter their differentiation in vitro

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Background: The efficacy of traditional Chinese medicine (TCM) as a treatment for atopic dermatitis has been evaluated in clinical trials. Until now, the underlying mechanism of this treatment has remained completely elusive; this is particularly true of its putative effects on dendritic cells (DCs), which might play a pivotal role in the disease.

Objective: We investigated the influence of a standardized extract from 10 Chinese herbs that was successfully used in clinical trials on the generation of monocyte-derived DCs from atopic donors.

Methods: Detailed phenotypic and functional exploration of DCs generated in the presence of IL-4 and GM-CSF and treated with different concentrations of TCM or a placebo control was performed.

Results: TCM profoundly affected the morphology and phenotype of the developing DCs. They lost their typical dendritic morphology and decreased their expression of CD1a as well as the low-affinity IgE receptor CD23. Most importantly, TCM-exposed DCs exhibited a diminished stimulatory activity toward autologous antigen-specific and allogeneic T cells while secreting high amounts of IL-10.

Conclusion: TCM induces immunopharmacologic alterations on DCs from atopic donors in vitro. These alterations might account, at least in part, for the therapeutic effect of this treatment in AD in vivo. (J Allergy Clin Immunol 2001;108:588-93.)

Key words: Dendritic cells, atopic dermatitis, Chinese herbal therapy, CD23, interleukin 10

Traditional Chinese medicine (TCM) predates the interest currently being taken in it in Western countries by more than 2000 years. At present, data from double-blind, placebo-controlled trials of TCM for atopic dermatitis (AD) were exclusively obtained with a formulation of 10 standardized herbs (Zemaphyte, Phytopharm plc, London, United Kingdom).¹⁻⁸ On the basis of current

Abbreviations used

AD: Atopic dermatitis
APC: Antigen-presenting cell
DC: Monocyte-derived dendritic cell
sCD23: Soluble CD23
TCM: Traditional Chinese medicine
TT: Tetanus toxin

knowledge, different modes of action of the single components have been proposed.^{3,8,9} The formulation, ingested as tea, led to a significant decrease in surface damage to the patients' skin in most of the clinical studies⁶⁻⁸; this was in contrast to the findings of other authors, who did not detect any significant improvement of the disease in patients with recalcitrant AD.^{10,11}

AD, a chronic skin disorder with numerous cellular and humoral defects, is often associated with other allergic diseases, such as hay fever and asthma.¹² Inasmuch as the etiology of AD is only partially understood, treatment is empirical and is based on the use of topical steroids aimed at reducing the skin inflammation.¹³ In recent years, alternative approaches have been used to target specific defects in AD,¹⁴⁻¹⁶ and there has been considerable interest in TCM as a treatment.^{1-8,15,16}

Because they express the high-affinity receptor for IgE (FcεRI) and exhibit strong stimulatory activity, it is assumed that antigen-presenting cells (APCs), particularly monocyte-derived dendritic cells (DCs), play a pivotal role in the pathophysiology of allergic diseases such as AD.¹⁷⁻²³ Thus, inasmuch as the mode of action of TCM remains completely elusive, we postulated that TCM could act on DCs in AD, and we investigated its impact on immunocompetent DCs of atopic donors and healthy volunteers in vitro.

MATERIAL AND METHODS

Reagents

The mAb W6/32 against the MHC class I-producing hybridoma cell line was kindly provided by Dr N. Koch (Institute of Immunobiology, University of Bonn, Germany). The anti-CD1a mAb (T6RD1)(IgG1) was from Coulter Clone (Miami, Fla). The mAbs against CD80 (BB1)(IgG1), CD86 (IT2.2)(IgG2b), and CD40 (5C3)(IgG1) and the PE-labeled mAbs against IL-8, IL-10, and their isotype-matched controls were from Pharmingen (Hamburg, Germany). The mAbs reacting with CD23 (9P25)(IgG1) and HLA-DR (BL2)(IgG2b) were from Immunotech (Marseille, Cedex,

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France). F(ab')² goat antimouse IgG (GaM-IgG) was from Jackson Immuno-Research Laboratories (West Grove, Pa), and mouse and rat sera were from Dianova (Hamburg, Germany). FITC-labeled mAbs against CD1a and CD14 were from Becton-Dickinson (San Jose, Calif). The neutralizing antibody against IL-10 mAb and IL-1 β mAb was from R&D Systems (Minneapolis, Minn). IL-4 was from Life Technologies (Eggenstein, Germany), and GM-CSF was from Genzyme (Cambridge, Mass). Final maturation was obtained by culturing the cells in the presence of IL-1 β (10 ng/mL; R&D Systems, Wiesbaden, Germany), IL-6 (1000 U/mL; R&D Systems), and TNF- α (10 ng/mL, R&D Systems).

Preparation of extract and placebo control

The TCM used in this investigation was identical to that used in a double-blinded, placebo-controlled clinical trial published previously (trade name, Zemaphyte P01P [97/116/C171]) and was a gift from Phytopharm plc. The placebo control (lot no. 0355R) was also obtained from Phytopharm plc and was tested for in vitro assay use. The granules were dissolved in VLE-1640 RPMI Medium (Biochrom, Berlin, Germany) in increasing concentrations (0.0001 to 0.1 mg/mL). TCM was added at the beginning of culture and supplemented on days 2 and 4 of culture, as the medium was changed and fresh cytokines were added at these time points.

The endotoxin level (in EU/mL) of the stock solution of TCM and the placebo control were tested through use of the Limulus amoebocyte-lysate E-Toxate test (Sigma, St Louis, Mo) according to the manufacturer's protocol. The presence of either phytosteroids or human steroids in those extracts was excluded by detailed HPLC analysis (data not shown).

In vitro generation of DCs

A total of 15 patients with AD and 15 healthy volunteers were recruited according to the criteria of Hanifin and Rajka²⁴; each of the patients had an elevated IgE serum level (>150 kU/L). The protocol was approved by the local ethics committee.

Monocytes were isolated from peripheral blood by density gradient centrifugation, and the purity of the cell preparation was evaluated by flow cytometric analysis through use of anti-CD14 staining (>85% viable monocytes). Monocytes were resuspended in cell culture medium consisting of VLE-RPMI with Glutamax supplemented with 10% heat-inactivated FCS (Gibco BRL, Eggenstein, Germany) and 1% antibiotic/antimycotic (Gibco BRL). Finally, IL-4 (500 U/mL) and GM-CSF (500 U/mL) were added to generate DCs. Every second day, one half of the medium was removed and replaced with fresh medium containing both cytokines in equal amounts, as described above.

Immunolabeling and flow cytometric analysis

Immunolabeling for phenotyping was performed as reported previously.²⁰ Intracellular staining for cytokines was carried out with a Cytofix/Cytoperm kit (Pharmingen), used according to the manufacturer's instructions. After washing with PBS, cells were analyzed on a FACSCalibur (Becton-Dickinson), as described in detail elsewhere.²¹ Results are expressed as percents of positive cells and in terms of relative fluorescence intensity (rFI), calculated as follows:

$$rFI = (MFI_{\text{structure}} - MFI_{\text{control}}) / MFI_{\text{control}}$$

where *MFI* is the mean fluorescence intensity.

Proliferation assays

Heparinized blood was mixed with PBS (Gibco BRL; 1:1), and PBMCs were collected by Lymphoprep (Nycomed, Oslo, Norway) density centrifugation and then resuspended in RPMI-1640 Medium (Gibco BRL) containing 10% heat-inactivated FCS (Gibco BRL), 1%

antibiotic/antimycotic (Gibco BRL), 1% mercaptoethanol (Sigma), and 2 mmol/L L-glutamine; they were then washed twice. T cells were isolated by filtration through a 5-mL nylon wool column (45 minutes of incubation at 37°C). The purity of the T-cell preparations was evaluated by flow cytometric analysis through use of anti-CD3 staining and was consistently greater than 85%. For antigen-specific proliferation, DCs generated in the presence or absence of TCM were cultured for 6 days, then washed 3 times and seeded with autologous or allogeneic T cells at 2×10^4 (1:1) cells per well in 96-well culture plates at 37°C for 5 days with the following allergens: *Betula alba* (Bet v 1; Bencard, Munich, Germany) or recombinant Bet v 1 (1 to 10 μ g/mL; a kind gift of Dr R. Valenta, Department of Pathophysiology, University of Vienna, Austria) and tetanus toxin (TT; 10 μ g/mL; Boehringer Mannheim, Mannheim, Germany). Then 20 μ L ³H-thymidine (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) was added to each well and the preparations were incubated for another 16 hours. Culture plates were harvested, and the incorporated radioactivity was measured in a liquid scintillation counter. Relative stimulation indices (rSI) were calculated as follows:

$$rSI = (cpm_{\text{SMLR}} - cpm_{\text{T cell}}) / cpm_{\text{T cell}}$$

where *cpm* is counts per minute.

Detection of soluble CD23 and IL-10

Cell culture supernatants of DCs were tested at different time points for the presence of soluble CD23 (sCD23) through use of a sandwich ELISA (Pharmingen), according to the manufacturer's instructions. For IL-10 ELISA, analysis of supernatants was carried out in triplicate according to the manufacturer's instructions (R&D Systems, Minn). An EliSpot assay (Mabtech, Nacka, Sweden) for IL-10 was performed according to the manufacturer's protocol; quantitative analysis was done with an EliSpot reader (Biosys GmbH, Karben, Germany).

Amplification of mRNA and analysis of transcripts

Total RNA was extracted after 12 hours of culture from highly purified DCs through use of Trizol (Life Technologies) according to the manufacturer's instructions. RT reactions were performed through use of 1 μ g of total RNA. Denaturation at 94°C for 40 seconds was followed by annealing of the primers at 55°C for 30 seconds and extension at 72°C for 30 seconds. A final extension phase of 5 minutes was added. Specific primer sequences for each gene were as follows:

human β -Actin: sense, 5'-GAG CGG GAA ATC GTG CGT GAC ATT-3'; antisense, 5'-GAT GGA GTT GAA GGT AGT TTC GTG-3', yielding a fragment of 240 bp

human IL-10: sense, 5'-AAC TGA GAC ATC AGG GTG GC-3'; antisense, 5'-AAA GCG TGG TCA GGC TTG-3' (fragment of 384 bp).

Amplification was performed on a Perkin-Elmer Gene Amp PCR System 9600 thermocycler (Applied Biosystems, Weiterstadt, Germany). The PCR cycle numbers for the amplification of the respective cDNAs were 25 for β -Actin and 35 for IL-10. Specific PCR fragments were separated on a 1% agarose gel and visualized through use of ethidium bromide staining. The PCR products were evaluated semiquantitatively by comparing the ratio of the specific products and the β -Actin band by digital image analysis through use of the WinCam system (Cybertech, Berlin, Germany).

Statistical analysis

Statistical differences were determined by means of a paired Student *t* test through use of SPSS for Windows 8.5. A *P* value of less than .05 was considered statistically significant.

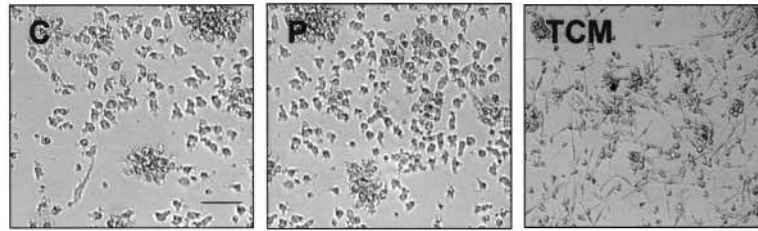


FIG 1. Morphologic alterations induced by TCM. Shown are cells cultured under control (C) or placebo (P) conditions and cells cultured with TCM (TCM). Magnification, 20 $\times$; bar, 100 μ m.

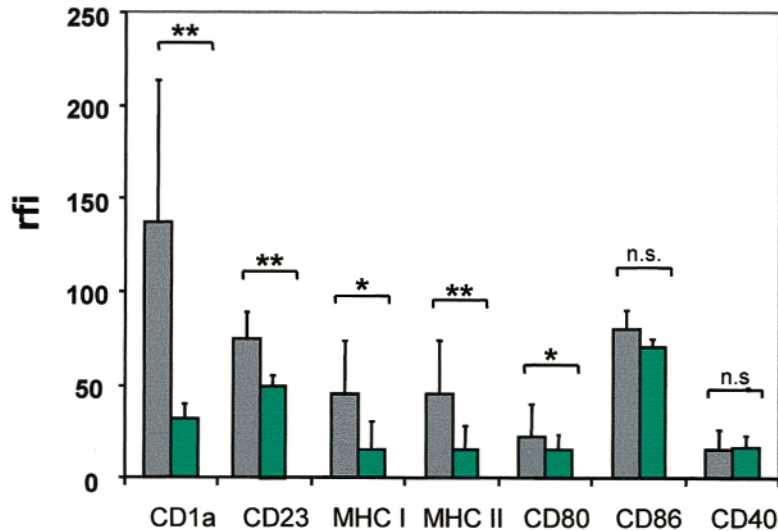


FIG 2. Influence of TCM on the phenotype of in vitro-generated DCs. The expression of the surface marker is shown for control cells cultured with placebo extract in comparison with DCs cultured with TCM for 6 days. The expression is indicated as rFI $\pm$ SD on the y-axis; n = 30. * P < .05. ** P < .001. n.s., Not significant.

RESULTS

TCM induces profound morphologic and phenotypic alterations in DC

DCs were generated in the presence of GM-CSF + IL-4, and TCM or placebo (both at 0.1 mg/mL) was added. In the presence of TCM, cells failed to display the typical cluster formation of DCs in the suspension. In contrast, the formation of adherent cells with elongated pseudopods and a fusiform body was observed. Under control conditions with or without placebo extracts for 6 days, DCs became nonadherent and clustered, showing the typical dendritic cell morphology (Fig 1).

Similarly, the immunophenotype of DCs was significantly altered in the presence of TCM. DCs generated with the placebo extract or under control conditions expressed high levels of CD1a ($92.4\% \pm 8.6\%$; rFI = 137.4 ± 76 ; Fig 2). In contrast, those generated in the presence of TCM expressed significantly lower levels of CD1a ($75.9\% \pm 9\%$; rFI = 31.8 ± 17.9). Addition of TCM after 48 to 72 hours, when most of the DCs already expressed CD1a, still led to a significant decrease in the CD1a surface expression (from an rFI value of $112 \pm$

70.2 in the control condition to an rFI value of 41 ± 9). However, this downregulatory effect on CD1a expression could not be achieved by addition of TCM at later time points of culture—ie, after day 4.

Upregulation of MHC I/II on the cell surface is a hallmark during the differentiation from monocytes into DCs. Cells generated in the presence of TCM exhibited a significantly reduced expression of MHC I in comparison with DCs generated with the placebo extract (rFI = 15.3 ± 14.4 and rFI = 44.7 ± 29.6 , respectively). Similarly, TCM led to a highly significant reduction of MHC II expression on day 6 in comparison with DCs generated with the placebo extract (rFI = 15.0 ± 12.5 and rFI = 45.2 ± 28 , respectively). In addition, the expression of the costimulatory molecule CD80 was significantly downregulated with TCM in comparison with the placebo control (rFI = 22 ± 17 and rFI = 16 ± 7.4 , respectively), whereas expression of CD86 and CD40 remained unaffected by TCM treatment (Fig 2). However, there were no significant differences in phenotype and function among control cells, placebo-cultured cells, and TCM-cultured cells when DCs were fully differentiated in the presence of IL-1 β , IL-6, and TNF- α on day 10.

Downregulation of IL-4 induced CD23 expression of TCM-treated DCs

The low-affinity receptor for IgE CD23 is expressed to a certain level on skin APCs of patients with AD. Because a downregulatory effect of TCM on the surface expression of CD23 on skin APCs has been reported *in vivo*, we wondered whether TCM might affect the CD23 expression of *in vitro*-generated DCs. Indeed, TCM significantly downregulated the IL-4-induced surface expression of CD23 on DCs (TCM, $49.3\% \pm 5\%$, $rFI = 9 \pm 6.4$; placebo, $74.8\% \pm 19\%$, $rFI = 23 \pm 14$; $n = 22$). This effect was dose-dependent and especially highly significant at early time points of culture (24–72 hours; data not shown). Interestingly, in contrast to the downmodulation of surface-bound CD23, the concentration of sCD23 in the supernatant was not altered by TCM (33.75 ± 15.2 ng/mL and 34.0 ± 12.4 ng/mL in the supernatants of the control and placebo-cultured DCs, respectively, versus 31.75 ± 16.7 ng/mL in the supernatant of the TCM-cultured DCs); this ruled out the possibility of an increased shedding and production of sCD23 under these conditions.

Cytokine production of DCs cultured with TCM

DCs have been assumed to be one of the cell types responsible for secreting cytokines that are responsible for initiating the inflammatory reaction and/or pivotal during the initial steps of the primary or secondary T-cell response. We therefore investigated the capacity of TCM to interfere with the cytokine production of DCs generated from monocytes from patients with AD. For this purpose, we analyzed the intracellular production of the proinflammatory cytokines IL-6, IL-8, and IL-12 and the anti-inflammatory cytokine IL-10, all of which are produced during the maturation process of DCs. Whereas the production of IL-6, IL-8, and IL-12 was not significantly altered by TCM (data not shown), the IL-10 production of cells cultured with TCM was significantly upregulated (TCM, 354 ± 25 pg/mL; placebo, 55 ± 44 pg/mL; $n = 6$) and confirmed by ELISpot assay (Fig 3, A and B). Most interestingly, the increased IL-10 production induced by TCM could be detected only in atopic donors; in nonatopic donors, IL-10 production was not altered by TCM. This was confirmed at the molecular level by an increased mRNA expression of IL-10 detected after 12 hours of culture with TCM in atopic donors, as revealed by semiquantitative PCR analysis (Fig 3, C).

TCM alters the stimulatory activity of DCs generated *in vitro*

A characteristic feature of DCs is their capacity to stimulate naive T cells, as seen in the model of allogeneic mixed lymphocyte reaction. To investigate whether the observed phenotypic alterations have any functional relevance, we tested the stimulatory activity of normal, placebo-treated, and TCM-treated DCs. As shown in Fig 4, A, compared with DCs generated under control conditions or with placebo, TCM-treated DCs exhibited a sig-

nificantly reduced stimulatory activity toward allogeneic T cells ($n = 5$). Most interestingly, when the endogenously synthesized IL-10 was neutralized by anti-IL-10 mAb, the suppressive property of TCM was partially reversed (Fig 4, B). As a control, the addition of anti-IL-1 β mAb and anti-IL-10 mAb to the standard condition did not reveal any effect on the phenotype and function of the DCs.

To evaluate whether TCM might exert similar downregulatory activity on DCs in an autologous antigen-specific T-cell response, monocytes were obtained from atopic donors sensitized to birch pollen—ie, exhibiting specific IgE for this allergen. All of these patients had also been vaccinated against tetanus. DCs were generated with or without TCM, and Bet v 1 or TT was added as an antigen in the culture system. Intriguingly, TCM failed to alter the stimulatory activity of TT-loaded DCs, whereas Bet v 1-loaded DCs were significantly impaired in their capacity to stimulate antigen-specific T cells (Fig 4, C; $n = 5$).

DISCUSSION

In the present study, we investigated the *in vitro* effect of TCM on human DCs, which are considered to be important in the pathophysiology of numerous inflammatory and allergic diseases. We have shown that (1) culture with TCM profoundly alters the morphology and phenotype of DCs, (2) TCM downregulates the expression of classic MHC I and MHC II and nonclassic MHC CD1a, as well as the expression of CD80 and the IL-4-induced CD23 surface expression on DCs, (3) the stimulatory activity of TCM-exposed DCs of atopic donors is significantly reduced, and (4) these functional modulations could result from an increased TCM-induced IL-10 production of DCs from atopic donors.

One of the main features of AD is an elevated serum IgE level and an increased expression of the high-affinity and low-affinity IgE receptors on the surfaces of APCs, including monocytes and epidermal Langerhans cells.^{23,25–27} CD23 exists in 2 forms: type A is constitutively expressed on B cells; type B can be induced by IL-4 on a variety of cells, including DCs.²⁸ In this study, we were able to show a significant downregulation of IL-4-induced CD23 surface expression on DCs by TCM, whereas the level of sCD23 measured in the cell culture supernatant remained unaffected. These findings suggest a modulatory effect of TCM at the transcriptional or translational level rather than induction of CD23 shedding.²⁹ Interestingly, our *in vitro* data are in accordance with the finding that CD23 is downregulated on APCs in the skin of TCM-treated patients.³

With regard to the role of IL-4 as a key cytokine in atopic disorders, our observations might be of *in vivo* relevance. In this context, it has been postulated that either anti-IL-4 strategies (eg, anti-IL-4 mAb)³⁰ or any strategy aimed at neutralizing IgE (eg, omalizumab) would arrest the efferent cellular response in AD.³¹ Given that the surface expression of Fc ϵ RI is known to be rapidly down-

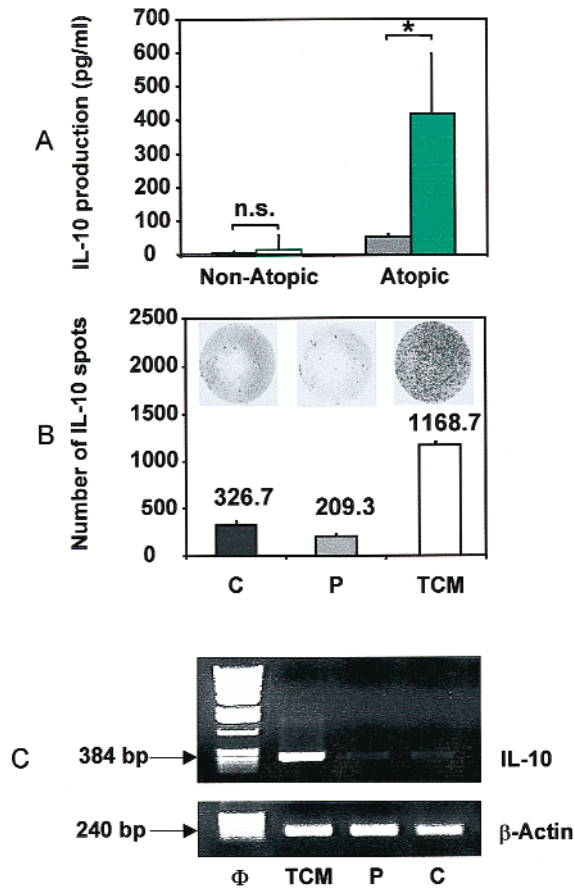


FIG 3. TCM induces IL-10 production. **A**, DCs from atopic and nonatopic donors were cultured in the presence or absence of TCM. After 24 hours, the culture supernatants were analyzed by an IL-10-specific ELISA according to the manufacturer's protocol. Values are expressed as means $\pm$ SDs from 6 independent experiments. * $P < .05$. n.s., Not significant. **B**, The number of IL-10-producing DCs after 18 hours of culture is measured in an ELISpot assay. The number of IL-10-producing, TCM-treated DCs is indicated on the y-axis; $n = 5$. **C**, Influence of TCM on the IL-10 mRNA level after 12 hours of culture.

regulated on in vitro-generated DCs, no reliable conclusion about the influence of TCM on FcεRI expression on DCs can be drawn at the moment.²¹ TCM profoundly affected the morphology and phenotype of the developing cells, leading to a marked decrease of CD1a expression. This reduction in CD1a-positive cells correlates with the reported decrease in such cells in skin biopsies from patients with AD who were treated with TCM.³ Most interestingly, the downregulatory effect of TCM on CD1a expression could also be induced when TCM was added to the culture on day 3, when most of the cells (>90%) already expressed CD1a. However, the addition of TCM at later time points—ie, after day 4—did not alter maturation of the cells. The MHC I and II molecules on the surfaces of the DCs were significantly downregulated by TCM in vitro, and downmodulation of the MHC II molecules on skin APCs has also been shown in vivo.³ TCM did not have any significant effect on the phenotype and

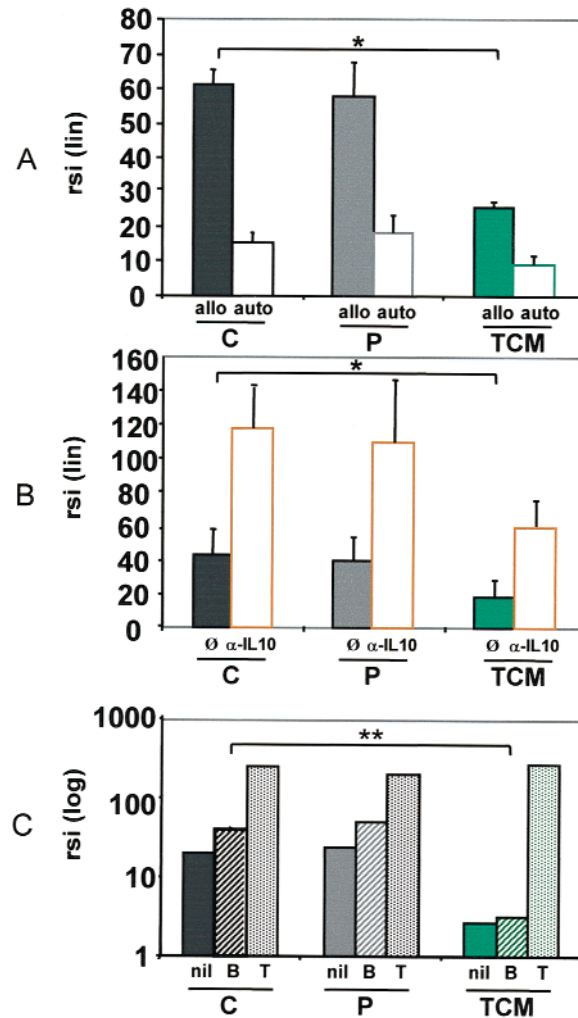


FIG 4. Suppression of the stimulatory activity of cells generated in the presence of TCM. **A**, DCs were cultured with or without TCM, washed, and then cocultured with purified responder autologous or allogeneic T lymphocytes. Values are expressed as linear (lin) relative stimulation indices (rsi) $\pm$ SDs of triplicate cultures; $n = 4$. * $P < .05$. **B**, Suppressive effect of TCM in allogeneic MLR is impaired by IL-10 neutralization. DCs from atopic donors were cultured in the presence of 10 μ g/mL anti-IL-10 mAb for 6 days and then used as stimulator cells. **C**, For antigen-specific proliferation assays, autologous T cells were seeded with DCs without any antigen (nil) or with *Betula alba* (B) 10 μ g/mL and tetanus toxin (T). Values are expressed as logarithmic (log) relative stimulation indices (rsi) $\pm$ SDs on the y-axis; $n = 5$. ** $P < .01$.

functional properties of fully mature DCs. This implies that most of the modulating properties of TCM in vitro could be achieved at the early maturation stage of DCs. IL-10 is known to modulate the differentiation of the cells at early stages—ie, within 48 hours of culture—whereas at later time points DCs seem to be insensitive to IL-10.

Our results demonstrate an impaired stimulatory capacity of DCs generated in the presence of TCM toward allogeneic and antigen-specific autologous T cells. Interestingly, TCM failed to significantly influence the stimulation of TT-specific T cells, whereas the prolifer-

eration of Bet v 1-specific T cells was dramatically reduced. This suggests that TCM might cause changes in the priming and expansion of different subsets of CD4⁺ T cells without leading to a general immunosuppression.

These results further indicate that the modulation of the immune response achieved by TCM treatment is a rather specific one. We have shown that TCM induces IL-10 production in DCs generated from monocytes of atopic donors and that most of the TCM-induced alterations described herein can be restored by anti-IL-10. Most interestingly, TCM-induced IL-10 production was observed only in DCs derived from atopic donors. This could explain, at least in part, the particular effectiveness of TCM in the management of patients with AD in clinical trials. With regard to the pivotal role of IL-10 in the induction of immune tolerance and its putative role in the so-called modified T_H2 response, our findings are of primary interest for understanding the mechanism of action of this therapy. Furthermore, inasmuch as IL-10-treated human DCs display antigen-specific suppressor activity by the induction of anergic T cells,³²⁻³⁴ this might be one mode of action of TCM *in vivo*.

To our knowledge, this is the first report showing a profound alteration of DCs induced by TCM on cells from atopic individuals. It suggests that TCM-induced IL-10 production could represent an important mode of action of this therapy.

REFERENCES

- Latchman Y, Banerjee P, Poulter LW, Rustin M, Brostoff J. Association of immunological changes with clinical efficacy in atopic eczema patients treated with traditional Chinese herbal therapy (Zemaphyte). *Int Arch Allergy Immunol* 1996;109:243-9.
- Banerjee P, Xu XJ, Poulter LW, Rustin MH. Changes in CD23 expression of blood and skin in atopic eczema after Chinese herbal therapy. *Clin Exp Allergy* 1998;28:306-14.
- Latchman Y, Whittle B, Rustin M, Atherton DJ, Brostoff J. The efficacy of traditional Chinese herbal therapy in atopic eczema. *Int Arch Allergy Immunol* 1994;104:222-6.
- Xu XJ, Banerjee P, Rustin MH, Poulter LW. Modulation by Chinese herbal therapy of immune mechanisms in the skin of patients with atopic eczema. *Br J Dermatol* 1997;136:54-9.
- Atherton DJ, Sheehan MP, Rustin MH, Whittle B, Guy G. Treatment of atopic eczema with traditional Chinese medicinal plants. *Pediatr Dermatol* 1992;9:373-5.
- Sheehan MP, Rustin MH, Atherton DJ, Buckley C, Harris DW, Brostoff J, et al. Efficacy of traditional Chinese herbal therapy in adult atopic dermatitis. *Lancet* 1992;340:13-7.
- Atherton DJ, Sheehan MP, Rustin M. Traditional Chinese plants for eczema. *Lancet* 1991;338:510.
- But P, Chang C. Chinese herbal medicine in the treatment of asthma and allergies. *Clin Rev Allergy Immunol* 1996;14:253-69.
- Tamaya T, Sato S, Okada HH. Possible mechanism of steroid action of the plant herb extracts glycyrrhizin, glycyrrhetic acid, and paeoniflorin: inhibition by plant herb extracts of steroid protein binding in the rabbit. *Am J Obstet Gynecol* 1986;155:1134-9.
- Fung AY, Look PC, Chong LY, But PP, Wong E. A controlled trial of traditional Chinese herbal medicine in Chinese patients with recalcitrant atopic dermatitis. *Int J Dermatol* 1999;38:387-92.
- Armstrong NC, Ernst E. The treatment of eczema with Chinese herbs: a systematic review of randomized clinical trials. *Br J Clin Pharmacol* 1999;48:262-4.
- Akdis CA, Akdis M, Trautmann A, Blaser K. Immune regulation in atopic dermatitis. *Curr Opin Immunol* 2000;12:641-6.
- Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000;105:860-76.
- Panhans-Groß A, Novak N, Kraft S, Bieber T. Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (FK506). *J Allergy Clin Immunol* 2001;107:345-52.
- Sheehan MP, Stevens H, Ostlere LS, Atherton DJ, Brostoff J, Rustin MH. Follow-up of adult patients with atopic eczema treated with Chinese herbal therapy for 1 year. *Clin Exp Dermatol* 1995;20:136-40.
- Sheehan MP, Atherton DJ. One-year follow up of children treated with Chinese medicinal herbs for atopic eczema. *Br J Dermatol* 1994;130:488-93.
- Bieber T. Role of Langerhans cells in the physiopathology of atopic dermatitis. *Pathol Biol (Paris)* 1995;43:871-5.
- Bieber T. Fc epsilon RI on antigen-presenting cells. *Curr Opin Immunol* 1996;8:773-7.
- Novak N, Haberkost J, Geiger E, Bieber T. Dendritic cells in allergy. *Allergy* 1999;54:792-803.
- Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin-4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 1994;179:1109-18.
- Kraft S, Wessendorf JH, Hanau D, Bieber T. Regulation of the high affinity receptor for IgE on human epidermal Langerhans cells. *J Immunol* 1998;161:1000-6.
- Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema. *J Invest Dermatol* 1996;106:446-53.
- Bieber T. Fc epsilon RII/CD23 on epidermal Langerhans' cells. *Res Immunol* 1992;143:445-7.
- Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol Suppl (Stockh)* 1980;92:44.
- Takigawa M, Tamamori T, Horiguchi D, Sakamoto T, Yamada M, Yoshioka A, et al. Fc epsilon receptor II/CD23-positive lymphocytes in atopic dermatitis. I. The proportion of Fc epsilon RII+ lymphocytes correlates with the extent of skin lesion. *Clin Exp Immunol* 1991;84:275-82.
- Dickensheets HL, Donnelly RP. IFN-gamma and IL-10 inhibit the induction of IL-1 receptor type I and II gene expression by IL-4 and IL-13 in human monocytes. *J Immunol* 1997;158:6226-33.
- Muller KM, Rocken M, Joel D, Bonnefoy JY, Saurat JH, Hauser C. Mononuclear cell-bound CD23 is elevated in both atopic dermatitis and psoriasis. *J Dermatol Sci* 1991;2:125-33.
- Gosset P, Lamblin-Degros C, Tillie-Leblond I, Charbonnier AS, Joseph M, Wallaert B, et al. Modulation of high-affinity IgE receptor expression in blood monocytes: opposite effect of IL-4 and glucocorticoids. *J Allergy Clin Immunol* 2001;107:114-22.
- Morinobu A, Kumagai S, Yanagida H, Ota H, Ishida H, Matsui M, et al. IL-10 suppresses cell surface CD23/Fc epsilon RII expression, not by enhancing soluble CD23 release, but by reducing CD23 mRNA expression in human monocytes. *J Clin Immunol* 1996;16:326-33.
- Park JS, Choi IH, Lee DG, Han SS, Ha TY, Lee JH, et al. Anti-IL-4 monoclonal antibody prevents antibiotics-induced active fatal anaphylaxis. *J Immunol* 1997;158:5002-6.
- Holgate ST, Corne J, Jardieu P, Fick RB, Heusser CH. Treatment of allergic airways disease with anti-IgE. *Allergy* 1998;53:83-8.
- Pretolani M, Goldman M. IL-10: a potential therapy for allergic inflammation? *Immunol Today* 1997;18:277-80.
- Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J Immunol* 2001;166:4312-8.
- Joss A, Akdis M, Faith A, Blaser K, Akdis CA. IL-10 directly acts on T cells by specifically altering the CD28 co-stimulation pathway. *Eur J Immunol* 2000;30:1683-90.