Andrographolide Interferes with T Cell Activation and Reduces Experimental Autoimmune Encephalomyelitis in the Mouse

Mirentxu I. Iruretagoyena, Jaime A. Tobar, Pablo A. González, Sofía E. Sepúlveda, Claudio A. Figueroa, Rafael A. Burgos, Juan L. Hancke, and Alexis M. Kalergis

Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Chile (M.I., J.A.T., P.A.G., S.E.S., C.A.F., A.M.K.); and Instituto de Farmacología, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Chile (R.A.B., J.L.H.)

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ABSTRACT

Andrographolide is a bicyclic diterpenoid lactone derived from extracts of Andrographis paniculata, a plant indigenous to South Asian countries that shows anti-inflammatory properties. The molecular and cellular bases for this immunomodulatory capacity remain unknown. Here, we show that andrographolide is able to down-modulate both humoral and cellular adaptive immune responses. In vitro, this molecule was able to interfere with T cell proliferation and cytokine release in response to allogenic stimulation. These results were consistent with the observation that T cell activation by dendritic cells (DCs) was completely abolished by exposing DCs to andrographolide during antigen pulse. This molecule was able to interfere with maturation of DCs and with their ability to present antigens to T cells. Furthermore, in vivo immune responses such as antibody response to a thymus-dependent antigen and delayed-type hypersensitivity were drastically diminished in mice by andrographolide treatment. Finally, the ability of andrographolide to inhibit T cell activation was applied to interfere with the onset of experimental autoimmune encephalomyelitis (EAE), an inflammatory demyelinating disease of the central nervous system that is primarily mediated by CD4+ T cells and serves as an animal model for human multiple sclerosis. Treatment with andrographolide was able to significantly reduce EAE symptoms in mice by inhibiting T cell and antibody responses directed to myelin antigens. Our data suggest that andrographolide is able to efficiently block T cell activation in vitro, as well as in vivo, a feature that could be useful for interfering with detrimental T cell responses.

Andrographis paniculata is a plant indigenous to South-east Asian countries that has been used as an official herbal medicine in China for many years. Whole-plant extracts have been used as a popular remedy for the treatment of various disorders and recently shown to have antitumoral (Rajagopal et al., 2003), anti-inflammatory (Gabrielian et al., 2002), and antiviral properties (Calabrese et al., 2000). Diterpenoid chemicals are the primary constituents present in the extracts of A. paniculata, where andrographolide, a bicyclic diterpenoid lactone, is the major constituent. Andrographolide has been reported to be particularly efficient at regulating immune responses (Calabrese et al., 2000; Rajagopal et al., 2003). This molecule has recently been shown to work as an anti-inflammatory agent by reducing the generation of reactive oxygen species in human neutrophils (Shen et al., 2002), as well as preventing microglia activation (Wang et al., 2004). However, the molecular and cellular mechanisms responsible for the immunomodulatory properties of andrographolide remain unknown, as well as the potential in vivo anti-inflammatory effects resulting from treatment with this drug.

Andrographolide could exert its immunomodulatory effects at different levels on the immune system. Considering that dendritic cells (DCs) play an important role in regulating adaptive immune responses, they represent a potential target for andrographolide. DCs are professional antigen-presenting cells (APCs) that have the unique abil-

ABBREVIATIONS: DC, dendritic cell; APC, antigen-presenting cell; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; MOG, mouse myelin oligodendrocyte glycoprotein; PBS, phosphate-buffered saline; OVA, chicken egg ovalbumin; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin; NP-BSA, 4-hydroxy-3-nitrophenyl-acetyl conjugated to bovine serum albumin; NP-CGG, 4-hydroxy-3-nitrophenyl-acetyl conjugated to chicken gamma globulin; DTH, delayed-type hypersensitivity.
ity to capture and present antigens to prime naive CD4+ and CD8+ T cells and are critical for the initiation of the adaptive immune response against infectious agents (Banchereau et al., 2000; Lanzavecchia and Sallusto, 2001). DCs have also been recently implicated in the pathogenesis of several autoimmune diseases (Hart and van Kooyk, 2004; Waldner et al., 2004). Besides their capacity to initiate adaptive immune response, DCs can also control immunity through their ability to induce antigen-specific lymphocyte unresponsiveness or tolerance to self-antigens (Yamazaki et al., 2003). Given the central role that DCs play as regulators of adaptive immunity, they represent interesting therapeutic targets for pharmacological modulation of immune responses. Recent evidence indicates that several established immunosuppressive drugs could interfere with immune responses by altering DC activity (Hackstein and Thomson, 2004). Thus, pharmacological modulation of DC function could be beneficial for interfering with deleterious immune responses such as hypersensitivity reactions and autoimmunity. According to this notion, it is possible that the immunomodulatory properties of andrographolide could be mediated by an effect of this molecule on DC function.

Importantly, therapeutic benefits have been observed in response to the administration of plant-derived molecules for several inflammatory diseases, such as rheumatoid arthritis and multiple sclerosis (MS) (Killestein et al., 2003; Shin et al., 2003; Soeken et al., 2003), providing encouragement for the potential use of these preparations to treat autoimmune disorders. MS is a chronic neuroinflammatory demyelinating disorder of the central nervous system that predominantly affects young adults (Nosworthy et al., 2000). Although the etiology of the progressive neurological loss has not yet been fully elucidated, evidence points toward an autoimmune pathogenesis, where myelin-specific CD4+ and CD8+ T cells are thought to play a central role by reacting against and destroying the myelin sheath (Wingerchuk et al., 2001). Due to the fact that it shows close similarity to clinical and histopathological aspects of human MS, experimental autoimmune encephalomyelitis (EAE) represents a suitable animal model for testing efficacy of potential therapeutic agents for MS (Sun et al., 2001; Kuchroo et al., 2002). In C57BL/6 mice, EAE can be induced by injection of a peptide derived from mouse myelin oligodendrocyte glycoprotein (MOG), which leads to chronic spinal cord demyelination and paralysis. EAE is characterized by focal areas of demyelination throughout the central nervous system, with axonal loss that results in ascending paralysis (Iglesias et al., 2001).

Because DC function and T cell priming may possibly be targets of the immunomodulatory activity of andrographolide, in this study we evaluated whether this molecule could interfere with these processes. Our data suggest that andrographolide can interfere with the ability of DCs to process and present antigens to T cells. Consistent with these findings, we observed that andrographolide was able to reduce T cell activation in vitro and in vivo. When this feature was applied for a potential favorable immune modulation of autoimmune diseases, using EAE as a model, it was observed that treatment with andrographolide reduced the severity of this disease. Our results support the notion that the immunosuppressive properties of this molecule could be considered for the treatment of autoimmune diseases.

**Materials and Methods**

**Animals.** Six- to eight-week-old female C57BL/6 mice were used in these experiments and kept under pathogen-free conditions at the animal core facility of the Pontificia Universidad Catolica de Chile. All animal work was performed according to institutional guidelines.

**Reagents and Synthetic Peptides.** Andrographolide was kindly provided by Amsar Private (Maharashtra, India). A stock solution for this molecule was prepared by dissolving andrographolide in dimethyl sulfoxide at 50 mM, which was then serially diluted in PBS immediately prior to experiments. Myelin oligodendrocyte glycoprotein-derived peptide (MOG35-55, MEWGYSRRFSRVRVHLYRNGK), chicken egg ovalbumin (OVA) peptide SIINFEKL (OVA257-264), for presentation on H-2Kd and OVA peptide TETWSSNVMEERKKIV (OVA265-280) for presentation on I-Ab were synthesized by solid-phase method using Fmoc chemistry on an automated 433A peptide synthesizer (Applied Biosystems, Foster City, CA) at the Peptide Synthesis Facility of the Albert Einstein College of Medicine. All peptides were purified to >98% homogeneity by reversed-phase high-performance liquid chromatography on a Vydac C-18 column (2.1 or 4.6 mm × 25 cm, 300 Å) using HP-1090M high-performance liquid chromatography (Hewlett Packard, Palo Alto, CA). The identity of the purified peptide was determined by a tandem quadrupole mass spectrometer (TSQ700, Thermo Finnigan, San Jose, CA).

**EAE Induction.** Six- to eight-week-old female C57BL/6 mice were injected subcutaneously with 50 μg of MOG35-55 peptide emulsified in Complete Freund’s Adjuvant (Invitrogen, Carlsbad, CA) supplemented with heat-inactivated Mycobacterium tuberculosis H37 RA (Difco, Detroit, MI). In addition, mice received intraperitoneal injections with 500 ng of pertussis toxin (Calbiochem, San Diego, CA) at the time of sensitization and 48 h later. Clinical signs of disease were seen usually between days 15 and 18 after sensitization and assessed daily according to the following scoring criteria: 0, no detectable signs of EAE; 1, flaccid tail; 2, hind limb weakness or abnormal gait; 3, complete hind limb paralysis; 4, paralysis of fore and hind limbs; and 5, moribund or death. To prevent unnecessary animal suffering, mice severely affected by the disease were euthanized with the supervision of a veterinarian. Mean clinical score was calculated by adding every day clinical score for all mice in a group and then divided by the total number of mice. Data shown are representative of four independent experiments.

**Andrographolide Treatment.** Mice were treated intraperitoneally with a daily dose equal to 4 mg/kg of andrographolide in PBS (total volume of 100 μl). This dose is not maximal and it is considerably under the LD50 for intraperitoneally administered andrographolide (11.6 g/kg) (Handa and Sharma, 1990). Treatment started 1 week before MOG sensitization and continued through all the experiment. As controls, age-matched female mice were sensitized with MOG but not treated with andrographolide. Treated and control mice were clinically evaluated on a daily basis. At the doses used, andrographolide was well tolerated by mice, and no evidence of toxicity was observed.

**DCs, Antigen Presentation Assay, and T Cell Hybridoma Activation.** Bone marrow-derived DCs were prepared as previously described (Iinaba et al., 1992; Lopez et al., 2000; Kalergis and Ravetch, 2002). Briefly, DCs were grown from bone marrow progenitors in RPMI 1640 containing 5% fetal calf serum (HyClone Laboratories, Logan, UT) supplemented with granulocyte/macrophage colony-stimulating factor (50 U/ml) (BD Biosciences PharMingen, San Diego, CA). Day 5 DCs were treated with 10 μM andrographolide for 24 h. After this time, DCs were pulsed for 16 h either with OVA protein or OVA peptide (OVA257-264 or OVA265-280) for presentation on I-Ab. After the pulse, DCs were washed and cocultured at different ratios with either 1 × 105 B3Z or...
1 × 10^5 OT4H T cell hybridomas. B3Z and OT4H are specific for H-2K^b/OVA_{257-264} and I-A^d/OVA_{265-270}, respectively, and secrete IL-2 upon T cell receptor stimulation (Shastri and Gonzalez, 1993). After 20 h of DC-T cell coculture, IL-2 from supernatants was measured by cytokine ELISA as previously described (Kalergis and Nathenson, 2000; Kalergis et al., 2000, 2001). DC viability was determined by trypan blue exclusion. For anti-CD3 T cell activation, B3Z and OT4H T cell hybridomas (2 × 10^5 cells/well) were stimulated with plate-bound anti-CD3ε, 500 ng/ml (clone 145-2C11, BD Biosciences PharMingen) in the presence of increasing concentrations of andrographolide (0–10 μM). After 24 h, culture supernatants were analyzed to determine IL-2 secretion by ELISA (Kalergis et al., 2001).

MOG-Specific T Cell Cytokine Release Assays. Inguinal and mesenteric lymph nodes were obtained on day 21 after EAE induction from control or andrographolide-treated mice (average clinical score was 2 for controls and 1 for andrographolide-treated mice). Cellular suspensions from these lymph nodes were cultured (5 × 10^5 cells/well) in RPMI 1640 containing 5% fetal calf serum with different concentrations of MOG_{35-55} peptide. Cultures were incubated in 96-well round bottom plates for 48 h at 37°C in a cell culture incubator. IL-2 release in response to MOG_{35-55} peptide was determined on culture supernatants by cytokine ELISA as previously described (Kalergis and Nathenson, 2000; Kalergis et al., 2000, 2001). IFN-γ release was also determined by cytokine ELISA but using purified anti-mouse IFN-γ (clone R4–6A2, BD Biosciences PharMingen) as capture antibody and biotin anti-mouse IFN-γ (clone XMG1.2, BD Biosciences PharMingen) as detection antibody.

Measurement of Anti-MOG Antibody Response. Mice sera were obtained on days 7 and 21 after sensitization with MOG_{35-55} peptide. At day 7, average clinical score for both groups was 0; at day 21, average clinical score was 3 for controls and 1.5 for andrographolide-treated mice. Sera were analyzed for the presence of MOG-specific IgG by ELISA. Briefly, ELISA plates (Falcon, Cowley, UK) were coated at 4°C overnight with 10 μg/ml MOG_{35-55} peptide in 0.1 M NaHCO3 buffer (pH 8.4) and then blocked with PBS-BSA 1% in PBS to 100 μl of PBS. Ear thickness was measured at different times after challenge with a micrometer (Mitutoyo, Tokyo, Japan). Increased ear thickness was expressed as the means of at least three measurements per mouse in millimeters × 10^-2 ± S.E.

Results

In Vitro T Cell Activation Is Inhibited by Andrographolide. The ability of andrographolide to interfere with T cell activation was evaluated in a mix lymphocyte reaction between C57BL/6 and BALB/c splenocytes. As shown in Fig. 1A, in this assay, T cell proliferation and IL-2 release were inhibited by andrographolide in a dose-dependent fashion. No measurable effect on background proliferation and IL-2 release was observed (data not shown).

To determine whether this was an effect on the T cells or the APCs, an antigen presentation assay was set up with bone marrow-derived DCs pulsed in vitro with OVA and cocultured either with H-2K^b/OVA_{257-264} or I-A^d/OVA_{265-270} specific T cell hybridomas (B3Z and OT4H, respectively). As shown in Fig. 1, B and C, treating DCs with andrographolide before OVA pulse prevented them from activating both CD4+ and CD8+ OVA-specific T cell hybridomas. This inhibition was only observed when andrographolide-treated DCs were pulsed with whole OVA protein and not when OVA_{257-264} or OVA_{265-270} peptides (for presentation on H-2K^b and I-A^b, respectively) were exogenously added to these cells (Fig. 1, B and C). Consistent with these findings, andrographolide treatment had no effect on APC-independent T cell activation with anti-CD3 (Fig. 1D). In addition, trypan blue exclusion assay shows that viability of DCs remains unaffected after treatment with 10 μM andrographolide (Fig. 1E). These data are supported by measurements of mitochondrial function showing that concentrations up to 50 μM andrographolide did not affect cell viability (Habtemariam, 1998; Chio et al., 2000).

Thus, our results suggest that, at the concentration tested, andrographolide inhibits the ability of DCs to process OVA and generate the peptide-MHC complexes required for T cell activation. To test this notion, the ability of andrographolide-treated DCs to process and present OVA-derived peptides on MHC-I was evaluated using H-2K^b/SIINFEKL complex. After washing, goat anti-mouse IgG-FITC (BD Biosciences PharMingen) was added to DCs. Cells were washed in PBS, fixed in paraformaldehyde (1% in PBS), and analyzed by fluorescence-activated cell sorting. To determine the densities of H-2K^b/OVA complexes on the surface of DCs, OVA-pulsed cells were stained with anti-CD11c-FITC (clone HL3; BD Biosciences PharMingen) plus anti-I-A^b–FITC (clone AF6-120.1; BD Biosciences PharMingen), anti-CD86–FITC (clone GL1; BD Biosciences PharMingen), or anti-CD40–FITC (clone 3/23; BD Biosciences PharMingen), fixed in paraformaldehyde (1% in PBS), and analyzed by fluorescence-activated cell sorting. To determine the densities of H-2K^b/OVA complexes on the surface of DCs, OVA-pulsed cells were stained with anti-CD11c-PE and 150 μl of 25-D1.16 supernatant (mouse κ-IgG1 mAb specific for the H-2K^b/SSINFEKL complex). After washing, goat anti-mouse IgG–FITC (BD Biosciences PharMingen) was added to DCs. Cells were washed in PBS, fixed in paraformaldehyde (1% in PBS), and analyzed by fluorescence-activated cell sorting.

Mixed Lymphocyte Reaction. Lymph node cell suspensions obtained from C57BL/6 and BALB/c mice were cocultured in 96-well round bottom plates with increasing concentrations of andrographolide (0–7.5 μM) at 1 × 10^5 cells per strain on each well for 2 h. After this time, supernatants were harvested and analyzed for IL-2 release by cytokine ELISA as described above. T cell proliferation was assessed using CellTiter Cell Proliferation Assay (Promega, Madison, WI) following the methodology provided by the manufacturer.

NP-Specific Antibody Response. Mice were immunized subcutaneously with 50 μg of 4-hydroxy-3-nitropheny lacetyl conjugated to bovine serum albumin (NP–BSA; Biosearch Technologies, Inc., Novato, CA) in alum (Pierce Chemical, Rockford, IL). Mice were treated intraperitoneally with a daily dose equal to 4 mg/kg andrographolide in PBS (total volume of 100 μl) since the day of immunization and continued for the duration of the experiment. Seven days after immunization, NP-specific IgG antibodies were measured in mice sera by ELISA. Briefly, plates were coated at 4°C overnight with NP_{23-65} CGG (0.5 μg/well) in 0.1 M NaHCO3 buffer (pH 8.4), and anti-NP IgG antibodies were detected as described above.

Delayed-Type Hypersensitivity Reaction. Mice were immunized subcutaneously with 100 μg of OVA emulsified in Complete Freund’s Adjuvant (Intrivogen). Mice were treated intraperitoneally with a daily dose equal to 4 mg/kg andrographolide in PBS since the day of sensitization. One week after immunization, animals were intracutaneously challenged in the ear with 50 μg of OVA dissolved in 20 μl of PBS. Ear thickness was measured at different times after challenge with a micrometer (Mitutoyo, Tokyo, Japan). Increased ear thickness was expressed as the means of at least three measurements per mouse in millimeters × 10^-2 ± S.E.

 Delayed-Type Hypersensitivity Reaction.
To further evaluate the effect that andrographolide could have on DC function, maturation of DCs was induced by LPS treatment in the presence of andrographolide. As shown in Fig. 2, B and C, andrographolide also inhibited up-regulation of the maturation markers I-Ab, CD40, and CD86 (B7.2) in response to LPS.

In Vivo T Cell Function Is Suppressed by Andrographolide Treatment. Data shown above suggested that andrographolide could be able to interfere with the initiation of an immune response by inhibiting antigen presentation by DCs, which is required for T cell priming. To test whether andrographolide was also able to affect in vivo immune responses, C57BL/6 mice were treated with the molecule and immunized with NP17-BSA (a thymus-dependent antigen) adsorbed to alum. Seven days postimmunization, anti-NP IgG titers were determined by ELISA using NP23-CGG as antigen. Compared with untreated controls, significantly reduced anti-NP IgG titers were observed for andrographolide-treated mice (Fig. 3A).
Reduced Antimyelin T Cell and Antibody Response in Andrographolide-Treated Mice. The diminished EAE incidence and severity resulting from andrographolide treatment in the mouse could be due either to an interference with autoreactive T cell activation and antibody production or to a nonspecific anti-inflammatory effect of this compound. To approach this issue, 3 weeks after EAE induction, lymph nodes were obtained from control and andrographolide-treated mice to evaluate cytokine release in response to MOG peptide. As shown in Fig. 5A, IFN-γ and IL-2 secretion was observed only in lymph node suspensions obtained from untreated mice suffering from EAE. In contrast, neither IFN-γ nor IL-2 could be detected in supernatants from MOG-stimulated lymph node cell suspensions derived from andrographolide-treated mice. Consistent with these observations, anti-MOG IgG could only be measured in sera from control animals suffering from EAE, whereas andrographolide-treated animals showed an almost complete absence of anti-MOG antibody titers (Fig. 5B). Thus, it seems likely that andrographolide treatment reduced EAE severity by impairing T cell priming by DCs, which could also indirectly affect antibody production against myelin antigens.

**Discussion**

New immunomodulatory therapeutic strategies are required to prevent or treat autoimmune diseases such as multiple sclerosis. Here, we provide evidence for a potential role of a bicyclic diterpenoid lactone, known as andrographolide, as an inhibitor of DC function able to down-modulate T cell-mediated immunity and ameliorate EAE in the mouse. When tested on a series of in vitro and in vivo assays, andrographolide was shown to interfere with the capacity of antigen-pulsed DCs to activate T cells.

Andrographolide was able to prevent OVA-pulsed DCs from activating either CD4+ or CD8+ T cell hybridomas. This result is consistent with the absence of H-2Kb/OVA257-264 complexes on the surface of DCs treated with andrographolide at the time of OVA pulse. Thus, it seems likely that treatment with this molecule prevented processing and presentation of OVA peptides on MHC molecules to OVA-specific T cells.

In addition to impairing generation of peptide-MHC complexes, andrographolide reduced the efficiency of DC maturation in response to LPS. Thus, when fold increase in surface expression was evaluated, reduced LPS-induced up-

### Table 1

Summary of EAE disease parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence</th>
<th>Maximum Score</th>
<th>Mean Maximum Score</th>
<th>Day of Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14/14 (100%)</td>
<td>5</td>
<td>3.7 ± 0.1</td>
<td>14.7 ± 0.2</td>
</tr>
<tr>
<td>Andrographolide</td>
<td>8/17 (47%)</td>
<td>3</td>
<td>1.9 ± 0.1*</td>
<td>17.8 ± 0.5*</td>
</tr>
</tbody>
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*p < 0.05, compared with the control, unpaired Student’s t test.*
Data shown are means of three independent experiments. Control mice are shown as black bars, andrographolide-treated mice as red bars. A, three weeks after EAE induction, lymph nodes were obtained from control (filled squares), andrographolide-treated (empty squares), and naive (empty circles) mice to evaluate cytokine release (IL-2 and IFN-γ) in response to MOG peptide (*, p < 0.05; **, p < 0.01; ***, p < 0.001, Student’s t test). B, sera were obtained from the same animals, and anti-MOG specific IgG titers were measured by ELISA. Control mice are shown as black bars, andrographolide-treated mice as shaded bars, and naive mice as white bars (**, p < 0.01, Student’s t test). Data shown are means of three independent experiments.

Although it was apparent that andrographolide slightly modulated the B7.2 molecule, it was observed as a result of andrographolide treatment. Thus, it is likely that andrographolide interferes with the inhibition of T cell activation observed in vivo after andrographolide treatment (see below).

Accordingly, the in vitro inhibition of T cell activation caused by andrographolide is consistent with the suppression of the immune response in the mouse, as shown on three different experimental assays designed to measure immune system function in vivo. Thus, antibody (IgG) secretion against the T cell-dependent antigen NP_{17}-BSA was significantly reduced by andrographolide treatment. Similarly, the DTH response against the antigen OVA was diminished to background levels by treatment with andrographolide. These results support the notion that T cell-mediated immune responses can be effectively impaired by this molecule.

Finally, we evaluated whether the capacity of andrographolide to impair T cell activation could be applied to prevent the onset of EAE in mice. As shown in Fig. 4 and Table 1, andrographolide treatment significantly reduced both the incidence and clinical severity of EAE in C57BL/6 mice during early phase of disease. Residual clinical signs were not significantly changed by treatment with this molecule. Clinical data were consistent with the observation that lymph node cellular suspensions derived from andrographolide-treated mice showed reduced IFN-γ and IL-2 release in response to MOG (Fig. 5A), two important pro-inflammatory cytokines that participate in EAE pathogenesis (Lassmann et al., 2001; Lucchetti et al., 2000; Wingerchuk et al., 2001). These data support the notion that the beneficial effects of andrographolide are mediated preferentially by specific interference with antigen presentation by DCs and, thus, with T cell activation, and they correlate with the clinical scores shown by the animals. Further research is required to evaluate the potential therapeutic capacity of andrographolide when administered after symptoms of EAE have started.

The reduced in vivo T cell priming, which is probably responsible for the decreased DTH and EAE responses observed in andrographolide-treated mice, could result from the impairment on DC maturation and generation of peptide-MHC complexes caused by andrographolide. However, whether this molecule is directly altering DC function in vivo remains to be evaluated.

In addition to activation of autoreactive T cells during the sensitization phase, EAE pathogenesis involves several inflammatory mediators, which are also responsible for myelin damage. Recent studies provide evidence suggesting that andrographolide could also interfere with the function of inflammatory cells such as neutrophils and microglia (Batkhuu et al., 2002; Shen et al., 2002; Wang et al., 2004). Because these inflammatory cells have been implicated in the pathogenesis of inflammation in MS (Calabrese et al., 2002; Smith and Lassmann, 2002; Hill et al., 2004), the relative contribution of andrographolide to diminish adaptive versus nonspecific inflammation needs to be defined. Furthermore, an antiapoptotic activity has been shown for andrographolide (Chen et al., 2004), which could also contribute to reduce severity of EAE in the mouse by increasing neuronal resistance to cell death induced by local inflammation. Thus, it is likely that andrographolide interferes with EAE by preventing activation of autoreactive T cells and by reducing inflammatory damage.

In summary, the data presented here suggest that andrographolide is able to modulate T cell activation both in vitro as well as in vivo. The exact mechanism by which andrographolide exhibits its beneficial effect on EAE is still not fully elucidated, but we provide evidence it could prevent initial T cell priming by interfering with DC maturation and antigen presentation capacity. Therefore, andrographolide may have utility as a therapeutic agent for the treatment of autoimmune diseases, such as multiple sclerosis.

**References**


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Address correspondence to: Dr. Alexia M. Kalgiris, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile. E-mail: kalgiris@bio.puc.cl