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ScienceDirect
Biochemical and Biophysical Research Communications 355 (2007) 603–610
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Diterpenes inhibit IL-12 production by DC and enhance Th2 cells polarization

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Received 26 January 2007
Available online 6 February 2007

Abstract

Sugiol and 12-hydroxy-6,7-secoabieta-8,11,13-triene-6,7-dial (Secoferruginol) are diterpenes isolated from the heartwood of *Cryptomeria japonica* and are pharmacologically active substances. Dendritic cells (DC) have a key influence on the differentiation of naïve T cells into Th1 or Th2 effector cells. We demonstrate that Sugiol and Secoferruginol activate human DC as documented by phenotypic and functional maturation and altered cytokine production. Human monocytes were exposed to Sugiol or Secoferruginol alone, or in combination with LPS and thereafter co-cultured with naïve T cells. The expression levels of CD83 on Sugiol-primed DC were enhanced. Sugiol dose-dependently inhibited IL-12p70 production by LPS-primed DC and to a lesser extent, the production of the proinflammatory cytokines. Naïve T cells co-cultured with Sugiol-primed DC, turned into typical Th2 which produced large quantities of IL-4 and released small amounts of IFN-γ and reduced Th1 cell polarizing capacity. Sugiol-primed DC induced the development of Th2 cells via the enhanced expression of OX40L and augmented the Th2 cell polarizing capacity of DC via the inhibition of IL-12p70. Similar results were obtained with Secoferruginol. These results suggest that some diterpenes modulate human DC function in a fashion that favors Th2 cell polarization and might have implication in autoimmune diseases.

Keywords: Dendritic cells; Diterpenes; Th2 response; Autoimmune diseases

DC are considered to be the principal antigen-presenting cells (APC), whose main function is to identify microbial structures and present these to naïve T cells in the lymph nodes [1]. The interaction of T cells with DC is crucial for directing T cell differentiation toward the Th1 or Th2 type and several factors determine the direction of T cell polarization [2–9], and the ratio of Th1 and Th2 is closely correlated with the outcome of many diseases [10,11]. Th1 responses predominate in organ-specific autoimmune disorders, acute allograft rejection, and in some chronic inflammatory disorders [12,13] and Th2 responses predominate in Omenn's syndrome, transplantation tolerance, chronic graft-*versus*-host disease, and allergic diseases [14]. Although different DC subsets may have some intrinsic potential to preferentially induce Th1 or Th2 responses, DC also display considerable functional plasticity in response to signals from microbes and the local microenvironment. Human monocyte-derived DC1 matured by either CD40-L, IFN-α, TNF-α, poly I:C (a synthetic double-stranded RNA), LPS or oligo CpG nucleotides *in vitro* produce high levels of IL-12 and induce Th1 cells [15–17]. On the other hand, PGE_2 and *Schistosoma mansoni* soluble egg antigen (SmEA) caused the generation of IL-12 deficient DC2, which induce the development of distinct DC phenotypes by provoking tissues to release the development of Th2-type responses [18]. Some support for a

Abbreviations: DC, dendritic cells; MLR, allogeneic mixed lymphocyte reaction; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; GM-CSF, granulocyte-macrophage colony-stimulation factor; IFN-γ, interferon-γ.

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doi:10.1016/j.bbrc.2007.01.157

specialized maturation program is provided by the finding that human DC conditioned by SmEA to induce Th2 responses express OX40L, whereas DC that are conditioned by microbial stimuli to induce Th1 responses do not [18]. IL-12 plays a central role in the immune responses and promotes the development of Th1 cells and IL-12 plays a critical role in the pathogenesis of rodent models of Th1-mediated autoimmune diseases such as type-1 diabetes, multiple sclerosis, rheumatoid arthritis (RA), inflammatory bowel disease, and acute graft-*versus*-host disease [19,20]. Some reports have demonstrated that exogenous factors such as lipids produced by parasites can modulate DC function for the purposes of evading host immunity [18]. These observations have been interested in defining how and which additional exogenous signals may regulate DC function in a fashion that may result in an altered generation of Th1- *versus* Th2-dominated immunity. Therefore, it is important to identify factors that might affect the differentiation, maturation, and function of DC.

Sugiol and Secoferruginol, which are phytochemically classified as diterpenes, are isolated from the black heartwood of *Cryptomeria japonica*. Sugiol has been shown to inhibit JNK1/2 and p38 phosphorylation in LPS-stimulated macrophages and anti-inflammatory effects [21,22]. Moreover, T-cadinol also is isolated from scented myrrh, which is the resin of the plant *Commiphora guidottii* Chiov., Burseraceae and this resin is widely used in Somalian traditional medicine as a remedy for treating various gastrointestinal disorders and diarrhea [23]. Thus, although some terpens have pharmacological activity, little is known about how Sugiol and Secoferruginol influence the initiation of the specific immune response at the level of DC, the highly professional APC for T cells. In the present study, the effects of Sugiol and Secoferruginol on human DC differentiation and function were investigated in detail.

Materials and methods

Culture medium, reagents, and monoclonal antibodies. The culture medium used in this study was serum-free AIM-V medium (Life Technologies, Paisley, UK). Recombinant human IL-4 (IL-4), recombinant human granulocyte-macrophage colony-stimulation factor (GM-CSF), CD40-L, rhIL-10, anti-IL10 mAb, rhIL-12, and anti-OX40L mAb were purchased from R&D Systems (Minneapolis, MN). LPS from *Escherichia coli* were purchased from Sigma–Aldrich (St. Louis, MO). For flow cytometry, monoclonal antibodies (mAbs) toward the following antigens were purchased from Becton–Dickinson (San Jose, CA): anti-CD14-FITC (fluorescent isothiocyanate), anti-CD1a-PE (phycoerythrin), anti-CD80-PE, anti-CD83-PE, anti-CD86-PE, and HLA-DR-FITC. Endotoxin levels in all agents were below 1.0 EU/ml.

Isolation of Sugiol and Secoferruginol from the Black Heartwood of C. japonica. Sugiol and Secoferruginol were prepared as previously described [24]. The purity of Sugiol and Secoferruginol was >99%. Sugiol and Secoferruginol were dissolved in dimethyl sulfoxide. The concentration of dimethyl sulfoxide in the culture medium was 0.1%, which had no effect upon the culture and the production of cytokines under the conditions used in this study. The endotoxin in Sugiol and Secoferruginol was removed using Endo Trap 5/1 (Profos AG, Regensburg, Germany). Leading to LPS concentrations below the detection limit of the assay (<0.05 EU/ml).

Generation of monocyte-derived DC. Generation of human monocyte-derived DC were carried out as previously [25,26].

Immunophenotype studies. Immunophenotype studies were carried out as previously [26]. The results were expressed as MFI.

Allo MLR. The allo MLR assay was carried out as previously [26].

Determination of naïve T cell polarization by DC. Determination of naïve T cell polarization by DC was carried out as previously [26].

Statistical analysis. Statistical significance was determined using a Student's *t*-test. Values of $P < 0.05$ were considered statistically significant.

Results and discussion

To investigate the direct effects of Sugiol or Secoferruginol on the function of human DC, immature monocyte-derived DC were exposed to Sugiol or Secoferruginol, and phenotypical and functional DC maturation were analyzed. Human monocytes were cultured with GM-CSF and IL-4 for 6 days under standard conditions, followed by another 2 days in the presence of various concentrations of Sugiol or Secoferruginol. The expression levels of CD1a, CD80, CD86, and HLA-DR as expressed by MFI on Sugiol- or Secoferruginol-primed DC were enhanced in a dose-dependent manner (Table 1). Viability of cells at 10 μM of Sugiol or Secoferruginol was >95%. The concentrations of Sugiol or Secoferruginol were used at 10 μM for subsequent experiments. The expression levels of CD1a, CD80, CD83, CD86, and HLA-DR as expressed by MFI on LPS (100 ng/ml)-primed DC were strongly enhanced (Table 1). As control, immature DC (with medium) were generated by cultivating human monocytes with GM-CSF and IL-4 for 8 days. The expression level of CD14 as expressed by MFI on day 8 was low or undetectable. When human monocyte-derived DC were stimulated simultaneously with LPS (100 ng/ml) plus Sugiol (10 μM) or Secoferruginol (10 μM), the presence of diterpenes

Table 1
Comparison phenotype of DC cultured with Sugiol, Secoferruginol or LPS alone, or in combination with LPS on day 8 (MFI)

	CDla	CD80	CD83	CD86	HLA-DR
Sugiol					
0.1 μM	76 ± 2	49 ± 2	10 ± 2	360 ± 18	689 ± 52
1.0 μM	88 ± 4	78 ± 3	15 ± 3	432 ± 20	742 ± 62
10 μM	99 ± 5	102 ± 3	25 ± 3	464 ± 19	846 ± 43
Secoferruginol					
0.1 μM	74 ± 3	45 ± 5	10 ± 3	345 ± 19	654 ± 42
1.0 μM	87 ± 3	66 ± 6	15 ± 4	428 ± 21	762 ± 48
10 μM	98 ± 4	99 ± 7	21 ± 5	466 ± 38	879 ± 52
LPS (100 ng/ml)	228 ± 19	248 ± 48	87 ± 18	618 ± 40	1349 ± 286
LPS + Sugiol (10 μM)	292 ± 15	301 ± 28	123 ± 9	762 ± 20	1873 ± 121
LPS + Secoferruginol (10 μM)	282 ± 21	284 ± 19	118 ± 8	748 ± 18	1842 ± 106
Immature DC	79 ± 8	47 ± 6	10 ± 3	348 ± 29	628 ± 96
Monocyte	5 ± 1	6 ± 2	7 ± 2	12 ± 3	48 ± 6

Data are means ± SEM of five independent experiments.