ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Biological Evaluation of Subglutinol A As a Novel Immunosuppressive Agent for Inflammation Intervention

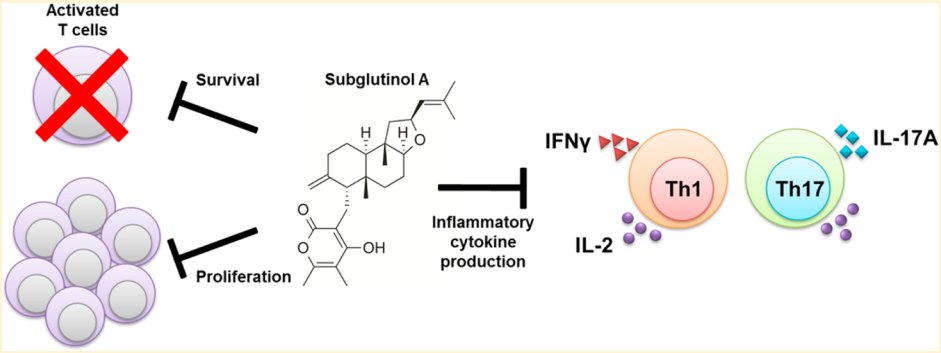
Regina Lin,[†] Hyoungsu Kim,[‡,∥] Jiyong Hong,*[,‡,§] and Qi-Jing Li*[,†]

[†]Department of Immunology, Duke University Medical Center, Durham, North Carolina 27710, United States

[‡]Department of Chemistry, Duke University, Durham, North Carolina 27708, United States

[§]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, United States

Supporting Information



ABSTRACT: Subglutinol A (**1**) is an immunosuppressive natural product isolated from *Fusarium subglutinans*, an endophytic fungus from the vine *Tripterygium wilfordii*. We show that **1** exerts multimodal immune-suppressive effects on activated T cells in vitro: subglutinol A (**1**) effectively blocks T cell proliferation and survival while profoundly inhibiting pro-inflammatory IFNγ and IL-17 production by fully differentiated effector Th1 and Th17 cells. Our data further reveal that **1** may exert its anti-inflammatory effects by exacerbating mitochondrial damage in T cells. Additionally, we demonstrate that **1** significantly reduces lymphocytic infiltration into the footpad and ameliorates footpad swelling in the mouse model of Th1-driven delayed-type hypersensitivity. These results suggest the potential of **1** as a novel therapeutic for inflammatory diseases.

KEYWORDS: *Subglutinol, immunosuppressive agent, T cell activation, inflammatory disease, mitochondrial dysfunction*

Autoimmunity is a misdirected immune response that occurs when the immune system goes awry and attacks the body itself. In many cases, it seems to be the result of a breakdown in T cell tolerance. Pathologically, this failure in tolerance will result in chronic lymphocyte activation, sustained leukocyte and lymphocyte tissue infiltration, massive production of inflammatory cytokines, and secretion of autoantibodies.[1] There are more than 80 human diseases currently classified as autoimmune, including multiple sclerosis, inflammatory bowel diseases, type 1 diabetes mellitus, rheumatoid arthritis, and systemic lupus erythematosus. Since they affect up to 8% of the US population[2] and often attack young adults, especially women, their social and economic impact is enormous.[3]

Immunosuppressive drugs are classical therapies to treat a wide range of autoimmune diseases.[4] In the past decade or so, a few new immunosuppression medications have been approved, increasing the number of options available to treat autoimmune diseases. However, currently available immunosuppressive drugs possess serious side effects.[4] Antiproliferative immunosuppressive drugs, such as methotrexate and cyclophosphamide, are limited in that they have nonspecific effects on various types of proliferating cells. They cause serious nonspecific bone marrow suppression, impair host resistance, and increase the incidence of infections. They also have a slow onset of action and a moderate efficacy that declines after several years of treatment. In comparison with these nonselective antiproliferative agents, cyclosporine A (CsA), FK506, and rapamycin act more selectively on different stages of the T- and B-lymphocyte activation cycles. However, even these more selective immunosuppressive drugs possess serious side effects, including acute neurological toxicity, chronic nephrotoxicity, biphasic effects to bone structure, and hypertriglyceridemia. Biologicals, such as antibody-based drugs, have faster onset of action and higher specificity than the existing small molecule-based therapies,[5,6] but they are expensive and some patients do not respond adequately. Moreover, they can also cause severe side effects such as the development of progressive multifocal leucoencephalopathy, idiopathic thrombocytopenic purpura, and lupus-like syndrome or vasculitis.[5] Thus, continued efforts

Received: November 21, 2013
Accepted: March 10, 2014
Published: March 12, 2014

must be made to develop novel immunosuppressive agents that lack undesirable side effects.

Subglutinols A (**1**) and B (**2**) (Figure 1) are immunosuppressive natural products isolated from *Fusarium subglutinans*,

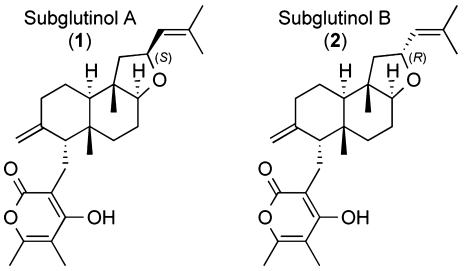


Figure 1. Structure of subglutinols A and B.

an endophytic fungus from the vine *Tripterygium wilfordii*.[7,8] *T. wilfordii* has long been used as an anti-inflammatory in traditional Chinese herbal medicine.[9,10] In addition, formulated extracts made from *T. wilfordii* are Chinese FDA approved drugs (Lei Gong Teng tablets, CFDA approval #Z42021534) for rheumatoid arthritis, psoriasis, lupus-associated autoimmune nephrotic syndrome, and autoimmune hepatitis. Subglutinols A (**1**) and B (**2**) were highly potent in the mixed-lymphocyte reaction (MLR) and thymocyte proliferation (TP) assays (IC_{50} = 0.1 μM).[7,8] Owing to the lack of toxicity,[7,8] **1** and **2** were expected to be promising new immunosuppressive drugs and have attracted a strong interest.[11−15] Our group reported the first total synthesis of subglutinols A (**1**) and B (**2**) and showed that **1** (IC_{50} = 25 nM) was indeed more potent than CsA (IC_{50} = 89 nM) in the MLR assay.[11,12]

Encouraged by the promising preliminary data, we embarked on more comprehensive evaluation of in vitro and in vivo immunosuppressive activity of **1**. Herein, we report the efficacy of **1** in eliminating Th1 and Th17 responses in vitro and in suppressing inflammation in vivo to demonstrate the potential of **1** as a novel immunosuppressive agent for autoimmune diseases.

As the commander controlling adaptive immune responses, T lymphocytes are tightly restricted to a quiescent state under normal conditions. During an infection, foreign antigen-specific T cells are rapidly activated and cycle to exponentially increase their numbers, which is essential for the efficiency of pathogen clearance. Upon resolution of the infection, most of these activated T cells are eliminated by apoptosis to keep the immune response in check and to prevent bystander tissue damage.[16] During the onset of autoimmune diseases, however, self-antigens are recognized as nonself and elicit immune responses from autoreactive T cells. Mirroring the immune responses toward foreign pathogens, massive clonal expansion and failure of contraction lead to the accumulation of pathogenic T cells responsible for mediating autoimmune diseases. In fact, defects in T cell apoptosis (e.g., resistance to Fas-induced cell death) are etiological causes of autoimmune lymphoproliferative syndrome (ALPS) and multiple sclerosis in humans.[17,18] Therefore, developing therapeutics capable of modulating the proliferation and cell death pathways of autoreactive T cells is a compelling strategy for the treatment of autoimmune disorders.[19,20]

To comprehensively evaluate the capacity of subglutinol A (**1**) in suppressing T cell effector responses, we used primary mouse T cells from the pMel-1 and LLO118 T cell receptor (TCR) transgenic mice. pMel-1 mice have CD8+ T cells that are specific for the melanoma antigen glycoprotein 100 ($hgp100_{25-33}$), while LLO118 mice bear CD4+ T cells recognizing the Listeria toxin listeriolysin ($LLO_{190-205}$).[21,22] Naïve pMel-1 CD8+ T cells and LLO118 CD4+ T cells were activated with their respective peptide antigens (5 μM $hgp100_{25-33}$ or 10 μM $LLO_{190-205}$) for 48 h in vitro, in the presence of 100 nM of **1** or CsA. DMSO was included as the vehicle control. T cell proliferation was assessed by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution, and the cell death was determined by live/dead staining. During the early phase of antigen-induced T cell activation, for both CD4+ and CD8+ T cells, **1** reduced their survival ratio from 80% to ∼10% (Figure 2). Among the remaining live T

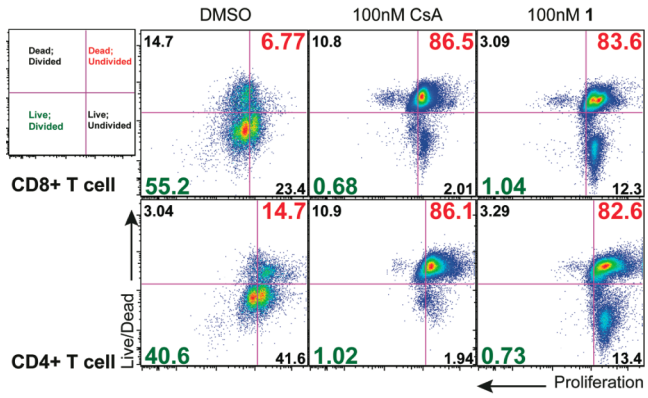


Figure 2. Subglutinol A (**1**) blocks antigen-induced T cell proliferation and induces massive apoptosis. CFSE-labeled naïve pMel-1 CD8+ (top) and LLO CD4+ transgenic T cells (bottom) were activated in vitro, in the presence of **1** (100 nM), CsA (100 nM), or DMSO vehicle control. After 48 h, T cell proliferation and cell death were assessed by CFSE dilution and live/dead staining, respectively. Data shown is representative of two independent experiments.

cells, cell division was completely halted. In a side-by-side comparison to CsA, **1** has a slightly lower cytotoxicity for undivided T cells but has a better efficacy in inhibiting T cell proliferation. Therefore, by intercepting these initial fundamental events of T cell activation, **1** has the potential to prevent the accumulation of autoreactive pathogenic T cells.

During an immune response, activated CD4+ T cells are the primary producers of IL-2, a pleitrophic cytokine that influences multiple immune cell subsets. Not only is IL-2 a potent growth factor essential for T cell proliferation and survival but it also drives the effector differentiation of Th1 cells, cytotoxic CD8+ T cells, and NK cells that perpetuate tissue destruction.[23,24] Therefore, we sought to investigate the influence of **1** on CD4+ T cell-derived IL-2 production. Naïve LLO118 CD4+ T cells were activated in vitro under a Th0 (nonpolarizing) condition for the first 4 days, during which they acquired IL-2-competence. In the 48 h that followed, these activated IL-2-competent T cells were then treated with various doses of **1**, CsA, or DMSO vehicle control. Cytokine production was evaluated by intracellular IL-2 staining and flow cytometry. Among DMSO-treated CD4+ T cells, over 30% of cells were capable of producing IL-2 (Supporting Information Figure A). Though **1** was less potent at inhibiting IL-2 production than CsA (Figure 3 and Supporting Information Figure A), **1** still effectively inhibited IL-2 expression by effector CD4+ T cells: IL-2 production fell

dx.doi.org/10.1021/ml4004809 | ACS Med. Chem. Lett. 2014, 5, 485−490

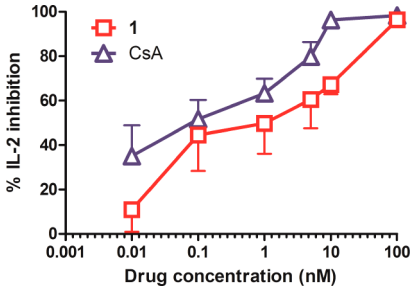


Figure 3. Subglutinol A (**1**) abrogates antigen-induced IL-2 production by activated CD4+ T cells. LLO CD4+ T cells were activated by the cognate antigen in vitro for the first 4 days in the absence of drugs, followed by 2 additional days in the presence of the indicated concentrations of **1**, CsA, or DMSO vehicle control (0 nM drugs). The percentages of IL-2-producing CD4+ T cells were then determined by intracellular staining and flow cytometry, and the percentage of inhibition was calculated. Data shown is mean ± SEM of three independent experiments.

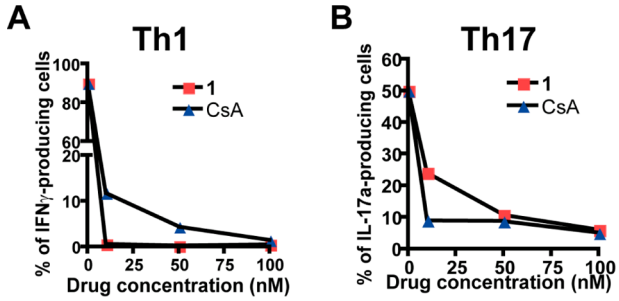


Figure 4. Subglutinol A (**1**) abolishes inflammatory cytokine production in fully differentiated Th1 and Th17 cells. LLO CD4+ T cells were activated in vitro and polarized into fully differentiated (A) IFNγ-producing Th1 or (B) IL-17A-producing Th17 cells for the first 4 days in the absence of drugs. This was followed by 2 additional days of culture in the indicated concentrations of **1**, CsA, or DMSO vehicle control (0 nM drugs). The percentages of cytokine-producing CD4+ T cells were then determined by intracellular staining and flow cytometry. Data shown is representative of two independent experiments.

around 50% upon treatment with 1 nM **1** and was completely abrogated at a higher dose of 100 nM (Figure 3 and Supporting Information Figure A).

As key drivers and perpetuators of inflammation,[25] self-antigen elicited IFNγ and IL-17 production by pathogenic Th1 and Th17 CD4+ T cells is a hallmark of autoimmune disorders (e.g., Type 1 diabetes,[25,26] rheumatoid arthritis,[27] multiple sclerosis,[28] systemic lupus erythematosus,[29] and others). It is therefore therapeutically desirable that immunosuppressive agents not only obstruct the initiation of antigen response but also achieve functional energy by blocking the production of these pro-inflammatory cytokines from full-fledged effector CD4+ T cells.[30] To assess whether **1** affects cytokine production by fully differentiated effector CD4+ T cells, naïve LLO118 CD4+ T cells were first polarized in vitro under Th1 or Th17 condition for 4 days, during which they acquired IFNγ- and IL-17-competence. In the 48 h that followed, these effector Th1 and Th17 cells were then treated with various doses of **1**, CsA, or the vehicle control DMSO. For fully differentiated Th1 cells, 10 nM **1** effectively diminished the percentage of IFNγ-producing cells from 90% to less than 1% (Figure 4A and Supporting Information Figure B). A similar level of suppression was achieved with 100 nM CsA. In terms of Th17 effector responses in the 10 nM range, **1** suppressed IL-17A production less effectively than CsA. However, both drugs exhibited comparable potency at the higher doses of 50 and 100 nM (Figure 4B and Supporting Information Figure B). Taken together, our data demonstrate that **1** not only restricts the accumulation of autoreactive T cells through its expansion-inhibitory effect but also dramatically attenuates inflammatory cytokine production by any remaining cells that survive.

We next sought to elucidate the mode of action by which **1** inhibits T cell activation. It is now increasingly appreciated that changes in T cell metabolism are necessary to fuel the functional and phenotypic changes associated with T cell activation. When a naïve T cell is activated, it undergoes a metabolic switch from quiescent catabolism to active anabolism, giving rise to key biosynthetic substrates critical for T cell clonal expansion and cytokine production.[31−33] Mitochondrion, the site of ATP-production by the mitochondrial electron transport chain and the source of biosynthetic precursors by the Krebs cycle, is at the heart of this critical metabolic switch.[33−35] In addition, the loss of mitochondrial integrity can also initiate the intrinsic apoptosis pathway to trigger cell death.[36−38] On the basis of the phenotype of **1**-treated T cells (impaired proliferation, increased cell death, and reduced cytokine production), we hypothesized that **1** may undermine T cell responses by compromising mitochondrial function. To test this hypothesis in fully differentiated Th1 and Th17 cells, the number of mitochondria was evaluated by MitoTracker Deep Red FM labeling for mitochondrial mass, while mitochondrial integrity was evaluated by MitoTracker Orange CMTMRos labeling for mitochondrial membrane potential. The total mitochondrial mass of fully differentiated Th1 was modestly reduced, while that of Th17 cells was unchanged across the doses of **1** tested, suggesting that **1** has little impact on mitochondrial replication or abundance in effector CD4+ T cells (Figure 5A). However, in both Th1 and Th17 cells, treatment with **1** induced mitochondrial depolarization in a dose-dependent manner, indicating that **1** disrupts mitochondrial membrane integrity (Figure 5B). On the contrary, CsA has previously been reported to inhibit mitochondrial permeability transition, thereby preventing mitochondrial depolarization.[39,40] Therefore, **1** may suppress inflammation though a distinct mechanism from CsA.

Mitochondria are essential organelles for all eukaryotic cells, and widespread mitochondrial dysfunction in normal tissues can result in severe adverse effects. To evaluate its target-cell specificity and potential as a therapeutic agent for auto-inflammation, we further interrogated whether **1** similarly affects the mitochondria of other cell types. To this end, we examined both resting and activated antigen-presenting cells of the innate immune system, as exemplified by immature bone marrow-derived dendritic cells (imBMDCs) and mature BMDCs (mBMDCs), respectively. Additionally, we also examined the impact on normal stromal cells, as exemplified by the 3T3 fibroblast cell line. Interestingly, 100 nM of **1**, the highest dose tested on activated T cells, altered neither mitochondrial mass nor depolarization in imBMDCs (Figure 5C, top panel), mBMDCs (Figure 5C, bottom panel), and 3T3 fibroblasts (Figure 5D). We postulate that activated T cells may have a heightened sensitivity to **1**-induced mitochondrial dysfunction than other cell types, a premise that warrants further dose−escalation studies in the future. Taken together, our data indicate that **1** preferentially exacerbates mitochondrial

dx.doi.org/10.1021/ml4004809 | ACS Med. Chem. Lett. 2014, 5, 485−490

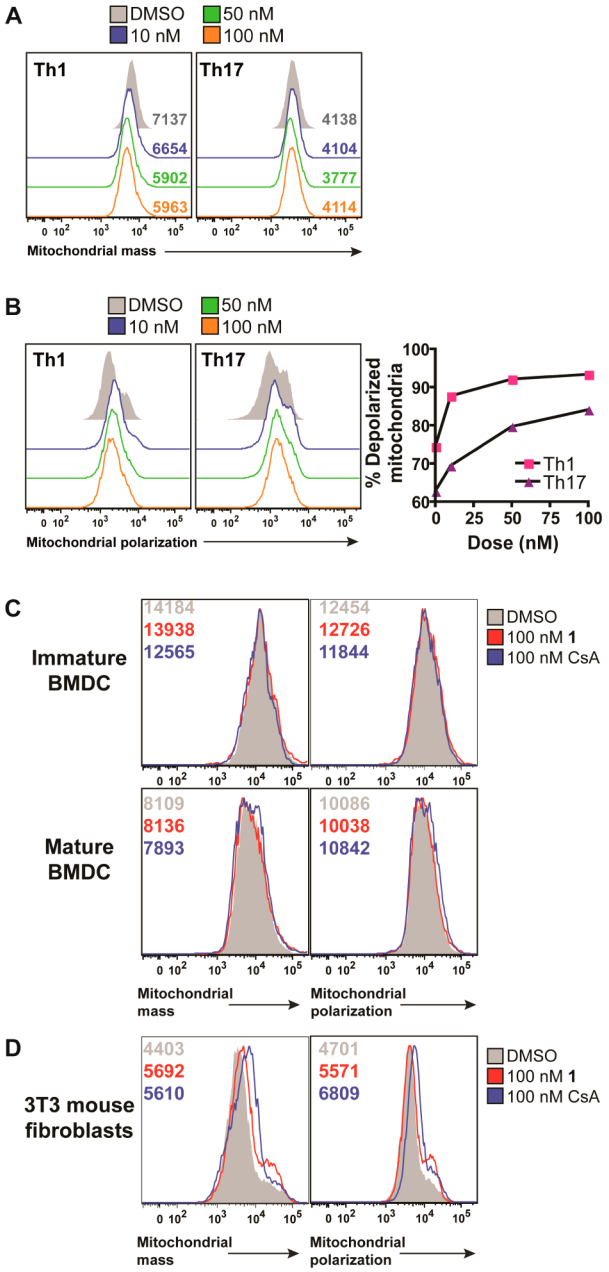


Figure 5. Subglutinol A (**1**) preferentially exacerbates mitochondrial depolarization in fully differentiated Th1 and Th17 cells. Naïve LLO CD4+ T cells were polarized in vitro under Th1- or Th17-skewing conditions for 4 days, followed by 2 additional days of culture in the indicated concentrations of **1** or DMSO vehicle control (0 nM). Th1 and Th17 cells were then labeled with (A) MitoTracker Deep Red FM to assess mitochondrial mass and (B) MitoTracker Orange CMTMRos to assess mitochondrial membrane depolarization in live CD4+ T cells by flow cytometry. (C) Immature and LPS-matured bone marrow-derived dendritic cells (BMDCs) generated from mice were treated for 1 day with **1** (100 nM), CsA (100 nM), or DMSO vehicle control. $CD11c^+$ BMDCs were analyzed for mitochondria mass and polarization by flow cytometry. (D) 3T3 mouse fibroblasts were treated for 2 days and analyzed for mitochondria mass and polarization by flow cytometry. Numbers in histograms indicate mean fluorescence intensity of MitoTracker Deep Red FM staining. Data shown is representative of two independent experiments.

damage in T cells, which may account for their impaired proliferation, death, and blunted cytokine production.

To interrogate the immune-suppressive effects of subglutinol A (**1**) in vivo, we utilized the classical Th1-driven inflammatory response of delayed-type hypersensitivity (DTH).[41] DTH is an antigen-specific inflammatory reaction in the skin that is triggered by repeated exposure to certain antigens, resulting in the activation and infiltration of skin-homing antigen-specific T cells.[42,43] The induction of the DTH response involves 2 stages: a sensitization phase and an effector phase. When T cells that have been previously sensitized by an antigen re-encounters the same antigen underneath the skin, the Th1-dominated effector response ensues, resulting in the manifestation of DTH. DTH is characterized by profound lymphocytic recruitment, Th1 CD4+ T cell-mediated cytokine (e.g., IFNγ) secretion, tissue damage, and local swelling at the site of antigenic stimulation.

We utilized a mouse model of DTH induced by the highly immunogenic protein, keyhole limpet hemocyanin (KLH).[44] C57BL/6J mice were first sensitized subcutaneously by introducing 100 μg of the KLH antigen emulsified in complete Freund's adjuvant (CFA). Seven days after the initial sensitization, each mouse was re-exposed on one footpad to 50 μg KLH and simultaneously treated with 16 nmol (0.273 mg/kg) of **1**, 16 nmol (0.769 mg/kg) of CsA, or DMSO vehicle control. As a negative control, PBS alone (antigen-free) was also injected into the other previously sensitized footpad. Forty-eight hours after antigenic re-exposure, we assessed swelling and lymphocytic infiltration into the footpads.

As compared to the footpad without re-exposure (PBS) that contained a few lymphocytes and maintained normal tissue integrity, mice that were re-exposed to KLH in combination with the DMSO vehicle control (KLH + DMSO) exhibited extensive lymphocytic infiltration into the dermis and severe disruption of tissue architecture. Consistent with earlier reports,[45−47] CsA did not alleviate DTH-induced footpad swelling at low doses (Figure 6); this was attributed to effects of CsA on non-T cells, which may counterproductively promote skin inflammation.[47] However, treatment with equimolar amounts of **1** (KLH + **1**) significantly blocked lymphocyte accumulation and ameliorated tissue injury (Figure 6). In addition, **1** also significantly reduced inflammation-induced footpad swelling. These data not only corroborate our findings from the in vitro assays, but more importantly demonstrate the in vivo immune-suppressive potency, efficacy, and target-cell specificity of **1** at low drug doses.

A comprehensive evaluation of the capacity of subglutinol A (**1**), a novel immunosuppressive natural product, was performed to demonstrate its potential as a novel therapeutic agent for autoimmune diseases. Subglutinol A (**1**) profoundly inhibited T cell proliferation, survival, and pathogenic cytokine production by fully differentiated Th1 and Th17 cells in vitro, potentially by aggravating mitochondrial damage in T cells. Importantly, immunosuppressive doses of **1** did not affect mitochondrial integrity in non-T cells, such as antigen-presenting BMDCs and stromal fibroblasts. Moreover, low-dose therapy with **1**, but not with CsA, was efficacious at attenuating the Th1-driven DTH response, demonstrating its anti-inflammatory, immunosuppressive efficacy, and target-cell specificity in vivo. These results suggest that **1** may provide opportunities for new, innovative, and efficacious therapies to treat autoimmune diseases, as well as better post-transplantation cares. Currently, structure−function relationship studies of **1** aimed at improving its efficacy and safety and further studies to identify the mode of action of **1** are in progress.

dx.doi.org/10.1021/ml4004809 | ACS Med. Chem. Lett. 2014, 5, 485−490

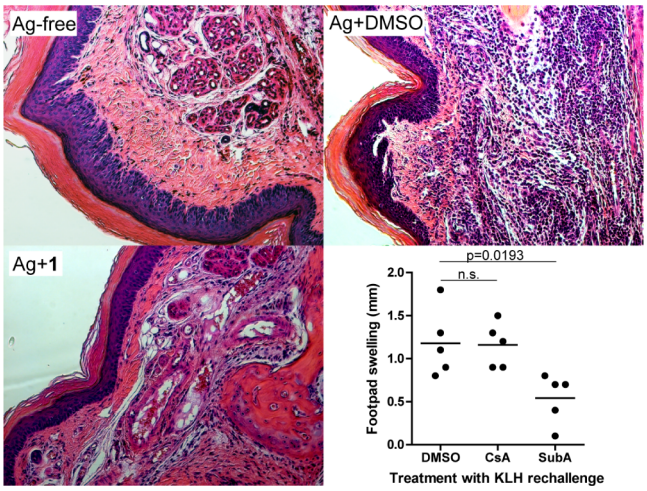


Figure 6. Subglutinol A (**1**) treatment suppresses antigen-induced DTH responses in vivo. C57BL/6J mice previously sensitized to the KLH antigen had their left footpads re-exposed to the KLH antigen and were treated with either 16 nmol (0.273 mg/kg) of **1** (KLH + **1**), 16 nmol (0.769 mg/kg) of CsA (KLH + CsA), or DMSO vehicle control (KLH + DMSO). As a control for background swelling, their right footpads were not re-exposed to KLH, but were injected with an equal volume of PBS (PBS). Two days after re-exposure, footpad swelling was read, mice were sacrificed, and their footpads excised for histological analysis by hematoxylin and eosin staining where indicated. Images of histological sections are representative of 5 mice per group; *p*-value is determined by the two-tailed unpaired *t*-test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

General experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(Q.-J.L.) E-mail: qi-jing.li@duke.edu.
*(J.H.) E-mail: jiyong.hong@duke.edu.

Present Address

‖(H.K.) College of Pharmacy, Ajou University, Suwon 443−749, Republic of Korea.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to American Diabetes Association (to Q.-J.L., 1-10-JF-28) and NIH/NIAID (to Q.-J.L., RAI091878), and Duke University (to J.H.) for funding this work. Q.-J.L. is a Whitehead Family Foundation Scholar.

■ ABBREVIATIONS

CsA, cyclosporine A; CFSE, carboxyfluorescein diacetate succinimidyl ester; IL-2, interleukin-2; IFNγ, interferon-γ; IL-17A, interleukin-17A; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin; CFA, complete Freund's adjuvant

■ REFERENCES

(1) Goodnow, C. C.; Sprent, J.; Fazekas de St Groth, B.; Vinuesa, C. G. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* **2005**, *435*, 590−597.
(2) *Progress in Autoimmune Disease Research*; U.S. Department of Health and Human Services, National Institute of Health, The Autoimmune Diseases Coordinating Committee: Washington, DC, 2005.
(3) Cooper, G. S.; Stroehla, B. C. The epidemiology of autoimmune diseases. *Autoimmun. Rev.* **2003**, *2*, 119−125.
(4) Stepkowski, S. M. Molecular targets for existing and novel immunosuppressive drugs. *Expert Rev. Mol. Med.* **2000**, *2*, 1−23.
(5) Balague, C.; Kunkel, S. L.; Godessart, N. Understanding autoimmune disease: new targets for drug discovery. *Drug Discovery Today* **2009**, *14*, 926−934.
(6) Sathish, J. G.; Sethu, S.; Bielsky, M. C.; de Haan, L.; French, N. S.; Govindappa, K.; Green, J.; Griffiths, C. E.; Holgate, S.; Jones, D.; Kimber, I.; Moggs, J.; Naisbitt, D. J.; Pirmohamed, M.; Reichmann, G.; Sims, J.; Subramanyam, M.; Todd, M. D.; Van Der Laan, J. W.; Weaver, R. J.; Park, B. K. Challenges and approaches for the development of safer immunomodulatory biologics. *Nat. Rev. Drug Discovery* **2013**, *12*, 306−324.
(7) Lee, J. C.; Lobkovsky, E.; Pliam, N. B.; Strobel, G.; Clardy, J. Subglutinols A and B: immunosuppressive compounds from the endophytic fungus *Fusarium subglutinans*. *J. Org. Chem.* **1995**, *60*, 7076−7077.
(8) Strobel, G. A.; Pliam, N. B. Immunosuppressant secondary metabolite diterpenes and method of production using a fungal organism. U.S. Patent 5648376, 1997.
(9) Tao, X.; Lipsky, P. E. The Chinese anti-inflammatory and immunosuppressive herbal remedy *Tripterygium wilfordii Hook F. Rheum. Dis. Clin. North Am.* **2000**, *26*, 29−50.
(10) Ho, L. J.; Lai, J. H. Chinese herbs as immunomodulators and potential disease-modifying antirheumatic drugs in autoimmune disorders. *Curr. Drug Metab.* **2004**, *5*, 181−192.
(11) Kim, H.; Baker, J. B.; Lee, S.-U.; Park, Y.; Bolduc, K. L.; Park, H.-B.; Dickens, M. G.; Lee, D.-S.; Kim, Y.; Kim, S. H.; Hong, J. Stereoselective synthesis and osteogenic activity of subglutinols A and B. *J. Am. Chem. Soc.* **2009**, *131*, 3192−3194.
(12) Kim, H.; Baker, J. B.; Park, Y.; Park, H.-B.; DeArmond, P. D.; Kim, S. H.; Fitzgerald, M. C.; Lee, D.-S.; Hong, J. Total synthesis, assignment of the absolute stereochemistry, and structure-activity relationship studies of subglutinols A and B. *Chem.—Asian J.* **2010**, *5*, 1902−1910.
(13) Kikuchi, T.; Mineta, M.; Ohtaka, J.; Matsumoto, N.; Katoh, T. Enantioselective total synthesis of (−)-subglutinols A and B: potential immunosuppressive agents isolated from a microorganism. *Eur. J. Org. Chem.* **2011**, 5020−5030.
(14) Duenes, R. A.; Morken, J. P. Catalytic, diastereoselective allylation of Oshima−Utimoto products. *Synlett* **2007**, 587−590.
(15) Lee, W.-G.; Kim, W.-S.; Park, S.-G.; Kim, H.; Hong, J.; Ko, H.; Kim, Y.-C. Immunosuppressive effects of subglutinol derivatives. *ChemMedChem* **2012**, *7*, 218−222.
(16) Vigano, S.; Perreau, M.; Pantaleo, G.; Harari, A. Positive and negative regulation of cellular immune responses in physiologic conditions and diseases. *Clin. Dev. Immunol.* **2012**, *2012*, 485781.
(17) Comi, C.; Fleetwood, T.; Dianzani, U. The role of T cell apoptosis in nervous system autoimmunity. *Autoimmun. Rev.* **2012**, *12*, 150−156.
(18) Fleisher, T. A.; Oliveira, J. B. Monogenic defects in lymphocyte apoptosis. *Curr. Opin. Allergy Clin. Immunol.* **2012**, *12*, 609−615.
(19) Pender, M. P. Treating autoimmune demyelination by augmenting lymphocyte apoptosis in the central nervous system. *J. Neuroimmunol.* **2007**, *191*, 26−38.
(20) Fife, B. T.; Pauken, K. E. The role of the PD-1 pathway in autoimmunity and peripheral tolerance. *Ann. N.Y. Acad. Sci.* **2011**, *1217*, 45−59.
(21) Overwijk, W. W.; Theoret, M. R.; Finkelstein, S. E.; Surman, D. R.; de Jong, L. A.; Vyth-Dreese, F. A.; Dellemijn, T. A.; Antony, P. A.;

Spiess, P. J.; Palmer, D. C.; Heimann, D. M.; Klebanoff, C. A.; Yu, Z.; Hwang, L. N.; Feigenbaum, L.; Kruisbeek, A. M.; Rosenberg, S. A.; Restifo, N. P. Tumor regression and autoimmunity after reversal of a functionally tolerant state of self-reactive CD8+ T cells. *J. Exp. Med.* **2003**, *198*, 569−580.
(22) Weber, K. S.; Li, Q. J.; Persaud, S. P.; Campbell, J. D.; Davis, M. M.; Allen, P. M. Distinct CD4+ helper T cells involved in primary and secondary responses to infection. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 9511−9516.
(23) Lan, R. Y.; Selmi, C.; Gershwin, M. E. The regulatory, inflammatory, and T cell programming roles of interleukin-2 (IL-2). *J. Autoimmun.* **2008**, *31*, 7−12.
(24) Boyman, O.; Sprent, J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat. Rev. Immunol.* **2012**, *12*, 180−190.
(25) Moudgil, K. D.; Choubey, D. Cytokines in autoimmunity: role in induction, regulation, and treatment. *J. Interferon Cytokine Res.* **2011**, *31*, 695−703.
(26) Leung, S.; Liu, X.; Fang, L.; Chen, X.; Guo, T.; Zhang, J. The cytokine milieu in the interplay of pathogenic Th1/Th17 cells and regulatory T cells in autoimmune disease. *Cell Mol. Immunol.* **2010**, *7*, 182−189.
(27) Boissier, M. C.; Semerano, L.; Challal, S.; Saidenberg-Kermanac'h, N.; Falgarone, G. Rheumatoid arthritis: from autoimmunity to synovitis and joint destruction. *J. Autoimmun.* **2012**, *39*, 222−228.
(28) Becher, B.; Segal, B. M. T(H)17 Cytokines in autoimmune neuro-inflammation. *Curr. Opin. Immunol.* **2011**, *23*, 707−712.
(29) Shin, M. S.; Lee, N.; Kang, I. Effector T-cell subsets in systemic lupus erythematosus: update focusing on Th17 cells. *Curr. Opin. Rheumatol.* **2011**, *23*, 444−448.
(30) Getts, D. R.; Shankar, S.; Chastain, E. M.; Martin, A.; Getts, M. T.; Wood, K.; Miller, S. D. Current landscape for T-cell targeting in autoimmunity and transplantation. *Immunotherapy* **2011**, *3*, 853−870.
(31) Fox, C. J.; Hammerman, P. S.; Thompson, C. B. Fuel feeds function: energy metabolism and the T-cell response. *Nat. Rev. Immunol.* **2005**, *5*, 844−852.
(32) Gerriets, V. A.; Rathmell, J. C. Metabolic pathways in T cell fate and function. *Trends Immunol.* **2012**, *33*, 168−173.
(33) Sena, L. A.; Li, S.; Jairaman, A.; Prakriya, M.; Ezponda, T.; Hildeman, D. A.; Wang, C. R.; Schumacker, P. T.; Licht, J. D.; Perlman, H.; Bryce, P. J.; Chandel, N. S. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* **2013**, *38*, 225−36.
(34) Fernandez, D.; Perl, A. Metabolic control of T cell activation and death in SLE. *Autoimmun. Rev.* **2009**, *8*, 184−189.
(35) van der Windt, G. J.; Everts, B.; Chang, C. H.; Curtis, J. D.; Freitas, T. C.; Amiel, E.; Pearce, E. J.; Pearce, E. L. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* **2012**, *36*, 68−78.
(36) Grimaldi, M.; Denizot, M.; Espert, L.; Robert-Hebmann, V.; Biard-Piechaczyk, M. Mitochondria-dependent apoptosis in T-cell homeostasis. *Curr. Opin. Invest. Drugs* **2005**, *6*, 1095−1102.
(37) Gupta, S.; Gollapudi, S. Susceptibility of naïve and subsets of memory T cells to apoptosis via multiple signaling pathways. *Autoimmun. Rev.* **2007**, *6*, 476−481.
(38) Gatzka, M.; Walsh, C. M. Apoptotic signal transduction and T cell tolerance. *Autoimmunity* **2007**, *40*, 442−452.
(39) Nieminen, A. L.; Petrie, T. G.; Lemasters, J. J.; Selman, W. R. Cyclosporin A delays mitochondrial depolarization induced by *N*-methyl-D-aspartate in cortical neurons: evidence of the mitochondrial permeability transition. *Neuroscience.* **1996**, *75*, 993−997.
(40) Liu, R. R.; Murphy, T. H. Reversible cyclosporin A-sensitive mitochondrial depolarization occurs within minutes of stroke onset in mouse somatosensory cortex in vivo: a two-photon imaging study. *J. Biol. Chem.* **2009**, *284*, 36109−36117.
(41) Kobayashi, K.; Kaneda, K.; Kasama, T. Immunopathogenesis of delayed-type hypersensitivity. *Microsc. Res. Tech.* **2001**, *53*, 241−245.
(42) Saint-Mezard, P.; Berard, F.; Dubois, B.; Kaiserlian, D.; Nicolas, J. F. The role of CD4+ and CD8+ T cells in contact hypersensitivity and allergic contact dermatitis. *Eur. J. Dermatol.* **2004**, *14*, 131−138.
(43) Vocanson, M.; Hennino, A.; Rozieres, A.; Poyet, G.; Nicolas, J. F. Effector and regulatory mechanisms in allergic contact dermatitis. *Allergy* **2009**, *64*, 1699−1714.
(44) Allen, I. C. Delayed-type hypersensitivity models in mice. *Methods Mol. Biol.* **2013**, *1031*, 101−107.
(45) Tchervenkov, J. I.; Diano, E.; Christou, N. V. Effect of cyclosporine on host defence. *Can. J. Surg.* **1985**, *28*, 525−527.
(46) Bussiere, J. L.; Mather, G. G.; Exon, J. H. Effect of cyclosporine on 3-methylcholanthrene-induced carcinogenesis and immune responses in the rat. *Immunobiology* **1991**, *182*, 205−215.
(47) Remitz, A.; Lauerma, A. I.; Erkko, P.; Reitamo, S. Delayed-type hypersensitivity in palmoplantar pustulosis: effect of cyclosporin A treatment on skin testing with recall antigens. *Acta Derm.-Venereol.* **1996**, *76*, 310−313.