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Farnesyltransferase inhibition: a novel method of immunomodulation

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Abstract

Farnesyltransferase inhibitors (FTIs) are anticancer compounds that inhibit Ras GTPases. Since Ras GTPases play key roles in T cell activation and function, we hypothesized that FTIs have immunomodulatory properties and are potential antirejection agents. An investigation was performed on a potent FTI to evaluate this hypothesis in the in vitro setting.

The in vitro effects of the FTI A-228839 were evaluated. Lectin- or antigen presenting cell (APC)-induced lymphocyte proliferation in the presence of A-228839 was measured. The effects of A-228839 on 1E5 T cell polarity were assessed by microscopy. Intracellular calcium ($[Ca^{2+}]_i$) kinetics of lectin-activated lymphocytes was monitored by flow cytometry. The effects of A-228839 on peripheral blood mononuclear cell (PBMC) cytokine production was assessed by a cytometric bead array method. Activation-induced apoptosis was measured with an annexin V staining assay.

A-228839 inhibited lectin-induced proliferation ($IC_{50} = 0.24 \pm 0.11$ μM). The inhibitory effects of A-228839 on lectin induced lymphocyte proliferation were additive to those of CsA. A-228839 was more effective in inhibiting APC-induced T cell proliferation ($IC_{50} = 0.10 \pm 0.09$ μM). A-228839 significantly disrupted the polarized shape of 1E5 T cells at physiologic concentrations. A-228839 altered PBMC baseline $[Ca^{2+}]_i$ but did not affect $[Ca^{2+}]_i$ kinetics during lectin-induced lymphocyte activation. A-228839 inhibited lymphocyte Th1 cytokine production at submicromolar levels and promoted apoptosis in lectin-activated lymphocytes.

A-228839 potently inhibits lymphocyte activation and function. Our results suggest that FTIs may represent a new class of clinically useful immunomodulatory agents. A-228839 has potent in vitro immunomodulatory properties that warrant in vivo evaluation as an antirejection agent.

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1. Introduction

The prototypic small GTPase Ras and its related small GTPase signal transduction proteins play pivotal roles in the regulation of cell activation and cell

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proliferation [1,2]. A significant number of malignancies contain a mutation in Ras that leads to constitutive activation and subsequent unchecked cellular proliferation [3,4]. Hence, Ras represents a logical target for the development of novel antineoplastic agents.

Ras and its related proteins are posttranslationally modified with hydrophobic isoprenyl groups (farnesyl- and geranylgeranylpyrophosphate) that allow their insertion into lipid membranes where they become activated and activate their effector signal transduction proteins [5]. Disrupting this posttranslational modification—farnesylation and geranylgeranylation—leads to the inability of these small GTPases to attach to the lipid membrane where they are activated. Farnesyltransferase inhibitors (FTIs) inhibit the enzyme farnesyltransferase that transfers farnesylpyrophosphate onto the carboxyl end of these signal transduction proteins, and thus this class of compounds represents promising antineoplastic agents [6–9]. Several FTIs are currently being evaluated in clinical trials as anticancer agents and have been found to have less toxicity than conventional cytotoxic chemotherapeutic agents [10,11].

Success of organ transplantation depends, in part, on the suppression of donor reactive lymphocytes in the recipient. The cyclophilin inhibitors, cyclosporine (CsA) and FK 506, effectively suppress lymphocyte activation and currently represent the backbone of maintenance antirejection regimens in organ transplant recipients. However, current antirejection agents have significant side effects that lead to significant morbidity, infections, neoplasia and even mortality [12,13]. Furthermore, these agents fail to prevent the development of chronic rejection, the major cause of long-term graft loss [12,14]. These shortcomings provide the impetus for the discovery and development of novel immunomodulatory agents to be used in clinical organ transplantation.

T lymphocyte activation and effector functions are a requisite upon the proper function of Ras and its related proteins [15–17]. It has been shown that Ras synergizes with calcineurin to activate the nuclear factor of activated T cells (NFAT) [18]. IL-2 production during T cell activation and signals from the IL-2, IL-3 and GM-CSF receptors are mediated by Ras [19,20]. Inactivation of Ras has also been shown in T cell anergy [21]. Furthermore, Ras-related proteins such as Rac and Rho have been implicated in the formation of the immunological synapse between T lymphocyte and antigen presenting cell [22,23]. Immunological synapse formation involves actin-driven migration of membrane microdomains that contain coreceptors to the synapse between T cell and APC [24,25]. In addition to Rac and Rho, Ras also affects actin polymerization and cellular morphology [26,27]. FTIs have also been shown to cause morphological reversion in malignant cells by affecting the actin cytoskeleton [28]. The inhibition of Ras with FTIs may inhibit these early critical events of the T cell activation pathway. Hence, FTIs are not only potential anticancer agents but may also be clinically useful immunosuppressive agents. Here we investigated and defined the in vitro immunomodulatory properties of a potent FTI, A228839. We analyzed the effects of this potent FTI on T cell proliferation, T cell polarized shape, intracellular calcium kinetics, cytokine production and apoptosis.

2. Materials and methods

2.1. Lectin induced lymphocyte proliferation assay

Lymphocytes were isolated from the lymph nodes of euthanized Wistar rats (Charles River Laboratories, Wilmington, MA) under an approved institutional animal subject protocol. Briefly, lymph nodes were procured using sterile microdissection technique and placed in RPMI 1640 media (Life Technologies, Carlsbad, CA). Lymph nodes were teased apart and cellular material was passed through a 70-μm nylon mesh to remove debris. Lymphocytes were washed several times in PBS before resuspending in RPMI supplemented with 10% fetal bovine serum (FBS, Life Technologies), 2 mM L-glutamine (Sigma, St. Louis, MO), 100 U/ml penicillin (Life Technologies), 100 μg/ml streptomycin (Life Technologies), 1% nonessential amino acids (Sigma), 1 mM sodium pyruvate (Sigma) and 100 mM β-mercaptoethanol (Sigma).

Lymphocytes were then dispensed into the wells of a 96-well microtiter plate at a density of 1.5×10^5 cells/well in a volume of 100 μl/well. A-228839 (Abbott Laboratories, Abbott Park, IL) and CsA (Novartis Pharma, Basel, Switzerland) were serially diluted from freshly made stock solutions and added to the wells. Treatments were performed in quadruplicate.

Phytohemagluttinin (PHA, Calbiochem-Novabiochem, San Diego, CA) was added to the wells to obtain a final concentration of 7 μg/ml after a 12-h preincubation with drug. Lymphocytes were allowed to proliferate for 72 h and proliferation was then quantitated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS)-based CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). A microplate reader (Bio-Rad Laboratories, Hercules, CA) was used to measure the absorbances of the wells at $\lambda = 490$ nm. The measured absorbances of the wells which are related to the number of viable cells were averaged and the percent inhibition was calculated as: $100 \times (1 - (OD_{exp} - OD_{-cont})/(OD_{+cont} - OD_{-cont}))$, where exp, −cont and +cont denote experiment, negative and positive control, respectively. Percent inhibitions were determined for all concentrations tested in every experiment and then entered in a simple E_{max} pharmacodynamic model to estimate the IC_{50} using the software WinNonlin version 3.0 (Pharsight, Mountain View, CA).

2.2. Antigen presenting cell: antigen-induced lymphocyte proliferation assay

The murine hen egg lysozyme (HEL)-restricted CD4+ T cell (1E5) [29] and MHC II-restricted B cell (2PK3) hybridomas (a gift from M.D. Cahalan, UC Irvine) were maintained in RPMI 1640 supplemented with the above supplements. 2PK3 APCs were loaded with 100 μg/ml HEL for 12 h. 1E5 T cells (0.5×10^5 cells/well) and HEL-loaded, mitomycin C (Sigma)-treated 2PK3 cells (0.5×10^5/well) were placed in the wells of a 96-well microtiter plate in a volume of 200 μl/well. A-228839 was added in serial dilutions to the wells in quadruplicate immediately after or 1 h after the addition of 2PK3 cells. Proliferation was carried out for 48 h and was then quantitated by the MTS proliferation assay. The IC_{50} was determined as described above for the lectin-induced proliferation assays.

2.3. T cell polarity

In physiological conditions the 1E5T cell has a characteristic polarized shape consisting of head and tail regions. Furthermore, it has been shown that intracellular calcium levels have been related to this polarized shape, and agents that abolish this polarized shape inhibit proper activation of these T cells [30]. To investigate the effects of A-228839 on T cell polarity, 1E5 T cells were dispensed into the wells of a 96-well plate at a density of 0.25×10^5 cells/well and incubated with various concentrations of A-228839 for 8 h. Cells were then fixed in 1% formaldehyde in PBS solution. An inverted microscope was used to count the total number of cells and cells without polarized shape per high-power field. Cells were considered round if they were circular in shape and had no tail; elliptical-shaped cells and cells with identifiable head and tail regions were counted as polarized. Cells from three high-power fields per well were studied, and thus at each drug concentration approximately 400 to 500 cells from a total of nine high-power fields were counted. The average number of cells with polarized shape per high power field for each concentration was compared with untreated controls using the Student's *t*-test.

2.4. T cell $[Ca^{2+}]_i$ kinetics

Lymphocytes were isolated from rat lymph nodes as described above. Lymphocytes were incubated with various concentrations of A-228839 and/or CsA for 8 h prior to assays. The calcium indicator dyes fluo-4 and Fura-red (Molecular Probes, Eugene, OR) were added to cells in PBS at a concentration of 3 μM each. Lymphocytes were incubated with these calcium indicators for 1 h at room temperature protected from light. Cells were then washed three times in PBS and resuspended in RPMI and placed in growth conditions for 15 min.

One million cells of each sample were then analyzed by a FacsCalibur flow cytometer (BD Biosciences Immunocytometry Systems, San Jose, CA) and the mean FL-1 and FL-3 channels recorded every 10 s for a total of 220 s. At least 8000 events were acquired during each 10-s period. The FL-1 and FL-3 detector voltages were initially set so that their values were approximately equivalent on a resting control sample. This arbitrarily set the resting FL-1/FL-3 ratio to be approximately equal to 1, and these settings were not changed for the remainder of the experiment. PHA (20 μg/ml) was added after the initial 30 s (resting period) to activate T cells. The $[Ca^{2+}]_i$ concentration is directly related to the ratio FL-1/FL-3. Data were analyzed using CELLQUEST software (BD Biosciences, Immunocytometry Systems).

2.5. PBMC cytokine production

Human PBMCs were isolated from fresh buffy coats obtained from the UCI Medical Center Blood Bank by Ficoll centrifugation under an approved institutional human subjects protocol. PBMCs (1×10^6 cells/ml) in complete RPMI 1640 media were dispensed into 24-well plates (0.5 ml/well) and incubated with varying concentrations of A-228839 (0–2 μM, 1 well/concentration) for 30 min prior to addition of PHA (7.5 μg/ml). Stimulated PBMCs were placed in growth conditions and supernatant fluids were collected 24 h later. Supernatant fluids were stored at − 80 °C until cytokine measurement.

Frozen supernatant fluids were thawed and centrifuged just prior to analysis. Cytokine measurement was performed with the Human Th1/Th2 Cytokine Cytometric Bead Array Kit (BD Biosciences Pharmigen, San Diego, CA) according to the manufacturer's directions. Cytokine-bound cytometric beads were analyzed on a FacsCalibur flow cytometer. Data output into listmode files was analyzed using the BD CBA Software (BD Biosciences Pharmigen). Cytokine concentration data were entered into an inhibitory effect pharmacodynamic model to estimate the IC_{50}s for each cytokine using the software WinNonlin.

2.6. Apoptosis assay

Lymphocytes were treated with A-228839 8 h prior to simulation with PHA. After 24 h of activation, lymphocytes were incubated with FITC-conjugated annexin V (0.5 μg/ml, BD Biosciences Clontech, Palo Alto, CA) for 15 min and then immediately analyzed on a FacsCalibur flow cytometer. Data were analyzed using CELLQUEST software (BD Biosciences, Immunocytometry Systems).

3. Results

3.1. A-228839 prevents lectin-induced lymphocyte proliferation

Lymphocytes were incubated with A-228839 for 12 h prior to addition of PHA and allowed to proliferate for 72 h. The FTI A-228839 was able to inhibit PHA-induced lymphocyte proliferation in a concentration-dependent manner. Fig. 1a is a representative MTS assay for A-228839. Addition of CsA (25 ng/ml) to varying concentrations of A-228839 results in increased inhibition of proliferation for this drug combination (Fig. 1a). The IC_{50} for A-228839 from three independent proliferation assays is 0.24 ± 0.11 μM (± S.E., Fig. 1b).

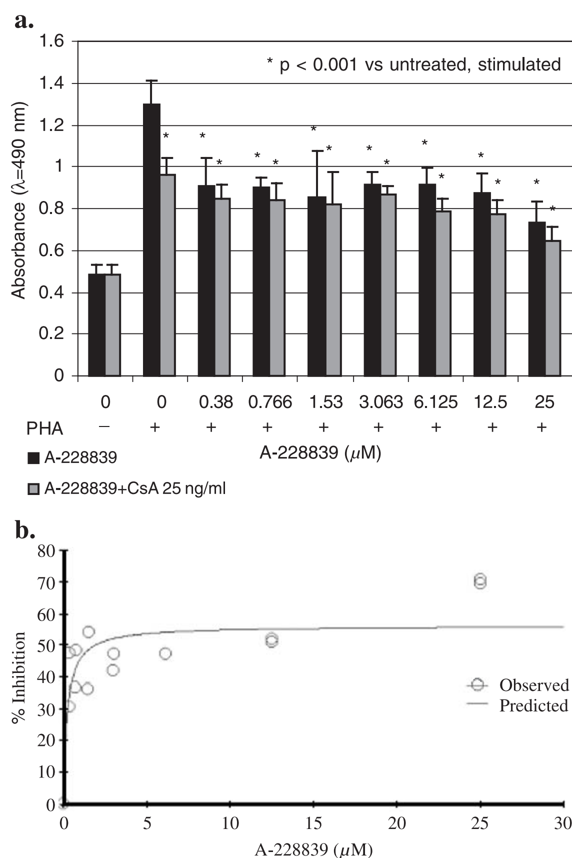

Fig. 1. FTI A-228839 inhibits lymphocyte proliferation. Lymphocytes were treated with A-228839, a potent FTI, prior to stimulation with PHA. Proliferation was carried out for 72 h and quantified with the MTS assay. Absorbance (λ = 490 nm, ± S.D.) is related to the number of viable cells. A representative proliferation assay is shown in (a). FTI and CsA combination results in greater inhibition of PHA induced lymphocyte proliferation. CsA (25 ng/ml) was added to A-228839-treated lymphocytes prior to stimulation with PHA. Data from three independent proliferation experiments are shown in (b). A predicted curve was generated using a simple E_{max} pharmacodynamic model (WinNonlin software) with an IC_{50} (μM, ± S.E.) of 0.24 ± 0.11 μM.

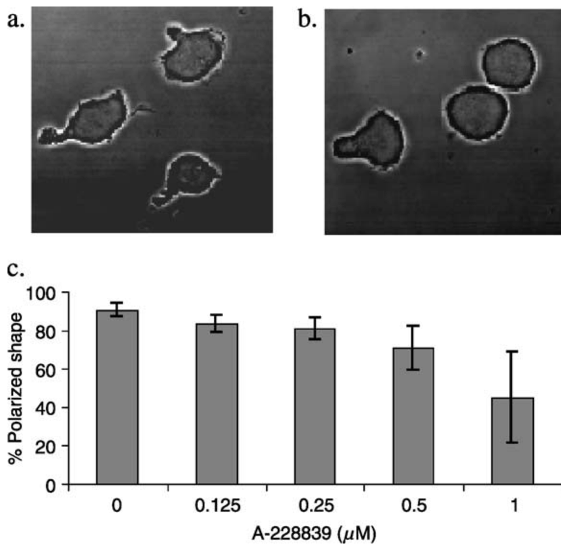

Fig. 2. A-228839 disrupts T cell polarized shape. Photomicrograph of phase contrast images of untreated and A-228839-treated 1E5 T cells (100 ×). Untreated 1E5 T cells have a characteristic polarized shape (a) while A-228839 (1 μM)-treated 1E5 T cells become round (b). Results are representative of three independent experiments. FTI causes a loss of polarized shape in 1E5 T cells in a concentration-dependent manner (c). 1E5 T cells were treated with varying concentrations of A-228839 for 8 h prior to fixation. Cells were inspected under the high power objective of an inverted microscope (100 ×) and only round cells were considered as cells with loss of polarized shape; elliptical cells and cells with tails were considered as polarized. At least a total of 500 cells were counted at each drug concentration. The percent of cells with polarized shape is calculated at each concentration, and Student's t test is used to compare the average percent polarized for each drug concentration to untreated controls. Error bars represent standard deviation. There was a significant difference between drug and control at all concentrations tested ($p < 0.05$). Results are representative of two independent experiments.

3.2. A-228839 prevents APC-induced T cell proliferation

Soluble mitogens such as PHA and anti-TCR antibodies activate T cells and stimulate proliferation; however, such activation does not approximate the in vivo activation by antigen and antigen presenting cells. To determine the effect of FTI on APC-induced T cell proliferation, murine 1E5 T cells were induced to proliferate with 2PK3 APCs loaded with HEL in the presence of varying concentrations of A-228839. A-228839 was able to inhibit APC-induced proliferation with an $IC_{50} = 0.10 \pm 0.09$ μM when added 24 h prior to the addition of 2PK3 APCs (data not shown).

3.3. A-228839 disrupts 1E5 T cell polarized shape

To determine if farnesyltransferase inhibition affected T cell polarized shape, 1E5 T cells were treated with varying concentrations of A-228839 for 12 h and

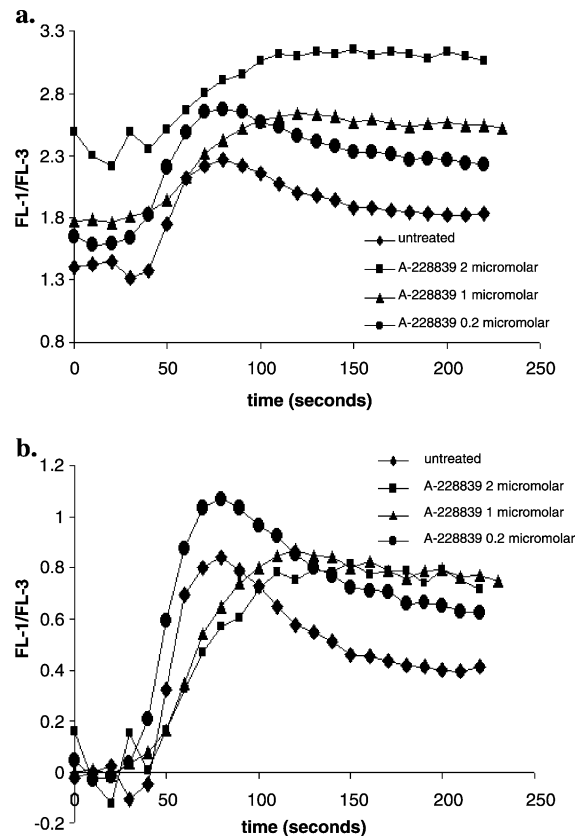

Fig. 3. A-228839 alters lymphocyte $[Ca^{2+}]_i$ homeostasis. Lymphocytes were treated with A-228839 for 12 h and then loaded with fluo-4 and Fura-red. Lymphocytes were stimulated with PHA and studied with a flow cytometer. The FL-1 and FL-3 channel signals were recorded at 10-s intervals and the ratio FL-1/FL-3 is directly related to the $[Ca^{2+}]_i$. Lymphocytes treated with A-228839 demonstrated an increase in their baseline $[Ca^{2+}]_i$ as compared to untreated controls (a). $[Ca^{2+}]_i$ kinetics of A-228839-treated lymphocytes (b). Baseline $[Ca^{2+}]_i$ is subtracted from each curve to allow graphical comparison of $[Ca^{2+}]_i$ change during activation. There was no significant difference in the $[Ca^{2+}]_i$ change in A-228839-treated lymphocytes. Results are representative of three independent experiments.

Table 1
A-228839 inhibits cytokine synthesis in PHA-activated PBMCs

Cytokine	IC_{50} (μM, ± S.E.)
IL-2	0.37 ± 0.08
IFN-γ	0.60 ± 0.22
TNF-α	0.78 ± 0.25
IL-4	0.78 ± 0.44
IL-5	1.0 ± 0.26
IL-10	6.8 ± 9.2

Human PBMCs in complete medium were treated with varying concentrations of A-228839 (0–2 μM) 30 min prior to stimulation with PHA in a 24-well plate. Cytokine levels were measured in supernatant fluids (1 well/concentration) after a 24-h incubation with a cytometric bead analysis method. Experiments were repeated three times and IC_{50}s were determined by inputting all data into an inhibitory effect pharmacodynamic model using WinNonlin software.

then inspected with light microscopy to assess cellular morphology. In Fig. 2a, untreated 1E5 T cells have a characteristic polarized shape containing head and tail regions while A-228839-treated cells have a round, nonpolarized appearance (Fig. 2b). Fig. 2c represents results of a typical polarity experiment; 1E5T cells significantly lose their polarized shape at the lowest concentration tested, 0.125 μM ($p < 0.005$).

3.4. A-228839 alters lymphocyte $[Ca^{2+}]_i$ homeostasis

$[Ca^{2+}]_i$ increases during T cell activation and is a very early event. To determine if farnesyltransferase inhibition affected $[Ca^{2+}]_i$ kinetics and homeostasis, lymphocytes were treated with varying concentrations of A-228839, loaded with fluo-4 and Fura-red, stimulated with PHA and studied on a flow cytometer. Fig. 3a shows representative $[Ca^{2+}]_i$ kinetics of both untreated and A-228839-treated lymphocytes. A-228839 pretreatment caused an increase in the baseline $[Ca^{2+}]_i$ and this increase was related to the concentration of A-228839. Fig. 3b represents a $[Ca^{2+}]_i$ kinetics plot with the average baseline (resting) $[Ca^{2+}]_i$ values subtracted from all data points so that changes in $[Ca^{2+}]_i$ during stimulation can be compared graphically. The changes in $[Ca^{2+}]_i$ from resting to peak $[Ca^{2+}]_i$ after stimulation with PHA were about the same for untreated and treated lymphocytes, suggesting that farnesyltransferase inhibi-

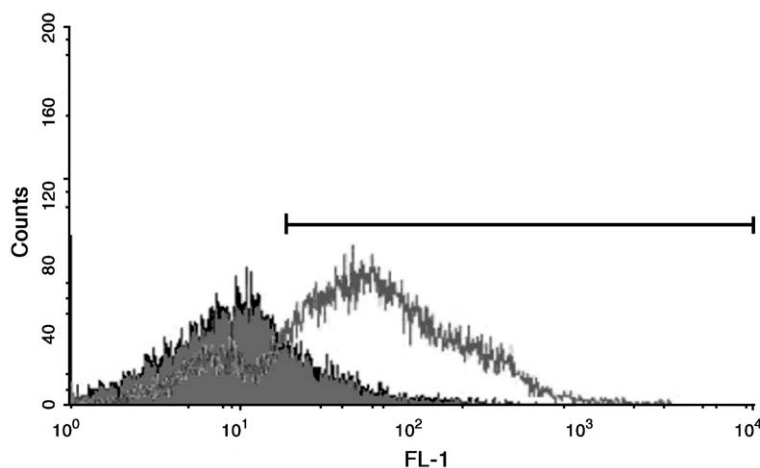

Fig. 4. A-228839 promotes apoptosis in PHA-activated lymphocytes. Lymphocytes were treated with A-228839 (0.5 μM) prior to stimulation with PHA. FITC-conjugated annexin V was then added to lymphocytes 24 h post stimulation and studied with a flow cytometer. At least 10,000 lymphocytes were analyzed on a forward and side scatter-based gate. Cells were considered apoptotic if their FL-1 values were within the linear region as shown. The position of the linear region was determined with unstained, unstimulated and untreated cells. A-228839-treated lymphocytes (overlay) demonstrated greater FL-1 channel signals reflecting increased staining with annexin V–FITC. Approximately 83% of A-228839-treated lymphocytes were apoptotic as compared to 31% of untreated controls. Results are representative of two independent experiments.

tion does not alter the kinetics of $[Ca^{2+}]_i$ during lymphocyte activation.

3.5. A-228839 potently inhibits PBMC cytokine production

To determine if FTIs could inhibit lymphocyte cytokine production, we investigated the effects of A-228839 on lectin-activated PBMC cytokine production. A-228839 inhibited Th1 cytokine production in a concentration-dependent fashion with significant inhibition even at the lowest concentration tested (Table 1). IL-2 and IFN-γ production seemed the most sensitive to A-228839 treatment followed by TNF-α. A-228839 decreased PBMC production of the Th2 cytokines IL-4, IL-5 and IL-10 to a more variable degree (Table 1).

3.6. A-228839 promotes apoptosis in lectin-activated lymphocytes

$[Ca^{2+}]_i$ can regulate both cellular proliferation and apoptosis in T cells [31]. Since our results from the $[Ca^{2+}]_i$ kinetics studies showed that A-228839 raised baseline $[Ca^{2+}]_i$, we determined if this compound could induce apoptosis in lymphocytes. Lymphocytes were treated with A-228839 for 8 h prior to stimulation with PHA, and apoptosis was measured by FITC-conjugated annexin V staining 24 h post-stimulation. A-228839 treatment (0.5 μM) caused a significant increase in the number of activated lymphocytes stained with FITC-conjugated annexin V as demonstrated by a signal shift in the treated lymphocytes to higher FL-1 channel values (Fig. 4). This shift to higher FL-1 signal values reflects a greater fraction of all lymphocytes undergoing apoptosis as compared to the stimulated control (83% vs. 31%).

4. Discussion

In this study we have demonstrated the immunomodulatory properties of a potent FTI, A-228839. This agent is able to inhibit T cell activation signals by the CD2 (PHA induced activation and proliferation) pathway and seems to be more potent in inhibiting T cell activation by the CD3/CD4 (APC-induced activation and proliferation) pathway [30]. A possible explanation for this observation is that T cell activation by soluble mitogens such as PHA bypasses the immunological synapse formation. On the other hand, APC-induced proliferation requires immunological synapse formation, which we hypothesize to be inhibited by Ras inhibitors such as the FTI A-228839. We are currently performing live imaging cell studies to investigate the effects of FTI on immunological synapse formation.

Intracellular calcium increase is a very early event during T cell activation. Ineffective immunological synapse formation, such as that between APC and the tail region of the 1E5 T cell [31], does not result in $[Ca^{2+}]_i$ spikes. On the other hand, robust immunological synapse formation, such as that between APC and the head region of the 1E5 T cell, results in $[Ca^{2+}]_i$ spikes [31,32].

Our observation that A-228839 did not alter $[Ca^{2+}]_i$ kinetics during T cell activation suggests that this agent acts downstream of the $[Ca^{2+}]_i$ signal. Ras is downstream of $[Ca^{2+}]_i$ signals and is indeed dependent on $[Ca^{2+}]_i$ for successful activation [33]. We observed that A-228839 increases the baseline $[Ca^{2+}]_i$, which is suggestive of early apoptosis [34]. It may be that the $[Ca^{2+}]_i$ levels achieved in A-228839-treated lymphocytes after activation program these cells for an apoptotic in lieu of a proliferative fate. The results of our apoptosis studies agree with this hypothesis.

Another possible explanation for the increase in apoptosis in A-228839-treated lymphocytes is that signals from the IL-2 receptor that prevent apoptosis in T cells are mediated by Ras, and it has been shown that T cells transfected with a dominant negative Ras underwent apoptosis when stimulated with IL-2 [35]. Hence, Ras inhibition by A-228839 may prevent rescue signals from the IL-2 receptor in lymphocytes.

The effect of A-228839 on 1E5 T cell polarity indirectly supports our hypothesis that this agent disrupts the actin cytoskeleton. We have shown previously that perillyl alcohol, a geranylgeranyltransferase inhibitor, caused 1E5 T cells to lose their polarized shape and that these round cells failed to activate when placed in contact with an anti-CD3 antibody-coated bead [36]. This loss of polarized shape induced by perillyl alcohol was identical to the effects of cytochalasin D, an anti-actin agent, and dissimilar to the effects of colchicine, an antimicrotubule agent. We

are currently investigating the direct effects of A-228839 on actin polymerization in T cells.

The combination of CsA and A-228839 resulted in an increased inhibition of PHA induced lymphocyte proliferation. Unexpectedly, the inhibition of proliferation by the combination of A-228839 and CsA appeared to be additive but not synergistic. It is not known why A-228839 does not synergize with CsA to inhibit T cell activation since it has been shown that Ras synergizes with calcineurin to regulate NFAT [18].

Finally, as further proof that FTIs inhibit lymphocyte activation and function, we showed that A-228839 alone potently inhibited lectin-induced PBMC cytokine production. The differential inhibitory effects on PBMC cytokine production suggest that the small GTPases that A-228839 inhibits may play a more important role in signaling pathways controlling Th1 versus Th2 cytokine synthesis.

Transplantation allograft rejection and graft versus host disease is an immune process driven by alloreactive lymphocyte activation, proliferation and Th1 cytokine production [37]. Autoimmune-based rheumatologic, vasculitic, nephritic and gastrointestinal diseases also derive from unchecked lymphocyte activation, proliferation and cytokine production [38]. Our in vitro results with the FTI A-228839 suggest that farnesyltransferase inhibition may be a novel target for immunomodulation in these and other clinical settings. We are currently evaluating the efficacy of FTIs such as A-228839 in preventing allograft rejection and other immunological diseases in animal models.

In conclusion, we have shown that the FTI A-228839 potently inhibits lymphocyte activation and function. These immunomodulatory characteristics are distinct from those of CsA and require further investigation to delineate the mechanism of actions of this compound's various effects on lymphocytes. Our findings with A-228839 suggest that FTIs may represent a novel class of clinically relevant immunomodulatory agents.

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References

[1] Pruitt K, Der CJ. Ras and Rho regulation of the cell cycle and oncogenesis. Cancer Lett 2001;171:1–10.
[2] Crespo P, Leon J. Ras proteins in the control of the cell cycle and cell differentiation. Cell Mol Life Sci 2000;57:1613–36.
[3] Li DH. Molecular epidemiology of pancreatic cancer. Cancer J 2001;7:259–65.
[4] Ayllon V, Rebollo A. Ras-induced cellular events (review). Mol Membr Biol 2000;17:65–73.
[5] Sinensky M. Recent advances in the study of prenylated proteins. Biochim Biophys Acta, Mol Cell Biol Lipids 2000;1484: 93–106.
[6] Prendergast GC, Rane N. Farnesyltransferase inhibitors: mechanism and applications. Expert Opin Investig Drugs 2001;10: 2105–16.
[7] Tahir SK, Gu WZ, Zhang HC, Leal J, Lee JY, Kovar P, et al. Inhibition of farnesyltransferase with A-176120, a novel and potent farnesyl pyrophosphate analogue. Eur J Cancer 2000; 36:1161–70.
[8] Tamanoi F, Gau CL, Jiang C, Edamatsu H, Kato-Stankiewicz J. Protein farnesylation in mammalian cells: effects of farnesyltransferase inhibitors on cancer cells. Cell Mol Life Sci 2001;58:1636–49.
[9] Prendergast GC, Oliff A. Farnesyltransferase inhibitors: antineoplastic properties, mechanisms of action, and clinical prospects. Semin Cancer Biol 2000;10:443–52.
[10] Karp JE, Kaufmann SH, Adjei AA, Lancet JE, Wright JJ, End DW. Current status of clinical trials of farnesyltransferase inhibitors. Curr Opin Oncol 2001;13:470–6.
[11] Karp JE, Lancet JE, Kaufmann SH, End DW, Wright JJ, Bol K, et al. Clinical and biologic activity of the farnesyltransferase inhibitor R115777 in adults with refractory and relapsed acute leukemias: a phase 1 clinical-laboratory correlative trial. Blood 2001;97:3361–9.
[12] Valantine-von Kaeppler HA. Prevention of chronic heart allograft rejection. Transplant Proc 1999;31:1288–9.
[13] Jain A, Khanna A, Molmenti EP, Rishi N, Fung JJ. Immunosuppressive therapy. Surg Clin North Am 1999;79:59–76.
[14] U.S. Department of Health and Human Services, Health Resources and Services Administration Office of Special Programs Division of Transplantation Rockville MD and United Network for Organ Sharing, Richmond VA. Annual Report of the U.S. Scientific Registry for Transplant Recipients and the Organ Procurement and Transplantation Network: Transplant Data: 1990–1999; 2002.
[15] Downward J, Graves JD, Warne PH, Rayter S, Cantrell DA. Stimulation of p21ras upon T-cell activation. Nature 1990; 346:719–23.
[16] Franklin RA, Tordai A, Patel H, Gardner AM, Johnson GL, Gelfand EW. Ligation of the T cell receptor complex results in activation of the Ras/Raf-1/MEK/MAPK cascade in human T lymphocytes. J Clin Invest 1994;93:2134–40.

[17] Ohtsuka T, Kaziro Y, Satoh T. Analysis of the T-cell activation signaling pathway mediated by tyrosine kinases, protein kinase C, and Ras protein, which is modulated by intracellular cyclic AMP. Biochim Biophys Acta 1996;1310:223–32.
[18] Woodrow M, Clipstone NA, Cantrell D. P21(Ras) and calcineurin synergize to regulate the nuclear factor of activated T-cells. J Exp Med 1993;178:1517–22.
[19] Rayter SI, Woodrow M, Lucas SC, Cantrell DA, Downward J. P21(Ras) mediates control of Il-2 gene promoter function in T-cell activation. EMBO J 1992;11:4549–56.
[20] Satoh T, Nakafuku M, Miyajima A, Kaziro Y. Involvement of ras p21 protein in signal-transduction pathways from interleukin 2, interleukin 3, and granulocyte/macrophage colony-stimulating factor, but not from interleukin 4. Proc Natl Acad Sci U S A 1991;88:3314–8.
[21] Fields PE, Gajewski TF, Fitch FW. Blocked Ras activation in anergic CD4+ T cells. Science 1996;271:1276–8.
[22] Villalba M, Bi K, Rodriguez F, Tanaka Y, Schoenberger S, Altman A. Vav1/Rac-dependent actin cytoskeleton reorganization is required for lipid raft clustering in T cells. J Cell Biol 2001;155:331–8.
[23] Dustin ML, Cooper JA. The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. Nat Immunol 2000;1:23–9.
[24] Viola A, Schroeder S, Sakakibara Y, Lanzavecchia A. T lymphocyte costimulation mediated by reorganization of membrane microdomains. Science 1999;283:680–2.
[25] Wulfing C, Davis MM. A receptor/cytoskeletal movement triggered by costimulation during T cell activation. Science 1998;282:2266–9.
[26] Rodriguez-Viciana P, Warne PH, Khwaja A, Marte BM, Pappin D, Das P, et al. Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. Cell 1997;89:457–67.
[27] Prendergast GC, Gibbs JB. Pathways of Ras function: connections to the actin cytoskeleton. Adv Cancer Res 1993;62: 19–64.
[28] Prendergast GC, Davide JP, de Solms SJ, Giuliani EA, Graham SL, Gibbs JB, et al. Farnesyltransferase inhibition causes morphological reversion of ras-transformed cells by a complex mechanism that involves regulation of the actin cytoskeleton. Mol Cell Biol 1994;14:4193–202.
[29] Adorini L, Sette A, Buus S, Grey HM, Darsley M, Lehmann PV, et al. Interaction of an immunodominant epitope with Ia molecules in T-cell activation. Proc Natl Acad Sci U S A 1988;85:5181–5.
[30] Owens T, Zeine R. The cell biology of T-dependent B cell activation. Biochem Cell Biol 1989;67:481–9.
[31] Negulescu PA, Krasieva TB, Khan A, Kerschbaum HH, Cahalan MD. Polarity of T cell shape, motility, and sensitivity to antigen. Immunity 1996;4:421–30.
[32] Wei X, Tromberg BJ, Cahalan MD. Mapping the sensitivity of T cells with an optical trap: polarity and minimal number of receptors for Ca(2+) signaling. Proc Natl Acad Sci U S A 1999;96:8471–6.
[33] Zippel R, Balestrini M, Lomazzi M, Sturani E. Calcium and calmodulin are essential for Ras-GRF1-mediated activation of the Ras pathway by lysophosphatidic acid. Exp Cell Res 2000; 258:403–8.
[34] Krebs J. The role of calcium in apoptosis. BioMetals 1998;11: 375–82.
[35] Gomez J, Martinez A, Fernandez B, Garcia A, Rebollo A. Critical role of Ras in the proliferation and prevention of apoptosis mediated by IL-2. J Immunol 1996;157:2272–81.
[36] Wei X, Si MS, Imagawa DK, Ji P, Tromberg BJ, Cahalan MD. Perillyl alcohol inhibits TCR-mediated [Ca(2+)](i) signaling, alters cell shape and motility, and induces apoptosis in T lymphocytes. Cell Immunol 2000;201:6–13.
[37] Le Moine A, Goldman M, Abramowicz D. Multiple pathways to allograft rejection. Transplantation 2002;73:1373–81.
[38] Kroemer G, Martinez C. Cytokines and autoimmune disease. Clin Immunol Immunopathol 1991;61:275–95.