Rapid inhibitory effect of Tacrolimus on T cell migration by suppressing CD29-related functions

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Abstract

Objective
To clarify the direct effect of Tacrolimus (FK506) on T cell function in relation to CD29.

Methods
Human T cell line H9 and phytohemagglutinin (PHA)-activated T cells were incubated with or without Tacrolimus. The cells underwent cell migration assay by using fibronectin-coated trans-wells, and at the same time the degree of adherence by cultured cells to fibronectin-coated plastic wells was measured. For H9 cells, intracellular filamentous actin formation and the cell surface expression of CD3, CD11a, CD25, CD26, CD44, CD29 were measured by using flow cytometry. Intracellular tyrosin-phosphorylation induced by fibronectin by CD29 stimulation in H9 cells was analyzed by immunoblotting.

Results
The ability of H9 cells and PHA-activated Tcells incubated with Tacrolimus for 2 hours (hrs) to migrate and to adhere to fibronectin was significantly suppressed. However, the inhibiton was transient, because the ability of cells incubated with Tacrolimus for 24 hrs to migrate was not affected despite the suppression of cell replication. Tacrolimus showed slight but significant reduction of cell surface expression of CD29 within 4 hrs, but CD3, CD11a, CD25, CD26 and CD44 were not affected. Tacrolimus rapidly inhibited intracellular filamentous actin formation; the maximum inhibition was within 2 hrs and the effect was not observed at 6 hrs. Intracellular tyrosin-phosphorylation induced by CD29 stimulation was also inhibited by Tacrolimus in H9 cells.

Conclusion
Tacrolimus appeared to have transient early phase inhibitory effects on CD29-related function that may be associated with T cell migration.

Key words
Tacrolimus, T cell, H9, CD29, fibronectin, migration.

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Introduction

Rheumatoid arthritis (RA) is a disease accompanied by polyarthritis, synovitis and joint destruction, and whose etiology is unknown. Accumulating evidence suggests that T cells have an important role in establishing and promoting the disease (1-4). Some T cell populations may be related to RA pathophysiology (3,4). T cells, which preferentially accumulate in the synovial fluid of inflamed joints in patients with RA, have high levels of CD29 on their surface (5). Inhibition of signal transduction by CD29 results in the suppression of interleukin-2 (IL-2) mRNA transcription in T cells from synovial fluid of patients with RA *in vitro* (6). CD29 may have some pathological roles in RA. Thus, CD29-related functions of T cells could be the target for RA treatment. CD29 is 1-integrin, which forms a heterodimer with -subunits. The 1-integrins are major adhesion molecules in T cells, and they also act as costimulatory molecules (7,8). Signals by 1-integrins induce various biological events, such as cell migration (9) and cytoskeleton organization (10).

Tacrolimus (FK506) is a derivative of macrolide compounds (11). It is used as an immunosuppressant for graft rejection (12). Its clinical application is now being expanded to treatment of RA (13). The pharmacological effects of Tacrolimus on T cells have been described mainly from its inhibition of T cell activation *in vitro* (14-16). Tacrolimus binds to the FK506-binding protein (FKBP) in T cells and inhibits IL-2 production by inhibiting nuclear factor of activated T cells (NF-AT) transition into the nucleus by inhibiting the dephosphorylation activity of calcineurin (17,18). The net effect of Tacrolimus is a marked inhibition of T cell proliferation. Tacrolimus is almost 100-fold more effective in inhibiting T cell proliferation than cyclosporin A, a calcineurin inhibitor with documented efficacy in treating patients with severe RA (19, 20). However, the mechanism of action of Tacrolimus reported until now is still insufficient to understand its effect in immune systems *in vivo*. Experimental evidence is needed to support its application in RA treatment.

In this study, we examined the direct effect of Tacrolimus on T cells in relation to CD29, and found that it appeared to have rapid and transient inhibitory effects on T cell migration associated with the CD29 function of T cells.

Materials and methods

Cells and reagents

Human peripheral blood mononuclear cells (PBMC) were isolated from healthy volunteer donors by using Ficoll-Hypaque (Pharmacia Biotech Inc., Piscataway, NJ) density gradient centrifugation. Unfractionated mononuclear cells were separated into an E rosette-positive (E+) population and were used as T cells. Monocytes were depleted by adherence to plastic plates for 4 hrs at 37°C and then by incubation with 5 mM L-leucine methyl ester HCL (Sigma, St. Louis, MO) for 1 hr. Purified human T cells were incubated with 10% Fetal bovine serum (FBS)-RPMI media in the presence of 0.5 μg/ml of phytohemagglutinin (PHA) for 24 hrs before assays. Monoclonal human T cell line H9 was purchased from American Type Culture Collection (ATCC). Monoclonal antibodies against CD3, CD11a, CD25, CD26, CD29 and CD44 were purchased from PharMingen (San Diego, CA). Monoclonal anti-phosphotyrosine antibody PT-66 was purchased from Sigma. Tacrolimus was kindly provided from Fujisawa Pharmaceutical Co. (Tokyo, Japan).

Cell migration assay

PHA-activated T cells or H9 cells (100,000 each) were pre-incubated with 0, 1.0 or 10 nM of Tacrolimus at 37°C for 2 or 24 hrs in 5% CO_2/95% air. The H9 cells were cultured with 100 ml of assay medium consisting of 0.6% bovine serun albumin (BSA) in RPMI medium, on 6.5 mm diameter cell culture inserts of trans-wells (pore size 8.0 mm; Costar, Corning, NY) with 0, 1.0 or 10 nM of Tacrolimus at 37°C incubation for 4 hrs in 5% CO_2/ 95% air. For PHA-activated T cells, trans-wells of 3.0 μm pore size were used with the same conditions as for H9 cells. Cells that had migrated into the outer chamber were counted. The assays were done in triplicate.

Cell adhesion assay

Wells in a 24-well plastic plate were coated with 5 µg/ml of fibronectin and left overnight. The wells were washed with phosphate buffered saline (PBS) 3 times and were used for the cell adhesion assay. H9 cells were incubated with 0, 1.0 or 10 nM of Tacrolimus at 37°C for 2 hrs in 5% CO_2/95% air, and non-adherent cells were gently washed out with PBS twice. Adherent cells were collected by adding PBS containing 5 mM ethylenediamine tetraacetic acid (EDTA), and were counted. The assays were done in triplicate.

Flow cytometry

H9 cells or PHA-activated T cells were incubated with 0 or 1.0 nM of Tacrolimus at 37°C for 4 hrs in 5% CO_2/95% air, and then were washed 3 times with PBS. The cells were suspended in 100 µl of 1% BSA-PBS and were further incubated with 5 µg/ml of monoclonal antibodies on ice for 30 minutes. The cells were then washed 3 times with PBS, and further incubated with FITC-conjugated goat anti-mouse IgG (Sigma) at 1:200 on ice for 30 minutes. The cells were washed 3 times with PBS and then underwent flow cytometric analysis. The assays were done in triplicate.

To analyze intracellular filamentous actin formation, H9 cells exposed to 0.1 nM, 0.5 nM, 1.0 nM and 10 nM concentrations of Tacrolimus for 2 hrs, or H9 cells exposed to 1.0 nM of Tacrolimus for 0.5 hr, 1.0 hr, 2.0 hr, 4.0 hr and 6.0 hr durations were stained with 50 ng/ml tetramethylrhodmine isothiocyanate (TRITC)-conjugated phalloidin (Sigma) that specifically stains the filamentous actin cytoskeleton and were analyzed by using a FACS Caliber[21] (Becton Dickinson, San Jose, CA).

Immunoprecipitation study of phosphotyrosine

Wells in a plastic plate were coated with 5 µg/ml of fibronectin (Sigma) and were left overnight. The wells were washed with PBS 3 times and were used for H9 cell stimulation. H9 cells were incubated with 0 or 1.0 nM of Tacrolimus on the fibronectin-coated wells

at 37°C for 30 minutes in 5% CO_2/95% air. Cells were lysed by RIPA lysis buffer, and phosphorylated tyrosine in lysates were immunoprecipitated with PT-66 and protein A-Sepharose (Bio-Rad, Hercules, CA). The immunoprecipitates were separated by sodium dodecyl sulfate – polyacrylamide gel electrophoresis and were electrophoretically transferred onto polyvinylidene membranes (NEN, Boston, MA). Immunoblotting was done with PT-66 diluted with 1% BSA-PBS at 1:2000, and horse radish peroxidase-conjugated anti-mouse antibodies (Amersham, Arlington Height, IL) and chemiluminescence reagents (ECL, AmershamPharmacia Biotech, Piscateway, NJ) according to the instructions provided. Tyrosine-phosphorylated proteins were visualized by using a Midnight-Sun Camera (Fuji Film, Tokyo, Japan).

Cell viability

A trypan blue dye exclusion test was used to assess the cell viability. In all experiments, the viability was over 90% at each point measured.

Statistical analysis

A comparison of the difference between the results in the presence and absence of Tacrolimus was done using the Student's t-test. P values less than 0.05 were significant.

Results

Effect of Tacrolimus on fibronectin-mediated cell migration of T cells

To understand the biological effect of Tacrolimus in relation to CD29, we tested the effect of Tacrolimus on fibronectin-mediated cell migration of PHA-activated T cells prepared from healthy volunteers. The migration ability of PHA-activated T cells without Tacrolimus differed from donor to donor: the proportion of migrated cells was 2.5–42%. The ability of T cells from each donor, pre-incubated with Tacrolimus for 2 hrs, to migrate was suppressed in a dose-dependent manner (Table I).

Tacrolimus (10nM) gave a maximum inhibition of migration of T cells from three of four donors tested, and the inhibition of migration of T cells from the fourth donor (Donor 3) was maximum at 1.0 nM. A marked inhibition of migration was seen for T cells from Donors 2 and 3, at 10 nM and 1.0 nM respectively, but migration of T cells

Table I. Cell migration assay of PHA-activated T-cells from healthy donors.

Donor	Incubation time with Tacrolimus	Migrated cell number (x 10^3) Concentration of Tacrolimus		
		0 nM	1.0 nM	10 nM
Donor 1	2 hrs.	12.0 ± 2.0 (0)*	10.5 ± 0.9 (12.5)	4.6 ±0.8 (61.7)
Donor 2	2 hrs.	42.0 ± 1.5 (0)	41.5 ± 1.8 (1.2)	14.0 ±0.7 (66.7)
Donor 3	2 hrs.	2.5 ± 0.3 (0)	1.5 ± 0.3 (40.0)	1.7 ±0.5 (32.0)
Donor 4	2 hrs.	8.3 ± 1.0 (0)	4.5 ± 0.8 (45.8)	2.7 ±0.6 (67.5)
Donor 3	24 hrs.	13.3 ± 0.9 (0)	10.7 ± 0.9 (19.5)	not done
Donor 4	24 hrs.	27.7 ± 3.3 (0)	37.3 ± 5.6 (0.0)	36.3 ±4.0 (0.0)

* % Inhibition
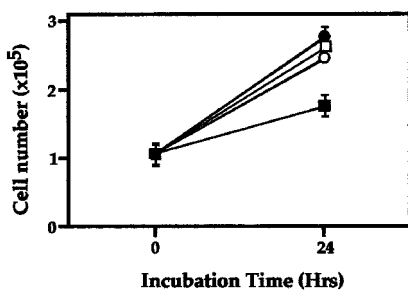
Table II. Cell migration assay of H9 cells.

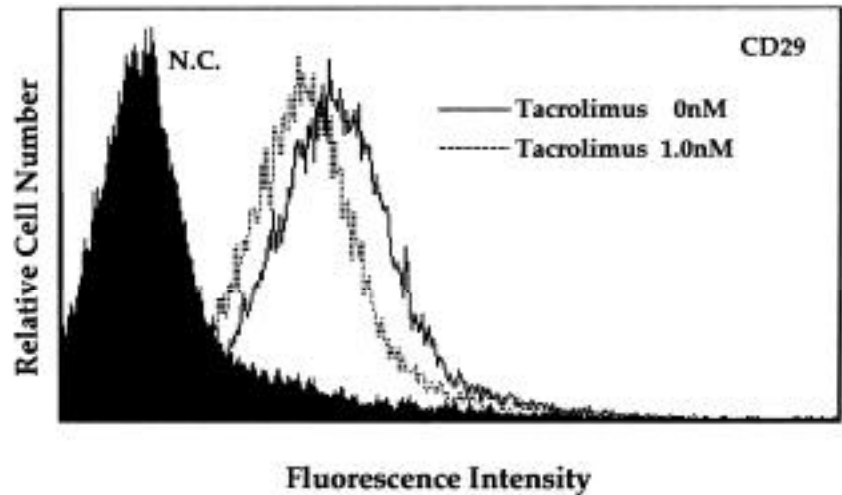| Incubation time with Tacrolimus	Migrated cell number Concentration of Tacrolimus		
	0 nM	1.0 nM	10 nM
2 hrs.	720 ± 80 (0)*	240 ± 22 (66.7)	60 ± 4 (91.7)
24 hrs.	600 ± 53 (0)	560 ± 61 (6.7)	605 ± 45 (0.0)

* % Inhibition

pre-incubated with Tacrolimus for 24 hrs was not affected (Table I). For H9 cells, the basic migration ability without Tacrolimus was less than 1% (0.6 – 0.7%). The migration of cells pre-incubated with Tacrolimus for 2 hrs was suppressed dose dependently and a marked inhibition was at 1.0 nM (Table II), but the migration of cells pre-incubated with Tacrolimus for 24 hrs was little affected (Table II). Tacrolimus suppressed the growth of H9 cells incubated for 24 hrs (Fig. 1): the percentage inhibition of H9 cell growth in 0.1 nM, 1.0 nM, and 10 nM Tacrolimus was 5.6%, 16.7%, and 65.6%, respectively. Asignificant suppression of H9 cell growth by Tacrolimus was at a concentration of 10 nM (p<0.01).

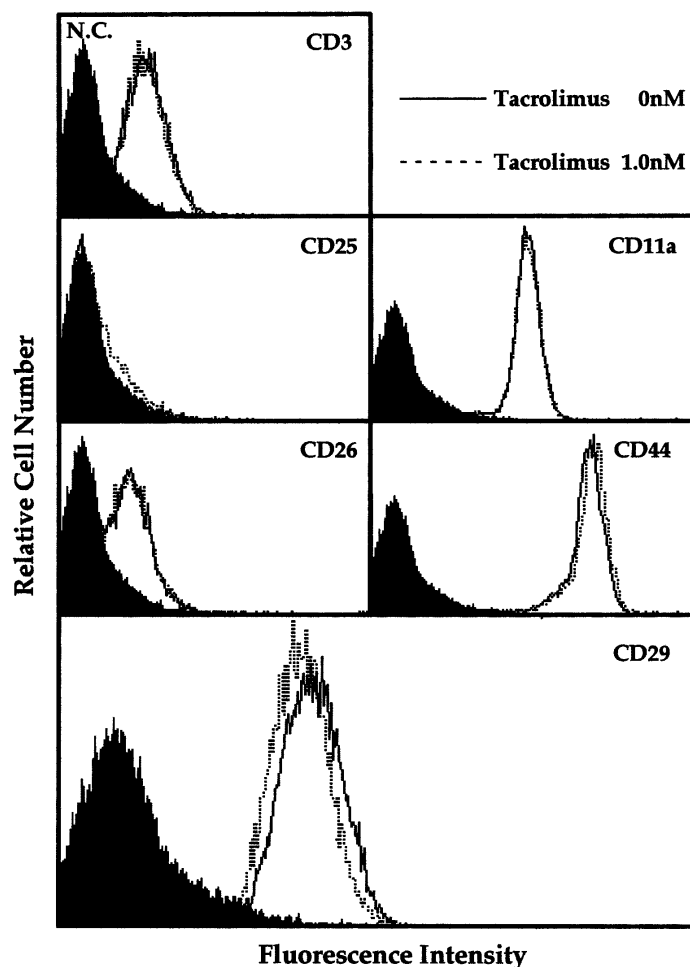*Effect on cell surface expression of CD29 in T cells*

To investigate the effect of Tacrolimus on cell surface expression of CD29, which is the receptor for fibronectin, in PHA-activated T cells or H9 cells, we analyzed by FACS with monoclonal antibodies against CD29. Tacrolimus (1nM) showed a slight but significant inhibition of cell surface expression of CD29 both on PHA-activated T cells and H9 cells (Figs. 2, 3). The mean fluorescence intensity (MFI) of CD29 expression in PHA-activated T cells with and without Tacrolimus was 17.1 ± 1.0 and 12.9 ± 1.8, respectively, and the inhibition proportion was 26.5% (p < 0.05). For H9 cells, the MFI of CD29 expression with and without Tacrolimus was 22.1 ± 1.3 and 26.4 ± 2.1, re-

Fig. 2. Effect of Tacrolimus on the cell surface expression of CD29 in PHA-activated T cells. Flow cytometric analysis was carried out as described in the Materials and Methods. The cell surface expression of CD29 was examined on PHA-activated T cells from 5 healthy individuals, and a representative result is shown. Profiles for the presence and absence of 1.0 nM of Tacrolimus are shown. A negative control (N.C.) was the profile examined in the absence of the primary antibody.

Fig. 1. Effect of Tacrolimus on the cell growth of H9 cells. H9 cells (100,000) were cultured with various concentrations of Tacrolimus (● 0 nM, ❏ 0.1 nM, ◯ 1.0 nM, ■ 10 nM) . The cell number was counted after 24 hrs of incubation. Values are the mean ± SD of 3 independent experiments.

Fig. 3. Effect of Tacrolimus on the expression of cell surface molecules in H9 cells. Flow cytometric analysis was carried out as described in the Materials and Methods. Results from the H9 cells are shown for CD3, 11a, 25, 26, 29 and 44 molecules examined. Profiles for the presence and absence of 1.0 nM of Tacrolimus are shown. A negative control (N.C.) was the profile examined in the absence of the primary antibody.

spectively, and inhibition proportion was 16.3% (p<0.05). To verify the specific effect of Tacrolimus on CD29, we further examined the effect of Tacrolimus on other cell surface molecules on H9 cells. Only the expression of CD29 on H9 cells was markedly affected after incubation with 1.0 nM of Tacrolimus for 4 hrs (Fig. 3), but the expression levels of CD3, CD25, CD26, CD11a, and CD44 were not affected.

Effect on fibronectin-mediated cell adhesion

As cell adhesion is necessary for the initial process of cell migration, we measured the effect of Tacrolimus on fibronectin-mediated cell adhesion of PHA-activated T cells and H9 cells. Fibronectin is the major ligand for CD29, and mediates T cell adhesion in tissues *in vivo* (22, 23). The ability of fibronectin-mediated cell adhesion was inhibited by Tacrolimus in a dose-dependent manner in PHA-activated T cells and H9 cells (Fig. 4). PHA-activated T cell numbers that adhered to fibronectin-coated plastic wells in the presence of 0 nM, 1.0 nM, and 10 nM of Tacrolimus were 58333 ± 5773, 43333 ± 6737, and 25000 ± 1060, respectively (Fig. 4A). H9 cell numbers that adhered to fibronectin-coated plastic wells in the presence of 0 nM, 1.0 nM, and 10 nM of Tacrolimus were 45000 ± 5000, 30000 ± 200, and 17500 ± 2886, respectively (Fig. 4B).

Effect on intracellular filamentous actin formation

CD29 has an integral role in cell adhesion *in vivo*, and the formation of intracellular filamentous actin is associated with cell adhesion. Thus, we examined the effect of Tacrolimus on intracellular filamentous actin formation. Tacrolimus (1 nM) significantly inhibited intracellular filamentous actin formation dose dependently within 2 hrs (20.8% inhibition, p<0.05) (Fig. 5A). The inhibition proportions of intracellular filamentous actin formation in H9 cells detected by phalloidin in the presence of 0 nM, 0.1 nM, 0.5 nM, 1.0 nM and 10 nM of Tacrolimus were 0%, 0%, 13.1%, 20.8% and 12.6%, respectively. The time course showed that the maxi-
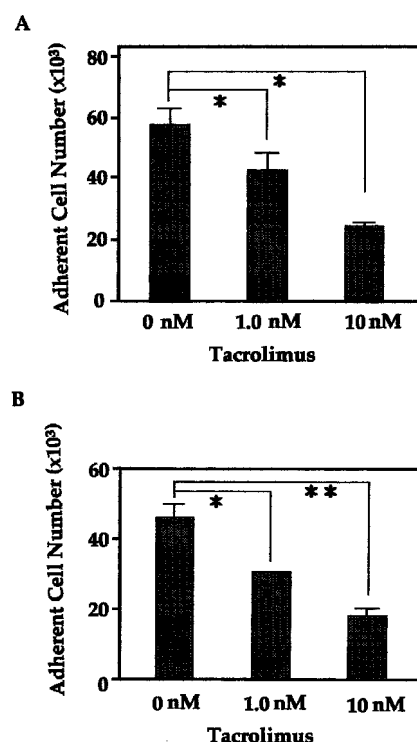
Fig. 4. Effect of Tacrolimus on PHA-activated T cell and H9 cell adhesion to fibronectin. PHA-activated T cells (**A**) and H9 cells (**B**) were cultured on a dish with solid-phase immobilized fibronectin in the presence or absence of Tacrolimus as described in the Materials and Methods. Data are from 3 independent experiments. The means and SDs of 3 experiments are shown (*p < 0.05; **p < 0.01).

mum inhibitory effect of Tacrolimus was within 2 hrs of incubation, and then the inhibitory effect gradually diminished. The inhibition proportions of intracellular filamentous actin formation in H9 cells in the presence of 1.0 nM of Tacrolimus at 0 hr, 0.5 hr, 1 hr, 2hr, 4hr and 6hr were 0%, 25.6%, 27.0%, 36.0%, 22.2% and 1.9%, respectively (Fig. 5B).

Effect on tyrosine phosphorylation in H9 induced by fibronectin stimulation

CD29 forms very late antigen (VLA) with -subunits, and is the receptor for extracellular matrix proteins such as fibronectin and is a co-stimulatory molecule transducting signals for cell activation(22,23). We used immunoprecipitation and immunoblotting to examine the effect of Tacrolimus on signaling mediated by CD29 using fibronectin-coated culture plates. Tyrosine phosphorylation (pp105 and pp59) induced

by fibronectin was partially inhibited by the presence of 1 µg/ml of anti-CD29 antibody in our system (Fig. 6). Phosphorylated-tyrosine in H9 was markedly reduced by the presence of Tacrolimus.

Discussion

Tacrolimus is widely used as an immunosuppressant in organ transplantation. It is beneficial and has a lifesaving effect for patients who receive organ transplantation (12). Tacrolimus is a 23-member ring macrolide lactone and belongs, together with cyclosporin A and rapamycin, to a unique class of immunosuppressive natural products whose major pharmacological attributes stem from an ability to perturb certain intracellular signal transduction processes of T cell activation. Tacroli-
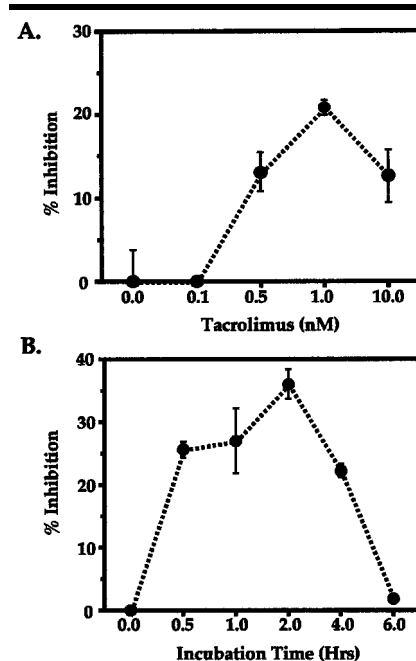
Fig. 5. Effect of Tacrolimus on intracellular filamentous actin formation in H9. Intracellular filamentous actin detected by phalloidin was examined by FACS analysis as described in the Materials and Methods. To observe the dose effect of Tacrolimus, concentrations 0, 0.1, 0.5, 1.0 and 10 nM of Tacrolimus were tested for the study (**A**). For a time course study, H9 cells were cultured with 1.0 nM of Tacrolimus for incubation times 0, 0.5, 1.0, 2.0, 4.0 and 6.0 hrs (**B**). The results are shown as the proportion of inhibition calculated by the mean fluorescence intensity of 0 nM of Tacrolimus and 0.0 hour incubation time as 0% inhibition in **A** and **B**, respectively. The means and SDs of triplicate cultures at each point are shown.

Fig. 6. Tyrosine-phosphorylation of H9 induced by fibronectin stimulation. H9 cells were stimulated by solid-phase immobilized fibronectin, as described in the Materials and methods. After 30 minutes of incubation with or without 1.0 nM of Tacrolimus, cells were collected and underwent immunoprecipitation followed by immunoblotting to detect phosphorylated-tyrosine. For a contrast study, we detected phosphorylated-tyrosine in the presence of anti-CD29 antibody as a competitor for fibronectin stimulation (2nd lane). The heavy chain of antibody PR-66 was shown as Ig.
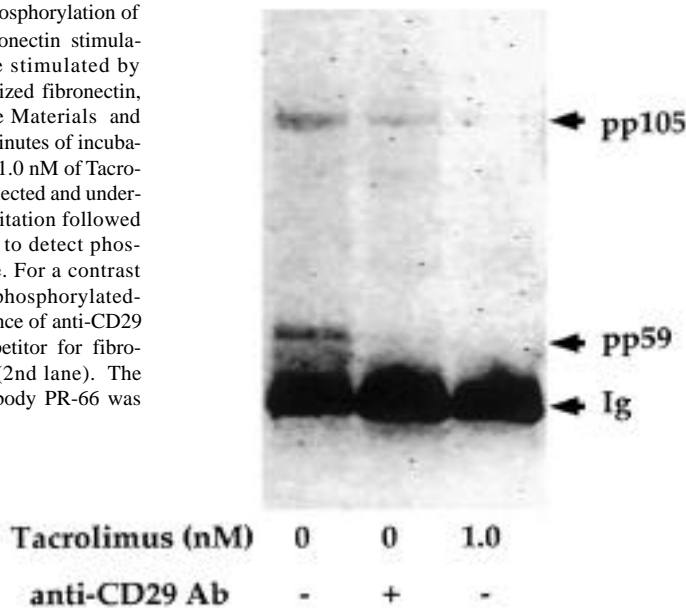
mus binds to FKBP and is a calcineurin inhibitor(17, 18). Calcineurin dephosphorylates NF-AT and results in the inhibition of mRNAtranscription of lymphokine genes, such as IL-2. Thus, the unique immunosuppressive effect of Tacrolimus is anticipated to apply to the treatment of autoimmune diseases(13, 24, 25). The efficacy of Tacrolimus for severe RA has been reported(26, 27), and its therapeutic usage for intractable RA will be approved in Japan and the US. However, its mechanism of effectiveness is unclear. We need to clarify the mechanism of action of Tacrolimus in relation to RA pathophysiology and verify the effectiveness of using Tacrolimus to treat RA.

CD29 is a major cell adhesion molecule expressed on T cells. Its function has been thoroughly investigated and it is indispensable to the immune system, especially for cell adhesion and cell migration (7-10). However, its pathological meaning has been discussed in autoimmune diseases such as RA(5, 6). T cells, which preferentially accumulate in the synovial fluid of inflamed joints in patients with RA, have high levels of CD29 on their surface(5). The suppression of CD29-positive T cell function, which may be cell migration or cytokine production, could lead to a cure of RA.

In our study, 1.0 – 10 nM of Tacrolimus suppressed the fibronectin-mediated migration ability of PHA-activated T cells and H9 cells. Tacrolimus at 1.0 nM – 10 nM inhibits of IL-2 production *in vitro* (28, 29), and our results showed almost the same concentration of Tacrolimus. To clarify the mechanism of migration suppression of these cells, we investigated the effect of Tacrolimus focusing on CD29-related functions. The cell migration process in our assay consisted of two phases: adhesion to a fibronectin-coated membrane and cell migration into a lower chamber.

First, we examined the effect of Tacrolimus on CD29-related cell adhesion. Tacrolimus (1-10 nM) inhibited cell adhesion to plastic wells coated with fibronectin, which is the ligand for CD29. Tacrolimus (1-10 nM) also suppressed the expression of CD29 in PHA-activated T cells and in H9 cells within 4 hrs of incubation. Karlsson described the down-regulation of CD-29 expression by Tacrolimus (30). However, he did not observe a direct effect of Tacrolimus on T cells. He observed the down regulation of CD29 expression in peripheral lymphocytes by Tacrolimus in an *in vitro* coculture system with activated endothelial cells. Our results clearly indicated that Tacrolimus has a direct inhibitory effect on CD29 expression in T cells without endothelial cells. Tacrolimus specifically suppressed the expression level of CD29 but it did not affect the cell surface expression levels of CD3, CD11a, CD25, CD26, and CD44. The inhibition of cell adhesion to fibronectin by Tacrolimus may be shown by suppression of the cell surface expression of CD29, but this suppression was slight and is not fully explainable as a mechanism of suppression of CD29-fibronectin-mediated T cell migration. Next, we examined the qualitative effect of Tacrolimus on CD29 by measuring the change in the cytoskeletal organization. Tacrolimus inhibited filamentous actin formation in H9 cells. For cell adhesion and migration, cells need to alter their conformation of the intracellular cytoskeleton (21). Tacrolimus suppressed CD29-fibronectin-mediated cell adhesion and migration also by affecting the cytoskeleton organization. Interestingly, this inhibition by Tacrolimus was in the early phase of incubation and it was reversible. The inhibition was within 4 hrs of incubation, and it was not observed later than 6 hrs. However, Tacrolimus inhibited cell growth at 24 hrs of incubation. We do not know the precise mechanism of the inhibition by Tacrolimus of filamentous actin formation at the moment. The transient inhibitory effect may suggest that an intracellular active form of Tacrolimus persists for a short period. Tacrolimus binds to FKBP and inhibits dephosphorylation of NF-AT transcription factor. If our results depend on the FKBP process, the turnover of the Tacrolimus-FKBP complex may be rapid. However, the intracellular kinetics of Tacrolimus is not clear, and we cannot deny the possibility that Tacrolimus affects cells independent of the FKBP process. Our study also showed that Tacrolimus inhibits tyrosine-phosphorylation in H9 cells induced by CD29-fibronectin stimulation. Phosphorylated tyrosine detected by PT-66 seems to correspond to Cas-L for pp105 (31, 32) and fyn for pp59 (33, 34) according to their putative molecular size. Thus, the inhibition by Tacrolimus of H9 cell adhesion and its suppression of filamentous actin formation is compatible with the results of our previous study in which Cas-L was essential for CD29-mediated signal trans-

duction that induces T cell migration (35).

In this study, we showed that Tacrolimus inhibits T cell migration by suppressing CD29 expression on the cell surface and inhibiting the signal transduction produced by CD29 stimulation. As CD29-positive T cells are preferentially attracted to the inflamed joints in RA, Tacrolimus appeared to be one of the rational therapeutic options caused by RA. Furst *et al*. (26) and Kremer *et al*.(27) assessed the efficacy of Tacrolimus in patients with RA. Furst *et al*. showed that patients resistant to or intolerant to methotrexate received 1, 3 or 5 mg/day of Tacrolimus as a single oral dose once a day for six months after discontinuation of methotrexate. Tacrolimus reduces the disease in methotrexate-resistent or methotrexate–intolerant patients with RA, and a dose response was observed when efficacy and toxicity were assessed at different doses. Kremer *et al*. showed that patients received 3 mg/day of Tacrolimus as a single oral dose once a day for six months, and they suggested a clinical benefit of Tacrolimus in combination with methotrexate.

In this study, we found a transient dose-dependet inhibition by Tacrolimus of T cell migration, but the mechanism is unknown. We speculate that a frequent increase in serum concentration of Tacrolimus may effectively suppress T cell migration, which may support the idea that administration of tacrolimus should be divided in a day. Our data may also support the idea of tacrolimus as an anti-inflammatory drug. From this perspective Tacrolimus could be beneficial for use as a first line medicine, especially for patients with early stages of active RA.

Acknowledgments

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