FEBS Letters 430 (1998) 414–418 FEBS 20467

FK506 (tacrolimus) inhibits extravasation of lymphoid cells by abrogating VLA-4/VCAM-1 mediated transendothelial migration

Shinobu Tsuzuki[a,b], Noriko Toyama-Sorimachi[a], Fujiko Kitamura[a], Yoshimi Tobita[a], Masayuki Miyasaka[c,*]

[a] *Department of Immunology, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan*
[b] *First Department of Internal Medicine, Nagoya University, School of Medicine, Nagoya, Japan*
[c] *Department of Bioregulation, Biomedical Research Center, Osaka University Medical School, 2-2, Yamada-oka, Suita 565-0871, Japan*

Received 20 May 1998; revised version received 2 June 1998

Abstract Extravasation is a critical process for the physiological lymphocyte traffic as well as the hematogenous spread of malignant hemopoietic cells. Here we report that abrogation of calcineurin activity leads to in vitro transendothelial migration and in vivo infiltration of human lymphoma Nalm-6 cells, which are associated with the abrogation of the VLA-4/VCAM-1 mediated pathway. Rapamycin, which can antagonize FK506 but not CsA to inhibit calcineurin, abrogates FK-506 mediated but not CsA mediated inhibition of in vitro transendothelial migration. FK506 may exert its potent immunosuppressive action partly by inhibiting VLA-4/VCAM-1 mediated transendothelial migration or insinuation of lymphoid cells to tissues.

Key words: Transendothelial migration; Lymphocyte; FK506; Calcineurin; VLA-4; VCAM-1

1. Introduction

Extravasation is an essential process in lymphocyte homing as well as systemic spreading of malignant hemopoietic cells. It involves adhesion of hemopoietic cells to the vascular endothelium [1] and transmigration through intercellular junctions [2].

FK506 is known as a potent immunosuppressive agent, and its mechanism of action is thought to be due to inhibition of calcineurin phosphatase [3,4]. The inhibition of calcineurin phosphatase in T cells leads to impairment of transcription of the IL-2 gene, and thus results in immunosuppression. In addition, FK506 has also been reported to inhibit migration of neutrophils on coated vitronectin by affecting the affinity of vitronectin receptor [5,6]. Accordingly, we postulated that the potent immunosuppressive action of FK506 is partly due to integrin mediated migration of immunocompetent cells.

Here we report that FK-506 inhibits VLA-4/VCAM-1 mediated events in vitro and in vivo and suggest that this is at least partly relevant to the immunosuppressive action of this agent.

2. Materials and methods

2.1. Cell lines

Nalm-6, a human pre-B cell line, was maintained in RPMI 1640 medium (Flow Labs., Irvine, UK) containing 10% fetal calf serum, 10 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, 10^{-4} M 2-mercaptoethanol, 1% (v/v) 100×non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin (complete RPMI). KOP2.16, an endothelial cell line derived from mouse peripheral lymph nodes, was maintained as described previously [7].

2.2. Mice

SCID (CD-17 scid/scid) mice aged 7 weeks were purchased from Charles River Japan (Atsugi, Japan). They were used according to the guidelines of the Animal Ethics Committee of the Institute.

2.3. Reagents

FK506 in crystalline form or containing a carrier solvent (HCO-60 and D-mannitol) was obtained from Fujisawa Pharmaceutical (Osaka, Japan), rapamycin from Calbiochem (La Jolla, CA), and cyclosporin A (CsA) from Sandoz (Basel, Switzerland). FK506 in crystalline form (1 mg/ml), rapamycin (1 mg/ml) and CsA (10 mg/ml) were dissolved in dimethyl sulfoxide (DMSO) and diluted to the desired concentration immediately before use. FK506 containing carrier solvent was diluted in phosphate buffered serum (PBS) and used for in vivo treatment.

2.4. Monoclonal antibodies

Hybridomas producing SG/19 (anti-human integrin β1) [8], SG/73 (anti-human VLA-4α) [8], KH/33 (anti-human VLA-5α) [8], M/K-2 (anti-murine VCAM-1) [9], and KM201 (anti-murine CD44) [10] were generous gifts from Dr. K. Miyake, Saga University Medical School (Saga, Japan). GoH3 (anti-human VLA-6α) was purchased from Immunotech (Marseille, France). Leu-12 (anti-human CD19) conjugated with FITC was from Becton-Dickinson (Mountain View, CA). SG/19 and SG/73 were purified from ascitic fluid produced in nu/nu mice using caprylic acid, as described [11].

2.5. Assays for interactions between lymphoma cells and endothelial cells

Binding was assessed as described previously [2] at 15 min after Nalm-6 cells had been added on KOP2.16 cells. Transmigration was assessed microscopically at 90 min after Nalm-6 cells were added onto KOP2.16 cells, when the number of transmigrated Nalm-6 cells reached a plateau [2]. The effects of FK506, CsA, or rapamycin were examined as follows. Nalm-6 cells or KOP2.16 cells were preincubated with the agent alone or in combination at the indicated concentrations for 2 h at 37°C, then without washing, Nalm-6 cells were added onto the KOP2.16 monolayer, and binding or transmigration was assessed in the presence of the test agent. We also studied the effects of mAbs. Nalm-6 cells or KOP2.16 cells were incubated with 10 μg/ml of the indicated antibody for 30 min, then binding and transmigration were assessed in the continued presence of the mAb.

2.6. Calcineurin phosphatase assay

Phosphatase activity was measured as ^{32}P release from ^{32}P-labeled substrates as described previously [4,12].

2.7. Inoculation of Nalm-6 cells into SCID mice

Nalm-6 cells (1×10^7) in 0.1 ml of PBS were inoculated intravenously (i.v.) into SCID mice via the tail vein. Mice were killed at the indicated times, the gross anatomy was surveyed, and tissues were removed for flow cytometry and immunohistochemical analysis. In some experiments, SCID mice were inoculated i.v. with Nalm-6 cells treated with FK506 (100 ng/ml, 37°C, 2 h), and FK506 (3 mg/kg of body weight) was given subcutaneously (s.c.) every day from one day before Nalm-6 cells inoculation to the day of death. Control mice were inoculated i.v. with Nalm-6 cells treated with the equivalent

*Corresponding author. Fax: (81) (6) 879-3979.
E-mail: mmiyasak@orgctl.med.osaka-u.ac.jp

PII: S0014-5793(98)00703-0

concentration of diluent, and the equivalent dose of carrier solvent was given s.c. Since Nalm-6 cells express a high level of human CD19, cell suspensions obtained from the bone marrow and spleen were labeled with mAb Leu-12 (anti-CD19) conjugated with FITC, then evaluated by flow cytometry on an EPICS-Elite (Coulter, Hialeah, FL). We also recorded the day when mice became paraplegic, an indicator of the involvement of Nalm-6 cells in the central nervous system.

2.8. Statistical analysis

Differences in the percentage of Nalm-6 cells between FK506 treated mice and control mice were examined for statistical significance using a two-tailed non-parametric Wilcoxon's test. The proportion of mice that developed paraplegia was calculated by the Kaplan-Meier method [13], and the difference between proportion curves was analyzed by the log-rank test. We considered differences significant at $P < 0.05$.

3. Results

3.1. FK506 inhibits transmigration by affecting both Nalm-6 cells and KOP2.16 cells

When Nalm-6 lymphoma cells were added onto a confluent KOP2.16 endothelial cell monolayer, they initially bound to the surface of endothelial cells and then swiftly transmigrated underneath them [2]. To determine whether FK506 inhibits these processes, cells were first treated with FK506 (100 ng/ml, 2 h), and assessed for binding to and transmigration underneath endothelial cells in the continued presence of the agent. As shown in Fig. 1, only treatment with FK506 of both Nalm-6 cells and KOP2.16 cells inhibited transmigration. Treatment of either of the cell types with FK506 did not result in inhibition. Interestingly, FK506 did not reduce the number of Nalm-6 cells bound on the surface of KOP2.16 cells (Fig. 1), suggesting that FK506 inhibited events critical for transmigration after adhesion of the cells.

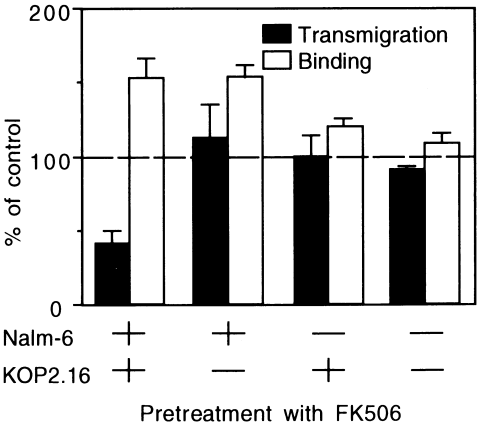

Fig. 1. FK506 inhibits transendothelial migration but not adhesion of Nalm-6 cells. Nalm-6 and KOP2.16 were pretreated with FK506, and transmigration or adhesion of cells was evaluated in the presence of FK506. Cells pretreated with an equivalent concentration of diluent were used as control. Data are results of three independent experiments and are expressed as mean ± S.D. of percent of control calculated as follows: % control = (migration or binding of FK506 pretreated cells in the presence of FK506/migration or binding of control cells in the presence of the diluent alone) × 100.

3.2. Correlation of calcineurin phosphatase activity with transmigration

We next examined if the observed inhibition of transmigration by FK506 was due to inhibition of calcineurin phosphatase activity. After treatment of Nalm-6 cells or KOP2.16 cells with various concentrations of FK506 or CsA for 2 h, cell lysates were prepared and calcineurin phosphatase activity was measured (Fig. 2B). We also studied the effects of such treatment on transmigration. Similar dose-response curves for inhibition of calcineurin activity and transmigration was observed using either FK506 or CsA (Fig. 2A,B), indicating that the inhibition of calcineurin phosphatase activity by FK506 or CsA closely correlated with the inhibition of transmigration. Such inhibition by FK506 was evident at a concentration of 1 ng/ml and reached a plateau at 10 ng/ml. Similarly, inhibition of calcineurin activity commenced at a comparable dose, and 70–80% inhibition was observed at 10 ng/ml in Nalm-6 cells or KOP2.16 cells. Similar results were obtained with CsA, although the concentrations necessary for inhibition of calcineurin phosphatase were about 10-fold higher than those of FK506. Combined, these results suggest that inhibition of calcineurin phosphatase activity by FK506 is causally related to inhibition of transendothelial migration of Nalm-6 cells.

To further investigate the relationship between calcineurin phosphatase activity and transmigration, we next used rapamycin (Fig. 2C,D), which is known to antagonize FK506 by competitively binding to FKBP (FK506-binding protein) [3,4]. When either Nalm-6 cells or KOP2.16 cells were treated with rapamycin (1 μg/ml) prior to the assay or when both cells were cultured with rapamycin during the assay, the FK506 mediated but not CsA mediated inhibition of transmigration was no longer observed (Fig. 2C), indicating that rapamycin specifically abrogated the inhibitory effect of FK506 on transmigration. The same dose of rapamycin also abrogated the FK506 mediated but not CsA mediated inhibition of calcineurin phosphatase activity (Fig. 2D), in line with previous reports indicating that rapamycin antagonizes FK506 but not cyclosporin [3,4]. These results suggest that FK506 inhibits calcineurin phosphatase activity of both Nalm-6 cells and KOP2.16 cells, and thereby inhibits transendothelial migration.

3.3. FK506 inhibits transmigration by abrogating VLA-4/VCAM-1 mediated transmigration

We then investigated the molecular mechanism of FK506 mediated inhibition of transmigration. Since integrins have been implicated in transendothelial migration of lymphoid cells [14,15], we first examined the involvement of VLA-4 and VLA-5 expressed in Nalm-6 [8] in transmigration. As shown in Fig. 3A, transmigration underneath KOP2.16 cells expressing VCAM-1 [7] was significantly inhibited by mAbs against VLA-4α, VLA-5α, anti-VLA β chain (anti-β1) and also by mAb against VCAM-1. However, none of these mAbs affected adhesion of Nalm-6 cells to KOP2.16 cells (Fig. 3B), indicating that VLA-4, VLA-5 and VCAM-1 were involved in the transmigration process but not in adhesion. In addition, the observations that anti-VCAM-1 inhibited transmigration to the same extent as did anti-VLA-4α and that the combination of the two mAbs resulted in no further inhibition (Fig. 3A) suggested that VLA-4 on Nalm-6 interacted with VCAM-1 on KOP2.16, leading to transmigration in cooperation with VLA-5. In line with this conclusion, anti-VLA-5 acted additively with anti-VLA-4 or anti-VCAM-1 in inhibiting transmigration (Fig. 3A).

We then investigated whether the FK506 induced inhibition

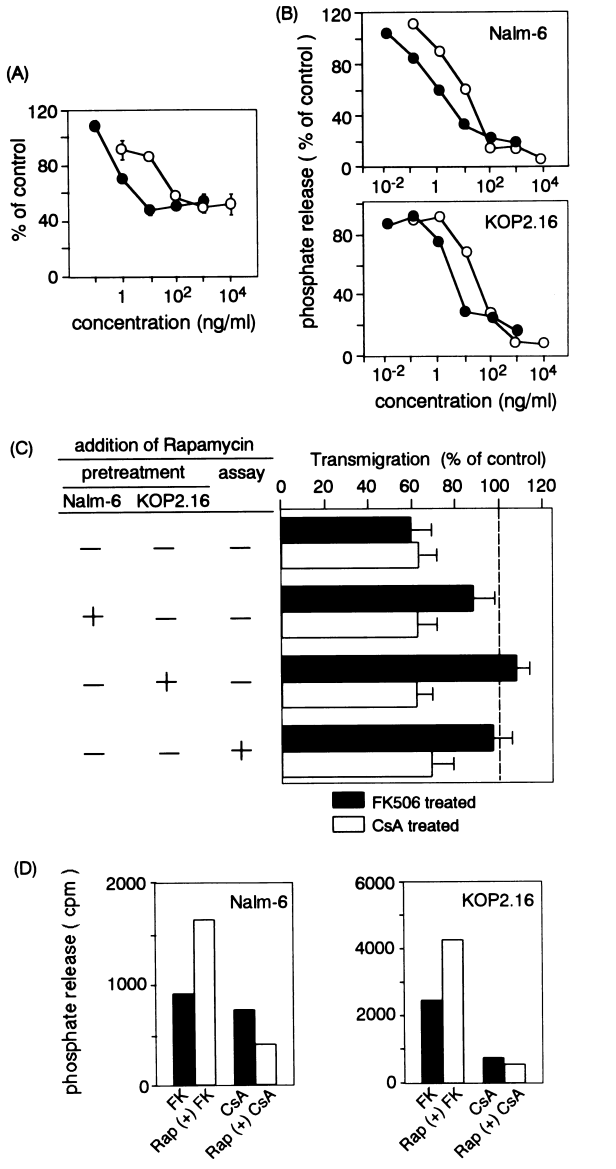

Fig. 2. Inhibition of transmigration of Nalm-6 closely correlated with inhibition of calcineurin phosphatase activity in Nalm-6 as well as in endothelial cells. A: Dose dependent inhibition of transmigration with calcineurin inhibitors. B: Dose dependent inhibition of phosphatase activity of calcineurin with FK506 or CsA in Nalm-6 (upper panel) or KOP2.16 endothelial cells (lower panel). Both Nalm-6 and KOP2.16 cells were treated with the indicated concentrations of FK506 (closed circles) or CsA (open circles). This was followed by determination of calcineurin phosphatase activities in Nalm-6 (upper panel) and KOP2.16 (lower panel). The extent of inhibition was calculated as described in the legend of Fig. 1. C: Abrogation of FK506 mediated inhibition of transmigration with rapamycin. Nalm-6 or KOP2.16 were pretreated with or without 1 μg/ml of rapamycin (indicated as + or − on the left side of the graph) before they were treated with FK506 (100 ng/ml) or CsA (1 μg/ml), and transmigration assay was performed in the presence or absence of rapamycin. When FK506 or CsA was used, the assay was performed in the presence of the corresponding reagent. D: Abrogation of FK506 mediated inhibition of calcineurin activity with rapamycin. Cells were pretreated with calcineurin inhibitors alone or in combination with 1 μg/ml of rapamycin, and then phosphatase activity of calcineurin was evaluated.

of transmigration of Nalm-6 could be explained by down-regulation of these integrin dependent pathways. Following treatment of cells with FK506, neither anti-VLA-4α nor anti-VCAM-1 inhibited transmigration of Nalm-6, whereas anti-VLA-5α induced inhibition did not change (Fig. 3C). Flow cytometric analysis showed that treatment with FK506 did not change the expression level of VLA-4 or VCAM-1 on respective cells (data not shown). These findings suggest that treatment with FK506 abrogated mainly the VLA-4/VCAM-1 mediated transmigration process without compromising the expression level of VLA-4 or VCAM-1.

3.4. FK506 inhibits infiltration of Nalm-6 cells to the bone marrow and spleen, but not to the central nervous system (CNS) in SCID mice

In the next series of experiments we examined whether FK506 can affect VLA-4/VCAM-1 dependent lymphoid cell

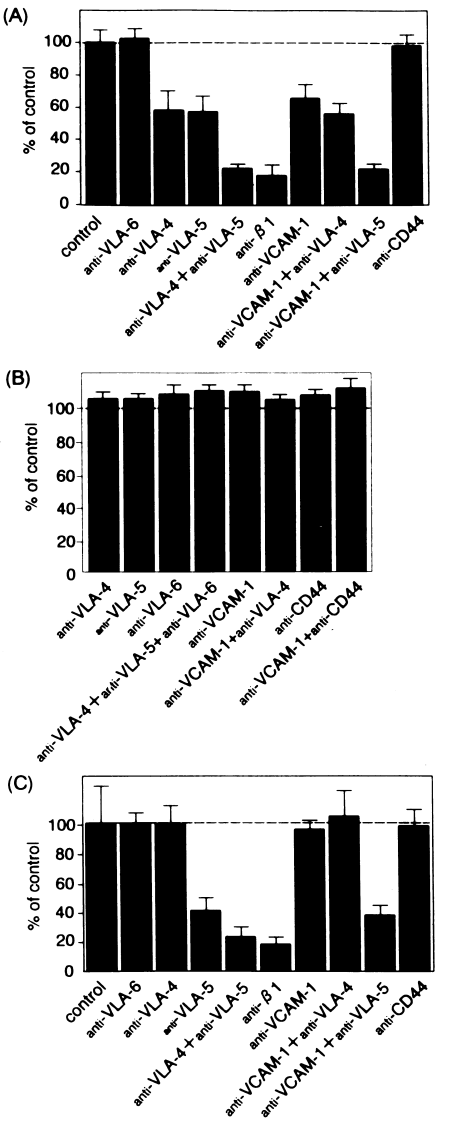

Fig. 3. Transendothelial migration but not adhesion of Nalm-6 is mediated by VLA-4/VCAM-1 interaction, and FK506 selectively downregulates VLA-4/VCAM-1 dependent transmigration. Antibody treatment was performed at a concentration of 10 μg/ml at 37°C for 30 min, and then transmigration (A) or binding (B) was assessed in the continued presence of the antibodies. The effect of FK506 on transmigration was then examined in the presence of various mAbs in the assay system (C) as described in Section 2.

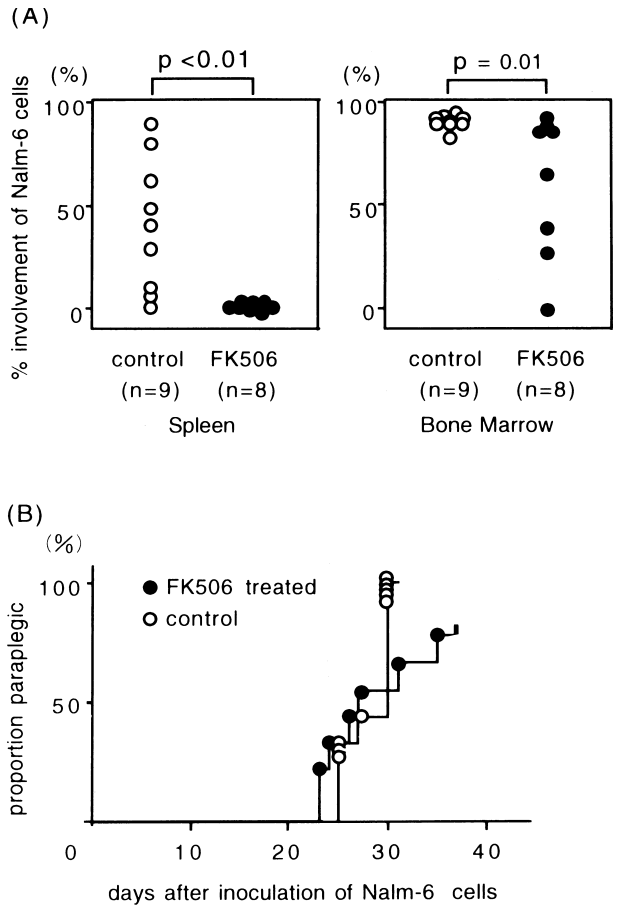

Fig. 4. FK506 inhibits infiltration of Nalm-6 cells into the spleen and bone marrow but not CNS in SCID mice. A: Infiltration of Nalm-6 cells into the spleen (left panel) and bone marrow (right panel) as assessed by flow cytometry. Results are presented as percentage of $CD19^+$ Nalm-6 cells among total nucleated cells of each organ. B: Comparison of the percentage of mice who developed paraplegia, among mice injected with Nalm-6 and later treated or untreated with FK506.

migration in vivo. SCID mice were i.v. inoculated with Nalm-6 cells (1×10^7) pretreated with or without FK506 (100 ng/ml, 2 h at 37°C) in vitro. The same animals were then treated with s.c. injections of FK506 (3 mg/kg) or vehicle alone every day. Mice were killed at 33–37 days after inoculation of Nalm-6 cells, and hematogenous spread of Nalm-6 cells to the bone marrow, spleen and CNS was examined (Fig. 4A,B). The selection of these tissues was based on the constitutive expression of a VLA-4 ligand, VCAM-1, in the bone marrow and spleen [16–19], while CNS does not express these molecules under physiological conditions [20,21]. In control mice, Nalm-6 cells invariably infiltrated into the bone marrow as well as CNS consistent with previous studies [22] and also infiltrated the spleen to various degrees. Infiltration into the bone marrow was particularly extensive, and more than 80% of the cells obtained from the marrow were identified as CD19 bright positive Nalm-6 cells at the time of death in all animals examined (Fig. 4A). Infiltration into CNS was also prominent, which caused progressive paraplegia in tumor inoculated mice (Fig. 4B). In contrast, treatment of mice with FK506 markedly reduced the number of Nalm-6 cells that infiltrated into the bone marrow and spleen relative to control mice, and in particular, colonization into the spleen was markedly and uniformly reduced (Fig. 4A). However, the proportion of FK506 treated mice that developed paraplegia was not different from control mice (Fig. 4B), indicating that the CNS involvement was little affected by FK506. Thus, FK506 substantially inhibited hematogenous spread of VLA-4 positive Nalm-6 cells to VCAM-1 expressing bone marrow and spleen but not that into the CNS which expresses little or no VCAM-1 under physiological conditions.

4. Discussion

The major finding of the present study was that FK506 inhibited transendothelial migration of Nalm-6 lymphoma cells and that this inhibition was not due to inhibition of cell adhesion but largely due to abrogation of the VLA-4/VCAM-1 dependent transmigration process. Inhibition of transmigration closely correlated with inhibition of calcineurin phosphatase activity.

That inhibition of calcineurin phosphatase activity was at least partly responsible for inhibition of transmigration was based on the following results. First, not only FK506 but also CsA, another inhibitor of calcineurin phosphatase, significantly inhibited transendothelial migration of Nalm-6 cells. Second, dose-response studies with FK506 and CsA showed that inhibition of transmigration closely correlated with inhibition of calcineurin phosphatase activity of Nalm-6 and KOP2.16 cells. Third, rapamycin, which antagonizes FK506 but not CsA by competitively binding FK506 binding protein [3,4], inhibited FK506 mediated but not CsA mediated inhibition of transmigration. However, the inhibitory effect of FK506 on transendothelial migration may not be explained solely by inhibition of calcineurin phosphatase. As shown in Fig. 1, to significantly inhibit transmigration both Nalm-6 and KOP2.16 cells had to be pretreated with FK506 which also had to be present throughout the assay. Removal of FK506 from the assay abrogated the inhibition of transmigration, although preliminary experiments indicated that calcineurin phosphatase activity of Nalm-6 cells or KOP2.16 cells remained inhibited at a comparable level after removal of FK506 for at least 90 min (data not shown). This period should have been sufficient for Nalm-6 cells to undergo transendothelial migration. Therefore, it appears that inhibition of calcineurin phosphatase alone may not be sufficient for inhibition of transmigration. A similar observation has been reported in FK506 mediated inhibition of neutrophil motility in that the motility on coated vitronectin was inhibited only in the continued presence of the agent and not when FK506 was removed before the addition of neutrophils on vitronectin [5,6].

Antibody blocking experiments indicated that transendothelial migration of Nalm-6 cells was largely ($\sim$80%) dependent on β1 integrins with VLA-4 and VLA-5 contributing equally and that VLA-4 on Nalm-6 was involved by recognizing VCAM-1 on endothelial cells. Since mAb against VLA-4, VLA-5, VCAM-1 and the integrin β1 chain inhibited transmigration without affecting cell adhesion, it was reasoned that the mAb mediated effects were specific and that the recognized molecules mediate transmigration preferentially. The molecule(s) principally involved in cell adhesion is currently unknown. Since FK506 treatment completely abrogated mAb induced inhibition of transmigration by anti-VLA-4 and/or anti-VCAM-1 without affecting anti-VLA-5 induced inhibi-

tion, it is likely that the inhibition was through selective downregulation of the VLA-4/VCAM-1 dependent pathway, although its precise mechanism is currently unclear. It is of note in this respect that a rise in intracellular Ca^{2+} is required to activate Ca^{2+}/calmodulin dependent calcineurin phosphatase [3] and that lymphocyte adhesion to endothelial cells can induce a rise in intracellular Ca^{2+} in both cell types [23]. Furthermore, VLA-4/VCAM-1 interaction has been actually shown to trigger Ca^{2+} mobilization in both lymphoid and endothelial cells [24]. Hence, one possibility is that interactions between VLA-4 and VCAM-1 elicited a rise in intracellular Ca^{2+} in both Nalm-6 cells and KOP2.16 cells, thus leading to activation of calcineurin phosphatase, which resulted in initiation of transmigration by an as yet undefined mechanism. In addition, it has been reported previously that the affinity/avidity of lymphocyte VLA-4 increases transiently during the transendothelial migration process, which apparently initiates transmigration [25]. This might also apply to Nalm-6 and that the affinity/avidity of VLA-4 on Nalm-6 cells could be controlled by a calcineurin dependent process where calcineurin of both Nalm-6 and endothelial cells are required, which may in part account for the need to treat both cell types with FK506 simultaneously to induce a significant inhibition of transmigration. Another possibility is that inhibition of transmigration was caused by a failure of reduction of integrin affinity, which would result in a decrease of de-adhesion, and hence reduction of cell motility [5,6].

Results of in vivo studies also supported the notion that FK506 can preferentially affect VLA-4/VCAM-1 dependent pathway. Thus, although Nalm-6 cells infiltrated into both VCAM-1 positive tissues (bone marrow and spleen) and VCAM-1 negative tissues such as CNS, FK506 selectively inhibited infiltration into VCAM-1 positive tissues. This inhibition was apparently not caused by inhibition of proliferation of Nalm-6 cells, since preliminary experiments indicated that up to 1000 ng/ml of FK506 did not inhibit proliferation of Nalm-6 during a 5 day culture period, and that concentrations in peripheral blood of FK506 treated mice were far lower than that (136.6 ± 17.0 ng/ml) at death ($n = 3$) (data not shown).

In summary, the results of our experiments indicate that FK506 inhibited calcineurin of both Nalm-6 cells and endothelial cells, which caused downregulation of VLA-4/VCAM-1 mediated transendothelial migration process. Although a molecular link between calcineurin and VLA-4/VCAM-1 dependent transmigration pathway remains unknown, we infer from the results of the present study that FK506 might exert its immunosuppressive effect partly by inhibiting the exit of lymphocytes from blood to the site of immunoreaction, where expression of VCAM-1 is constitutively expressed or possibly induced by cytokines.

Acknowledgements: This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, a grant from the Ministry of Science and Technology of Japan, and a grant from the Ministry of Health and Welfare of Japan.

References

[1] Miyasaka, M. (1995) Clin. Orthop. 312, 10–18.
[2] Tsuzuki, S., Toyama-Sorimachi, N., Kitamura, F., Tobita, Y., Tsuboi, H., Ando, J., Sakurai, T., Morii, N., Narumiya, S. and Miyasaka, M. (1998) Jpn. J. Cancer Res. 89, 571–577.
[3] Liu, J. (1993) Immunol. Today 14, 290–295.
[4] Fruman, D.A., Klee, C.B., Bierer, B.E. and Burakoff, S.J. (1992) Proc. Natl. Acad. Sci. USA 89, 3686–3690.
[5] Hendey, B., Klee, C.B. and Maxfield, F.R. (1992) Science 258, 296–299.
[6] Hendey, B. and Maxfield, F.R. (1993) Blood Cells 19, 143–164.
[7] Toyama-Sorimachi, N., Miyake, K. and Miyasaka, M. (1993) Eur. J. Immunol. 23, 439–446.
[8] Miyake, K., Hasunuma, Y., Yagita, H. and Kimoto, M. (1992) J. Cell. Biol. 119, 653–662.
[9] Miyake, K., Medina, K., Ishihara, K., Kimoto, M., Auerbach, R. and Kincade, P.W. (1991) J. Cell Biol. 114, 557–565.
[10] Miyake, K., Medina, K.L., Hayashi, S.-I., Ono, S., Hamaoka, T. and Kincade, P.W. (1990) J. Exp. Med. 171, 477–488.
[11] Russo, C., Callegaro, L., Lanza, E. and Ferrone, S. (1983) J. Immunol. Methods 65, 269–271.
[12] Mukai, H., Kuno, T., Chang, C.D., Lane, B., Luly, J.R. and Tanaka, C. (1993) J. Biochem. 113, 292–298.
[13] Kaplan, E.L. and Meier, P. (1958) J. Am. Statist. Assoc. 53, 457–481.
[14] Chan, P.Y. and Aruffo, A. (1993) J. Biol. Chem. 268, 24655–24664.
[15] Oppenheimer-Marks, N., Davis, L.S., Bogue, D.T., Ramberg, J. and Lipsky, P.E. (1991) J. Immunol. 147, 2913–2921.
[16] Jacobsen, K.J., Kravitz, J., Kincade, P.W. and Osmond, D.G. (1996) Blood 87, 73–82.
[17] Papayannopoulou, T., Craddock, C., Nakamoto, B., Priestley, G.Y. and Wolf, N.S. (1995) Proc. Natl. Acad. Sci. USA 92, 9647–9651.
[18] Schweitzer, K.M., Drager, A.M., van der Valk, P., Thijsen, S.F., Zevenbergen, A., Theijsmeijer, A.P., van der Schoot, C.E. and Langenhuijsen, M.M. (1996) Am. J. Pathol. 148, 165–175.
[19] Schweitzer, K.M., Vicart, P., Delouis, C., Paulin, D., Drager, A.M., Langenhuijsen, M.M. and Weksler, B.B. (1997) Lab. Invest. 76, 25–36.
[20] Greenwood, J., Wang, Y. and Calder, V.L. (1995) Immunology 86, 408–415.
[21] Yednock, T.A., Cannon, C., Fritz, L.C., Sanchez-Madrid, F., Steinman, L. and Karin, N. (1992) Nature 356, 63–66.
[22] Shah, S.A., Halloran, P.M., Ferris, C.A., Levine, B.A., Bourret, L.A., Goldmacher, V.S. and Blattler, W.A. (1995) Cancer Res. 53, 1360–1367.
[23] Pfau, S., Leitenberg, D., Rinder, H., Smith, B.R., Pardi, R. and Bender, J.R. (1995) J. Cell Biol. 128, 969–978.
[24] Ricard, I., Payet, M.D. and Dupuis, G. (1997) Eur. J. Immunol. 27, 1530–1538.
[25] Hourihan, H., Allen, T.D. and Ager, A. (1993) J. Cell Sci. 104, 1049–1059.