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ORIGINAL ARTICLE

Differential Effects of Calcineurin Inhibitors, Tacrolimus and Cyclosporin A, on Interferon-Induced Antiviral Protein in Human Hepatocyte Cells

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The premise of our study is that selective inhibition of interferon (IFN) by calcineurin inhibitors contribute to the increased severity of hepatitis C virus (HCV) posttransplantation. Therefore, we examined the influence of calcineurin inhibitors in the human hepatocyte cell line on IFN-α-induced phosphorylation of Janus kinase (Jak) and signal transducers and activators of transcription (STAT), nuclear translocation of IFN-stimulated gene factor 3 (ISGF-3), IFN-stimulated regulatory element (ISRE)-contained promoter activity, and the expressions of antiviral proteins. Tacrolimus (Tac), but not cyclosporin A (CyA), had an inhibitory effect on IFN-α-induced double-stranded ribonucleic acid (RNA)-dependent protein kinase (PKR) in a dose-dependent manner. STAT-1 also acted in a similar fashion to PKR. IFN-α combined with Tac attenuated the ISRE-containing promoter gene activity as compared with IFN-α alone. In contrast, its expression in pretreated CyA was slightly attenuated. In pretreated Tac, but not CyA, the levels of IFN-α-induced tyrosine phosphorylated STAT-1 and -2 were clearly lower than those induced by IFN-α alone. Tac and CyA did not decrease the IFN-α-induced JAK-1 phosphorylation. The nuclear translocation rate of tyrosine phosphorylated STAT-1 was inhibited by pretreatment of both Tac and CyA by western blotting and immunohistochemistry. In an HCV replicon system, pretreated Tac diminished the replication inhibitory effect of IFN-α. In this study, we show that calcineurin inhibitors, especially Tac, are the negative regulators of IFN signaling in the hepatocyte; the greatest cause of such inhibition is the phosphorylation disturbance of STAT-1, next to inhibition of the nuclear translocation of STAT-1. In conclusion, disturbance of tyrosine phosphorylation of STAT-1 resulted in diminished ISRE-containing promoter activity and a decline in antiviral protein expression. Moreover, the replication of HCV was activated. This phenomenon is detrimental to IFN therapy after liver transplantation, and the selection of calcineurin inhibitors may warrant further discussion depending on the transplant situation. ***Liver Transpl 14:292–298, 2008.*** **© 2008 AASLD.**

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Hepatitis C virus (HCV) infection is widespread worldwide. A major problem of chronic HCV infection is hepatocellular carcinoma. Currently, liver transplantation for HCV-related liver disease is an option worldwide.[1] Recently, it has been demonstrated that the prognosis for liver transplantation patients with HCV-related disease deteriorates with time,[2] resulting in a poorer out-

Abbreviations: CyA, cyclosporin A; HCV, hepatitis C virus; IFN, interferon; ISGF-3, IFN-stimulated gene factor 3; ISRE, IFN-stimulated regulatory element; Jak, Janus kinase; NF-AT, nuclear factor of activated T cells; PKR, double-stranded RNA-dependent protein kinase; RNA, ribonucleic acid; STAT, signal transducers and activators of transcription; Tac, tacrolimus.

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come than in the non-HCV course.[3,4] The transplanted liver in HCV-related disease undergoes a rapid progression of fibrosis and worsens to cirrhosis and graft failure.[5] The factors for a worsening outcome were speculated to be increased donor age,[3-5] stronger immunosuppression,[3] and high levels of HCV-ribonucleic acid (RNA) at transplantation.[4] These factors have no small effect on the reinfection and reactivation of HCV in the graft liver.

Reinfection of HCV in the graft liver is rapid after transplantation, and the virus immediately proliferates in the graft. In the natural course of reinfection, approximately 10 to 25% of recipients will develop cirrhosis, and a strategy for the prevention of reinfection has not been developed.[6] At present, treatment of HCV after transplantation is inadequate, and does not result in a cure.[7] Recently, pegylated interferon (IFN) and ribavirin combination therapy has been effective in the treatment of HCV genotype 1a chronic hepatitis, with a sustained viral response rate of 45%.[8] However, reinfection after transplantation is the norm despite combined therapy.[9,10] Meanwhile, the patients with a sustained viral response after transplantation show no progression or reversal of liver fibrosis.[11,12] The refractory nature of pegylated IFN and ribavirin combination therapy for liver transplantation patients contributes to a worsening outcome in HCV-related transplantation.

We speculated that posttransplantation immunosuppression is part of the reason for IFN resistance to HCV reinfection of the graft liver. Methylprednisolone pulse therapy is a risk factor for severe outcome after transplantation, and the treatment of acute cellular rejection using heavy immunosuppressive agents is also a risk factor.[3,4,6] Previous reports described the fact that glucocorticoid inhibits the expression of signal transducers and activators of transcription (STAT)-1, as a signal transduction factor of IFN, and diminishes the signaling of IFN.[13] However, the effects on HCV reinfection and IFN therapy by calcineurin inhibitors, the most frequently used immunosuppressants, have not been fully evaluated, until now. Therefore, we have attempted to evaluate the influences of calcineurin inhibitors on IFN signaling in the hepatocytes.

IFN-α and β, after binding to their receptors, stimulate the intracellular IFN-signaling cascade including the Janus kinase (Jak)-STAT tyrosine kinases, the phosphorylation of STAT-1 and -2, and the formation of IFN-stimulated gene factor 3 (ISGF-3), which consists of STAT-1, STAT-2, and p48.[14] ISGF-3 translocates into the nucleus and binds to the IFN-stimulated regulatory element (ISRE) in the promoter sequences of a variety of IFN-inducible genes, including antiviral proteins such as double-stranded RNA-dependent protein kinase (PKR).[15] Several negative regulation systems of Jak-STAT signaling, including the suppressor of cytokines signaling family, the protein inhibitor of activated STAT family, and the SH2-containing protein tyrosine phosphatase family, are notorious contributors to a state of inflammation and carcinogenesis in the hepatocyte.[16,17] In addition, the nucleus-cytoplasm transport of ISGF-3 was regulated by translocated specific proteins along with the phosphorylation of STAT.[18] We examined the influence of calcineurin inhibitors on IFN-induced phosphorylation of Jak and STAT, nuclear translocation of ISGF-3, ISRE contained promoter activity, and the expressions of antiviral proteins.

MATERIALS AND METHODS

Reagents and Cell Culture

Recombinant human IFN-α2b, tacrolimus (Tac), and cyclosporine A (CyA) were generous gifts from Schering-Plough KK (Tokyo, Japan), Astellas Co. (Tokyo, Japan), and Novartis Pharma Co. (Basel, Switzerland), respectively. Hc human hepatocyte cells (Applied Cell Biology Research Institute, Kirkland, WA) and HuH-7 human hepatoma cells (American Type Culture Collection, Rockville, MD) were maintained in a chemically-defined medium, CS-C completed (Cell Systems, Kirkland, WA) and RPMI (Invitrogen, Grand Island, NY), respectively, supplemented with 5% fetal bovine serum. In the pretreatment of calcineurin inhibitors, the cells were cultured in 5% RPMI containing varying concentrations of Tac and CyA, and then the medium was exchanged and the cells were treated with IFN 100 IU/mL at the indicated time.

HCV Replicon System

OR6 cells stably harboring the full-length genotype 1 replicon, ORN/C-5B/KE[19] were used to examine the influence on the anti-HCV effect of IFN of calcineurin inhibitors. The cells were cultured in Dulbecco's modified Eagle's medium (Gibco-BRL; Invitrogen) supplemented with 10% fetal bovine serum, penicillin, and streptomycin and maintained in the presence of G418 (300 mg/L; Geneticin; Invitrogen). This replicon was derived from the 1B-2 strain (strain HCV-o, genotype 1b), in which the *Renilla* luciferase gene is introduced as a fusion protein with neomycin to facilitate the monitoring of HCV replication. After the treatment, the cells were harvested with *Renilla* lysis reagent (Promega, Madison, WI) and subjected to luciferase assay according to the manufacturer's protocol.

Western Blotting and Antibody

Western blotting with anti-PKR, anti-STAT-1, anti-STAT-2 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-tyrosine-701 phosphorylated STAT-1, anti-tyrosine-689 STAT-2, anti-JAK-1 or anti-tyrosine 1022/1023 JAK-1 (New England Biolabs, Beverly, MA) was performed as described previously.[20] Briefly, Hc cells were lysed by the addition of lysis buffer (50 mmol/L Tris-HCl, pH 7.4, 1% Np40, 0.25% sodium deoxycholate, 0.02% sodium azide, 0.1% sodium dodecyl sulfate buffer, 150 mmol/L NaCl, 1 mmol/L ethylene diamine tetraacetic acid, 1 mmol/L phenylmethanesulfonylfluoride, 1 μg/mL each of aprotinin, leupeptin, and pepstatin, 1 mmol/L sodium o-vanadate, and 1 mmol/L NaF). Extraction of nucleus and cytoplasm were performed using the NE-PER Nuclear and Cyto-

plasmic Extraction kit (Pierce, France). Samples were analyzed by electrophoresis on 8 to 12% sodium dodecyl sulfate buffer polyacrylamide gel and electrotransferred to nitrocellulose membranes, and then blotted with each antibody. The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G or anti-mouse immunoglobulin G, and the immunoreactive bands were visualized by the ECL chemiluminescence system (Amersham Life Science, Buckinghamshire, England). The density of each band was quantified using the National Institutes of Health image analysis software program.

Reporter Gene Assay

pISRE-Luc containing 5 copies of the ISRE sequence and firefly luciferase gene and pRL-SV40 containing SV40 early enhancer/promoter and *Renilla* luciferase gene were obtained from Clontech (San Diego, CA) and Promega, respectively. The HuH-7 cells were grown in 24-well multiplates and transfected with 1 μg of pISRE-Luc and 10 ng of pRL-SV40 as a standard by the lipofection method. One day later, the cells were incubated in the absence or presence of varying concentrations of Tac, CyA, and IFN-α, and the luciferase activities in the cells were determined using a dual-luciferase reporter assay system and a TD-20/20 luminometer (Promega). The data were expressed as the relative ISRE-luciferase activity.

Fluorescence Immunohistochemistry

The Hc cells were seeded onto 11-mm glass coverslips in 24-well plates at 240,000 cells/well. The next day, the medium was replaced with serum-free medium, and the cells were pretreated with 10 μmol/L of Tac, 100 μmol/L of CyA, or vehicle, for 16 hours and then stimulated with 100 IU/mL of IFN-α for 10 minutes. Fluorescence immunohistochemistry was performed as described previously.[21] The cells were incubated with anti-tyrosine-701 phosphorylated STAT-1 antibody for 1 hour at room temperature, washed 3 times in phosphate buffered saline, incubated with rhodamine-conjugated donkey anti-rabbit immunoglobulin G (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 1 hour, washed in phosphate buffered saline, and mounted in Vectashield Mounting Medium (Vector Laboratories Inc., Burlingame, CA). Nuclear staining was performed using Hoechst 33258 (Invitrogen Japan K.K., Tokyo, Japan). Immunofluorescence analysis was done by an Olympus BX50 microscope (Tokyo, Japan) and the image was captured by a Nikon DXM 1200 digital camera (Tokyo, Japan).

RESULTS

Differential Effects of Tac and CyA on IFN-induced Antiviral Protein Expression

To elucidate how calcineurin inhibitors exert influence on IFN-induced antiviral protein, the Hc cells were incubated in the absence or presence of IFN-α after the presence or absence of pretreatment of Tac (Fig. 1A) or CyA (Fig. 1B) for 16 hours, and then were harvested for the western blot analysis. Pretreated Tac had an inhibitory effect on IFN-α-induced PKR expression, antiviral protein as messenger RNA translation inhibitor activated by double-stranded RNA dependent, in a dose-dependent manner, but no inhibitory effect of pretreatment CyA for PKR expression was recognized in our experiment. STAT-1 is an essential signal transmitter substance of IFN and IFN-inducible proteins.

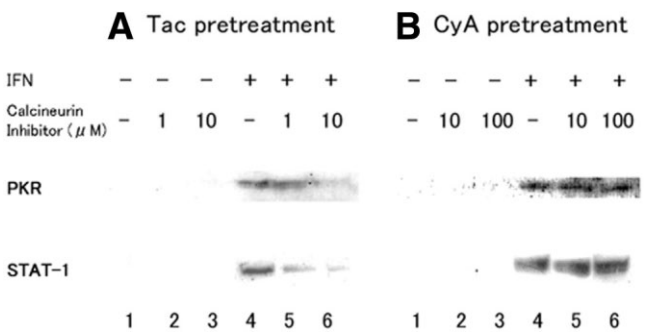

Figure 1. Effect of (A) Tac and (B) CyA on IFN-α-induced PKR and STAT-1. Hc cells were treated with 100 IU/mL of IFN-α in the absence (lane 4) or presence of pretreatment (lanes 5 and 6). Indicated concentration of calcineurin inhibitor alone was lanes 2 and 3, lane 1 was not treated with IFN-α and calcineurin inhibitors. One day later, PKR and STAT-1 were determined by western blotting.

The expression of IFN-inducible STAT-1 also decreased in a dose-dependent manner after the administration of Tac, but not after the administration of CyA.

Alterations of IFN-α-Stimulated Reporter Gene Expression by Tac and CyA

Because the formation of IFN stimulating gene factor (ISGF) 3 by IFN-α leads to transactivation of the ISRE in the promoter regions of the IFN-α-inducible genes, we performed the reporter gene transfection assay using plasmids containing ISRE in their promoter sequence. Because there were not enough Hc cells for reporter gene transfection, we used HuH-7 cells in the transfection assay. HuH-7 cells were transfected with pISRE-Luc containing 5 repeats of ISRE sequence and pRV-SV40 as a standard and then were treated with IFN-α after 16 hours in the presence or absence of pretreated Tac or CyA (Fig. 2). Tac and CyA alone did not influence the ISRE-luciferase activities. IFN-α combined with Tac and attenuated its expression compared with IFN-α alone. In contrast, there was a slight attenuation effect of its expression in 100 μmol/L of pretreated CyA.

Inhibitory Effect of Tac on IFN-α-Induced Tyrosine Phosphorylation of STATs

The activation of STAT-1 and -2 by phosphorylation of tyrosine-701 and 689 residues, respectively, is essential for the relay of IFN-α signal with the formation of ISGF-3. Therefore, we examined the effect of Tac and CyA on the IFN-α-induced tyrosine phosphorylation of

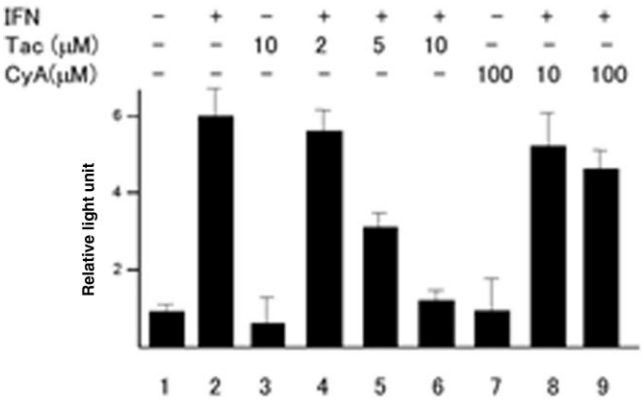

Figure 2. Suppression effect of calcineurin inhibitors on IFN-α-induced reporter gene assay. HuH-7 cells transfected with reporter gene (pISRE-Luc and pRL-SV40) were either untreated (lanes 1, 2) or pretreated with Tac (lanes 3-6) or CyA (lanes 7-9) for 16 hours, followed by IFN-α 100 IU/mL (lanes 2, 4-6, 8, and 9)or absence (lanes 3 and 7). Six hours later, the relative ISRE-luciferase activity (n = 4) was determined as described in Materials and Methods. The data are expressed as the mean ± SD and are representative examples of four similar experiments.

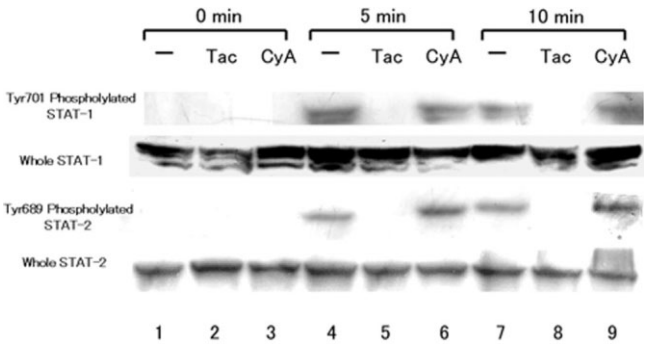

Figure 3. Effect of calcineurin inhibitors on STAT-1and STAT-2. After pretreatment with 10 μmol/L Tac (lanes 2, 5, and 8) and 100 μmol/L CyA (lanes 3, 6, and 9) for 16 hours, Hc cells were untreated (lane 1) or treated with 100 IU/mL IFN-α (lanes 4-9) for the indicated periods and phosphorylated STAT-1 at tyrosine-701 residue (first panel), expression of STAT-1 (second panel), phosphorylated STAT-2 at tyrosine-689 residue (third panel), and expression of STAT-2 (fourth panel) were analyzed by western blotting. The density of each band was quantified and the nuclear translocation rate was calculated by the following: nuclear intensity (N)/[nuclear intensity (N) + cytoplasmic intensity (C)].

STAT-1 and -2 (Fig. 3). IFN-α clearly induced the tyrosine phosphorylation of STAT-1 and -2, but Tac and CyA could not. However, when the Hc cells were pretreated with Tac, but not CyA, before IFN-α stimulation, the levels of tyrosine phosphorylated STAT-1 and -2 were clearly lower than those induced by IFN-α alone. In the case of pretreatment with CyA, the IFN-α-induced tyrosine phosphorylation levels were similar to IFN-α alone. Then, the cells were changed from Hc cells to HuH-7 cells and a similar experiment was done. The inhibitory effect of Tac to IFN-α-induced STAT-1 and -2 tyrosine phosphorylation was the same (data not shown).

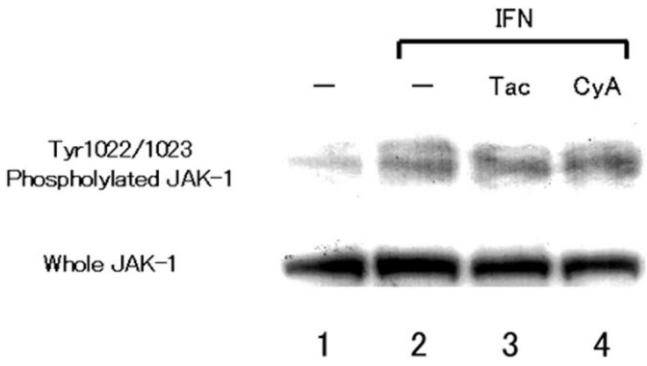

Figure 4. Evaluation of IFN-α-induced phosphorylated JAK-1 by calcineurin inhibitors. After pretreatment of 10 μmol/L Tac (lane 3) and 100 μmol/L CyA (lane 4) for 16 hours, Hc cells were untreated (lane 1) or treated with 100 IU/mL IFN-α (lanes 2-4) for 3 minutes, then phosphorylated JAK-1 at tyrosine-1022/1023 residue (first panel) and expression of JAK-1 (second panel) were analyzed by western blotting.

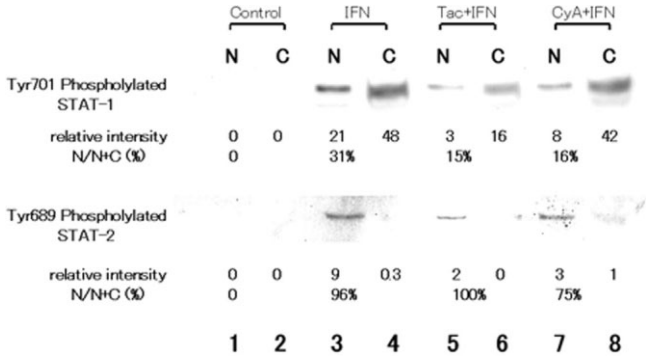

Figure 5. Alternation of distribution of IFN-α-induced phosphorylated STAT-1 and STAT-2 by calcineurin inhibitors. Hc cells were pretreated with absence (lanes 1-4) or presence of 10 μmol/L Tac (lanes 5 and 6) or 100 μmol/L CyA (lanes 7 and 8). And then, Hc cells were stimulated by 500 IU/L IFN-α (lanes 3-8) for 10 minutes. Hc cells harvested by extraction kit of nucleus (lanes 1, 3, 5, and 7) and cytoplasm (lanes 2, 4, 6, and 8). Phosphorylated STAT-1 at tyrosine-701 residue (upper panel) and phosphorylated STAT-2 at tyrosine-689 residue (lower panel) were analyzed by western blotting.

When we performed western blotting of phosphorylated JAK-1 under the same conditions, Tac and CyA did not decrease the IFN-induced JAK-1 phosphorylation (Fig. 4).

Influence of Calcineurin Inhibitors on IFN-α-Induced Nuclear Translocation of Tyrosine Phosphorylated STATs

For transcription of the IFN-α-induced antiviral gene, the ISGF-3 complex, including activated STAT-1, STAT-2, and p48, could be translocated to the nucleus. Initially, we detected tyrosine phosphorylated STAT-1 and -2 extracted it from the nucleus and cytoplasm by western blotting. In this experiment, detectable band intensities were quantified by National Institutes of Health image software and we evaluated the nuclear translocation rate of activated STAT-1 and -2 (Fig. 5).

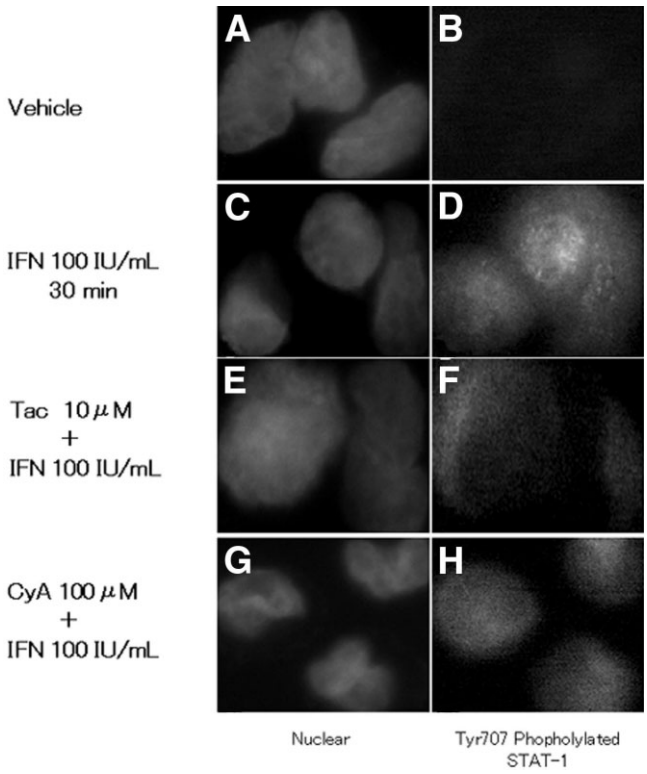

Figure 6. Inhibition of IFN-α-induced nuclear translocation of phosphorylated STAT-1 by calcineurin inhibitor. Hc cells were pretreated in the absence (A-D) or presence of 10 μmol/L Tac (E,F) or 100 μmol/L CyA (G,H). After pretreatment, Hc cells were stimulated by 100 IU/L IFN-α (C-H) for 30 minutes. Thereafter, the cells were fixed, permeabilized, processed for immunofluorescence (B,D,F,H) and Hoechst staining (A,C,E,G), and visualized with fluorescence microscopy. The results shown are from one representative experiment from a total of three performed.

The total IFN-α-stimulated tyrosine phosphorylated STAT-1 was decreased by pretreatment with Tac; furthermore, the nuclear translocation rate of activated STAT-1 was inhibited both by pretreatment with Tac and CyA. However, in the case of pretreatment with Tac and CyA, there was no effect on the nuclear translocation of tyrosine phosphorylated STAT-2. Secondarily, we evaluated the location of tyrosine phosphorylated STAT-1 by fluorescence immunohistochemistry of cultured Hc cells (Fig. 6).The IFN-α-induced nuclear translocation of tyrosine phosphorylated STAT-1 was observed, but its translocation was inhibited by pretreatment with Tac. Along with the nuclear translocation rate of activated STAT-1 by western blotting (Fig. 5), pretreatment with Tac also attenuated the nuclear staining of activated STAT-1 compared to IFN-α alone, but did not attenuate the expression of activated STAT-1 by immunohistochemistry.

Inhibitory Effect of Tac on IFN-α-Induced Anti-HCV Efficiency

To examine the effect of calcineurin inhibitors on IFN-α, we used the full-length HCV replication system, OR6 cells. The cells were treated with IFN-α after 16 hours in the presence or absence of pretreated Tac or CyA (Fig. 7). IFN-α or CyA alone repressed the *Renilla* luciferase activity, which is well correlated with HCV-RNA concentration in OR6 cells.[19] In contrast, Tac alone had little effect on *Renilla* luciferase activity. However, pretreatment with Tac attenuated the IFN-α-induced repression of *Renilla* luciferase activity (Fig. 7; lane 2 versus lanes 4 and 5), but pretreatment with CyA did not (Fig. 7; lanes 8 and 9).

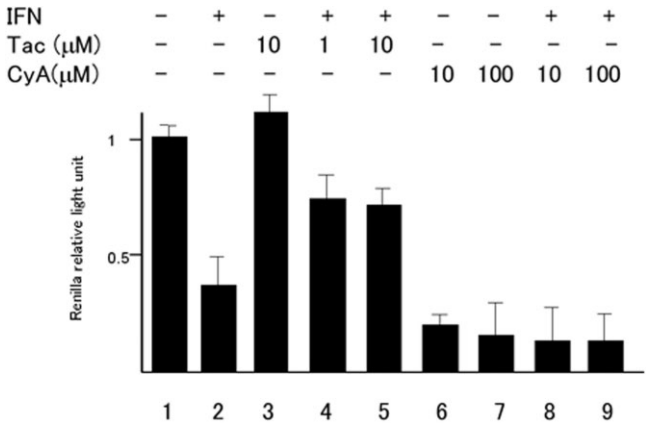

Figure 7. Alternation of IFN-α-suppressed HCV replication by Tac. OR6 cells, full-length replicon system, were treated with 100 IU/mL of IFN-α in the absence (lane 2) or presence of pretreatment (lanes 4, 5, 8, and 9). Indicated concentration of calcineurin inhibitor alone was lanes 3, 6, and 7, lane 1 was not treated with IFN-α and calcineurin inhibitors. One day later, *Rennila* luciferase activity was determined by luminometer.

DISCUSSION

We herein show that calcineurin inhibitors, especially Tac, are negative regulators of IFN signaling in the hepatocyte, and the greatest cause of this phenomenon is phosphorylation of STAT-1, next to inhibition of nuclear translocation of STAT-1. Disturbance of STAT-1 phosphorylation caused diminished ISRE-containing promoter activity, for example PKR and STAT-1, and antiviral protein expression declined. Pretreatment with Tac diminished the replication inhibitory effect of IFN-α. This phenomenon has a detrimental effect on IFN therapy after HCV-related liver transplantation. In our experiments, we speculated that Tac is not better suited for posttransplantation IFN therapy than CyA, but it did not report that IFN-a response is different between Tac and CyA in human study in previous time. When the alternative of potent immunosuppressant for prevention of rejection, or antiviral-activity for HCV reactivation is weighed, we might need to consider other factors in choosing between Tac and CyA. We had compared high concentration CyA with low concentration Tac, since rejection was controlled by serum trough values of tacrolimus of 5 ng/mL and of cyclosporin of 100 ng/mL in our hospital in the period of stability after liver transplantation.

Recently, the difference between Tac and CyA has been regarded in another function than immunosuppression, and we presume that this discrepancy de-

pended on differences of "immunophilins." Immunophilins are a ubiquitous family of proteins. All cells contain several members of this family, which bind specific calcineurin inhibitors and participate in many cellular functions.[22] Tac has been reported to have neuroprotection,[23] but CyA did not, whereas CyA had anti-HCV action,[24-26] but Tac did not. Tac binds specific FK506 binding protein members of the immunophilin family, whereas cyclosporin binds a different subset of immunophilins (cyclophilins). FK506 binding protein and CyP have the same function as peptidyl prolyl *cis-trans* isomerase and they inhibited the nuclear translocation of nuclear factor of activated T cells (NF-AT). Despite this common pathway, the cell protection activity has been reported to require the induction of heat shock protein 70 by Tac but not CyA,[27] and the anti-HCV activity contributed to a specific blockade of CyP B by CyA.[25] The differences in the medical effects for immunosuppression between Tac and CyA require attention, when these immunosuppressants are used in posttransplantation-related HCV infection.

In our study, the IFN-induced tyrosine phosphorylated STAT-1 and -2 both decreased after the administration of Tac, but Tac is known essentially for the inhibition of serine/threonine protein phosphatase. Calcineurin, regardless of independent Jak-1 tyrosine phosphorylation, and CyA did not have such a tyrosine phosphatase action against STAT-1 and -2. We could not resolve this phosphatase mechanism, but we speculated that Tac induced the tyrosine phosphatase kinase and inhibited tyrosine phosphorylation of STAT-1 and -2. Tac did not induce suppression of cytokines signaling-1 and 3, Jak inhibitors, by western blotting in our study (data not shown); however, we could not rule out the induction of other types of tyrosine phosphatase. Previous studies described that suppressor of cytokines signaling-1, 3 and SH2-containing protein tyrosine phosphatase inhibited NF-AT activation,[28-30] and therefore the relationship between Tac and tyrosine phosphatase might be reconsidered. Barat and Tremblay[31] and Zhu and McKeon[32] previously described the protein-tyrosine phosphatase inhibitor bisperoxovanadium as a potent activator of T cell receptor signaling, and SH2-containing protein tyrosine phosphatase-1, T cell protein-tyrosine phosphatase, Tac, and CyA are inhibitors of such activation. We were interested in the inhibition of protein-tyrosine phosphatase inhibitor by Tac and CyA, because Tac and CyA possessed the same action as SH2-containing protein tyrosine phosphatase-1 and protein-tyrosine phosphatase.[32] Furthermore, this action of Tac was stronger than CyA.[31] From these studies, we assume that Tac has tyrosine phosphatase action in the hepatocyte and inhibits tyrosine phosphorylation of STAT-1 and -2.

The inhibition of IFN-induced antiviral proteins by Tac, and the inhibition of nuclear trafficking of tyrosine phosphorylated STAT-1, is the common phenomenon between Tac and CyA in this study. This phenomenon was observed in the western blotting findings (Fig. 3) and immunohistochemistry of the cultured cells (Fig. 6).

NF-AT activation requires the suppression of Crm1-dependent export from nucleus to cytoplasm by calcineurin,[33] and the presence of importin, bounded to calcineurin, in the nucleus.[34] In IFN-induced Jak-STAT signaling, nuclear trafficking of ISGF-3 requires suppression of Crm1 and binding importin[18] in the same fashion as NF-AT. Calcineurin inhibitors bind to immunophilin and inhibit dephosphorylation of NF-AT, then they inhibit the transcription activity of NF-AT. In addition to such action, it might be considered that the nuclear trafficking of NF-AT is regulated by the calcineurin inhibitor and immunophilin complex. We speculated that the decrease of the nuclear import of tyrosine phosphorylated STAT-1 is the function, the calcineurin inhibitor and immunophilin complex modified Crm1 and importin in the same fashion as NF-AT. Then, we recognized that the mechanisms of diminished tyrosine phosphorylation STATs and nuclear translocation STAT-1 were different.

Presently, there is no definite opinion regarding the selection of calcineurin inhibitors for liver transplantation.[6] However, reports of inhibition of HCV replication by CyA in vitro were noted recently[24-26] and the result were same in our full-length replicon system (Fig. 7). In our data, we consider that CyA has the effect of, not only the previously reported anti-HCV replication action itself, but it creates much less interference with IFN treatment for HCV reactivated after liver transplantation than does Tac. It has been reported that CyA increased the chance of a sustained viral response after liver transplantation.[35] However, we used care with our data, because both Tac and CyA inhibit the nuclear translocation of tyrosine phosphorylated STAT-1. Our data revealed that when an excess of CyA was used after liver transplantation, it resulted in a decrease in the amount of IFN-induced antiviral protein, because of inhibition of nuclear transportation of tyrosine phosphorylation STAT-1 (Figs. 5 and 6). The immunosuppression levels of Tac and CyA have already been reported to decrease significantly in patients responding favorably to anti-HCV therapy post–liver transplantation.[36] In this study, we therefore considered it necessary to pay attention to an excess dose of CyA, when IFN treatment for reactivation of HCV is required.

In conclusion, Tac has been shown to influence the tyrosine phosphorylation of STAT-1, and the result was a decline in antiviral protein PKR. In addition, Tac and CyA have been shown to interfere with the translocation of STAT-1. We speculated that posttransplantation immunosuppression is part of the reason for IFN resistance to HCV reinfection of the graft liver. As the course, calcineurin inhibitors, especially Tac, were pointed out in this study, and we clarified a part of the IFN resistance. Although the mechanism of inhibition of IFN signaling has not yet been fully investigated, it is necessary to compare the antirejection action of Tac to the anti-HCV action of CyA when selecting calcineurin inhibitors.

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