In Vitro Evidence for a Direct Antifibrotic Role of the Immunosuppressive Drug Mycophenolate Mofetil

Nina Roos, Nicolas Poulhalon, Dominique Farge, Isabelle Madelaine, Alain Mauviel, and Franck Verrecchia

Institut National de la Santé et de la Recherche Médicale U697, Paris, France (N.R., N.P., D.F., A.M., F.V.); and Service de Médecine Interne (D.F.) and Pharmacie (I.M.), Hôpital Saint-Louis, Paris, France

Received November 14, 2006; accepted January 30, 2007

ABSTRACT

The immunosuppressive drug mycophenolate mofetil (MMF) is currently used to prevent organ transplant rejection (Mele and Halloran, 2000; Ciancio et al., 2005; Danovitch, 2005; Shapiro et al., 2005). MMF efficacy has been mostly attributed to its inhibitory activity on lymphocyte proliferation. Although the immunosuppressive xenobiotic drug mycophenolate mofetil (MMF) is currently used to prevent organ transplant rejection, and has shown some efficacy to prevent the fibrotic complications that occur during autoimmune diseases such as systemic sclerosis or during graft-versus-host disease (GVHD). We tested the hypothesis that MMF may exert direct effects on fibroblast extracellular matrix remodeling. Incubation of human lung fibroblast cultures with MMF led to dose- and time-dependent reduction in the synthesis and expression of type I collagen. Inhibition of COL1A1 and COL1A2 mRNA steady-state levels occurred at the level of transcription via repression of their promoters. In contrast, MMF significantly enhanced the expression and the synthesis of interstitial collagenase (matrix metalloproteinase-1). MMF was also found to diminish the capacity of fibroblast to contract mechanically unloaded collagen lattices and to reduce the synthesis of α-smooth muscle actin, a marker of the contractile myofibroblast phenotype. In addition, MMF diminished the fibroblasts motility. In conclusion, we provide novel mechanism by which MMF alters fibroblast functions that make MMF an effective immunosuppressive drug.

The immunosuppressive xenobiotic drug mycophenolate mofetil (MMF) is currently used to prevent organ transplant rejection, and has shown some efficacy to prevent the fibrotic complications that occur during autoimmune diseases such as systemic sclerosis (Stratton et al., 2001; Liossis et al., 2006) or graft-versus-host disease (GVHD) (Arai and Vogelsang, 2000; Zeiser et al., 2004). For example, in rodent models, oral administration of MMF alone or in combination with cyclosporin A inhibits the sclerotic manifestations of GVHD (Allison and Eugui, 2000). In humans, successful treatment of high-risk chronic GVHD with MMF has also been reported on a limited number of patients (Allison and Eugui, 2000; Kim et al., 2004; Krejci et al., 2005; Lopez et al., 2005).

Systemic sclerosis and GVHD are heterogeneous immune diseases characterized by predominant T cell activation, production of autoantibodies, and cytokine release. These events contribute to fibroblast activation and lead to increased deposition of collagen at various organ sites, ultimately producing localized fibrosis, largely leading to organ failure and death (Derk and Jimenez, 2003; Ruzek et al., 2004; Abraham and Varga, 2005; Hess, 2006; Verrecchia et al., 2006). The development of fibrosis also involves a series of distinct yet complementary pathophysiological events that include attraction of blood-born cells (e.g., leukocytes, platelets, activated lymphocytes), local alteration of the microvasculature....

This work was supported by the Programme National de Recherche Dermatologie 2006, by the Institut National de la Santé et de la Recherche Médicale, by the Groupe Français de Recherche sur la Sclérodermie, and by the Association des Sclérodermiques de France.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.106.117051.

ABBREVIATIONS: MMF, mycophenolate mofetil; MPA, mycophenolic acid; GVHD, graft-versus-host disease; ECM, extracellular matrix; MMP, matrix metalloproteinase; SMA, smooth muscle actin; FCS, fetal calf serum; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; RT, reverse transcriptase; TIMP, tissue inhibitor of metalloproteinase.
was obtained from Roche Pharmaceuticals (Nutley, NJ).

The effects of MMF were studied in wounds, called myofibroblasts, express high levels of a-smooth muscle actin (SMA) and consequently contracts the ECM, thereby allowing wound closure (Gabbiani, 2003). Myofibroblasts are present in abundance within fibrotic lesions and notably contribute to excessive ECM deposition and scarring (Gabbiani, 2003).

The aim of this work was to investigate whether MMF exerts direct effects on fibroblast functions. We demonstrate that MMF inhibits collagen gene expression, ECM contraction, and fibroblast migration, suggesting that it may exert direct antifibrotic activities independent from its role as an immunosuppressive drug.

**Materials and Methods**

**Cell Cultures.** Human lung fibroblasts (WI-26) were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, and antibiotics (100 U/ml penicillin, 50 mg/ml streptomycin-G, and 0.25 mg/ml amphotericin B (Fungizone)) in 5% CO₂ at 37°C. Myofibroblasts were obtained from Roche Pharmaceuticals (Nutley, NJ).

**Cell Viability Assay.** Confluent WI-26 fibroblasts were cultured in 96-well cell culture clusters and treated with various concentrations of MMF for 12, 24, and 48 h in the presence of 1% FCS. Cell viability was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium assay, according to the manufacturer’s protocol (Promega, Madison, WI).

**Collagen Matrix Contraction.** Fibroblasts were harvested from monolayer cultures with 0.25% trypsin and 1 mM EDTA. Trypsin was subsequently neutralized with fetal calf serum-containing medium. Collagen lattices in 60-mm dishes were prepared with 7 ml of collagen (Biocoat; BD Biosciences, San Jose, CA) in medium. Collagen lattices in 60-mm dishes were prepared with 7 ml of collagen (Biocoat; BD Biosciences, San Jose, CA) in medium supplemented with 1% FCS. When needed, MMF was added to the mixture before polymerization of the collagen matrix. Polymerization of collagen was subsequently neutralized with fetal calf serum-containing medium. Collagen lattices required 60 min at 37°C. To initiate lattice contraction, freshly polymerized matrices were released from the underlying culture dish with a few gentle taps on the dish (Javelaud et al., 2003).

**Scratch Wound Closure Assay.** Confluent cell monolayers were wounded by mechanical scraping with a pipette tip. Wound width was assessed at the time of scrapping to ensure that all wounds were the same width at the start of the experiment (see corresponding figures). Cell culture medium was then replaced with fresh medium, with or without MMF at various concentrations, and wound closure was recorded photographically over time using phase-contrast microscopy (Javelaud et al., 2003).

**Reverse Transcription-PCR Analysis.** Total RNA was extracted according to the manufacturer’s instructions using the RNeasy mini-kit (QIAGEN, Valencia, CA). DNase I treatment (25 U, 15 min) of total RNA was directly performed on the spin columns to eliminate genomic contamination of the RNA samples. One microgram of total RNA was used for first strand cDNA synthesis using a RT-for-PCR kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Real-time PCR was performed with an ABI PRISM 7700 instrument (Applied Biosystems, Foster City, CA) using SYBRGreen PCR Core Reagents (Applied Biosystems). Reaction mixtures were incubated for 2 min at 50°C, followed by 10 min at 95°C and 40 cycles of 15 s at 95°C, 45 s at 60°C, and 1.5 min at 68°C, and finally 15 s at 95°C, 20 s at 63°C, and 15 s at 95°C. For each mRNA, gene expression was corrected against glyceraldehyde-3-phosphate dehydrogenase mRNA content in each sample. Primers used are shown in Table 1.

**Transient Cell Transfections and Reporter Assays.** Transient cell transfections were performed with jetPEI according to the manufacturer’s protocol (Polyplus-transfection, Illkirch, France).

**Plasmid Constructs.** –3500COL1A1/CAT (a gift from Francesco Ramirez, Mt. Sinai School of Medicine, New York, NY) and –2300COL1A1/CAT (gift from John Varga, Northwestern University Feinberg School of Medicine, Chicago, IL) have been described previously (Verrecchia et al., 2002). Quantitations were made with a PhosphorImage (Amersham Biosciences, Uppsala, Sweden).

**Western Blot Analyses.** Total cell extracts (30 μg) in Laemmli buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride) were denatured by heating at 95°C for 3 min before resolution by SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to Hybond membranes (Amersham Biosciences), immunoblotted with anti-type I collagen (Southern Biotech, Birmingham, AL), anti-α-smooth muscle actin (Sigma-Aldrich, St. Louis, MO), or anti-actin (Sigma-Aldrich) antibodies, all at a dilution of 1:1000 in 1X phosphate-buffered saline/5% nonfat milk for 1 h. After incubation, filters were washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. Filters were stained with an ECL detection kit (Amersham Biosciences).

**TABLE 1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL1A1</td>
<td>5'-GGGCAAGACAGTGATTGAATA-3'</td>
<td>5'-ACCTCGAAGCGGAATTCCT-3'</td>
</tr>
<tr>
<td>COL1A2</td>
<td>5'-TCTCTACTGGCGGAAACCTGTA-3'</td>
<td>5'-TCTTACCAAGACGTATTTT-3'</td>
</tr>
<tr>
<td>MMP-1</td>
<td>5'-CCCCAAAGGGCTGTTGACATGA-3'</td>
<td>5'-GTTACAGGGATTTGGCC-3'</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>5'-CTCTGACATCGCCGGTGCT-3'</td>
<td>5'-GGTGTGTCACCTGAGAATTA-3'</td>
</tr>
<tr>
<td>α-SMA</td>
<td>5'-CCCCATCTATAGGGCTATGC-3'</td>
<td>5'-CGGCCACGAGATTCC-3'</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>5'-GCTCTCTCGTGTCAGTC-3'</td>
<td>5'-ACCTTCCATGGTTGTCGA-3'</td>
</tr>
</tbody>
</table>

**Reverse Transcription-PCR Analysis.** Total RNA was extracted according to the manufacturer’s instructions using the RNeasy mini-kit (QIAGEN, Valencia, CA). DNase I treatment (25 U, 15 min) of total RNA was directly performed on the spin columns to eliminate genomic contamination of the RNA samples. One microgram of total RNA was used for first strand cDNA synthesis using a RT-for-PCR kit (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. Real-time PCR was performed with an ABI PRISM 7700 instrument (Applied Biosystems, Foster City, CA) using SYBRGreen PCR Core Reagents (Applied Biosystems). Reaction mixtures were incubated for 2 min at 50°C, followed by 10 min at 95°C and 40 cycles of 15 s at 95°C, 45 s at 60°C, and 1.5 min at 68°C, and finally 15 s at 95°C, 20 s at 63°C, and 15 s at 95°C. For each mRNA, gene expression was corrected against glyceraldehyde-3-phosphate dehydrogenase mRNA content in each sample. Primers used are shown in Table 1.

**Transient Cell Transfections and Reporter Assays.** Transient cell transfections were performed with jetPEI according to the manufacturer’s protocol (Polyplus-transfection, Illkirch, France).

**Plasmid Constructs.** –3500COL1A1/CAT (a gift from Francesco Ramirez, Mt. Sinai School of Medicine, New York, NY) and –2300COL1A1/CAT (gift from John Varga, Northwestern University Feinberg School of Medicine, Chicago, IL) have been described previously (Boast et al., 1990; Chen et al., 1998).

**Western Blot Analyses.** Total cell extracts (30 μg) in Laemmli buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride) were denatured by heating at 95°C for 3 min before resolution by SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to Hybond membranes (Amersham Biosciences), immunoblotted with anti-type I collagen (Southern Biotech, Birmingham, AL), anti-α-smooth muscle actin (Sigma-Aldrich, St. Louis, MO), or anti-actin (Sigma-Aldrich) antibodies, all at a dilution of 1:1000 in 1X phosphate-buffered saline/5% nonfat milk for 1 h. After incubation, filters were washed and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h. Filters were stained with an ECL detection kit (Amersham Biosciences).
then washed, developed according to chemiluminescence protocols (ECL, Amersham Biosciences), and revealed with a PhosphorImager (Amersham Biosciences).

Quantitative Determination of MMP-1 Production. The MMP-1 production in cell supernates was determined using the Quantikine Human pro-MMP-1 Immunoassay kit from R&D Systems (Minneapolis, MN) according to the manufacturer’s instructions.

**Results**

**MMF Inhibits Type I Collagen Gene Expression.** To determine whether MMF had a direct effect on collagen production by fibroblasts, WI-26 human fibroblast cultures were incubated with various concentrations of MMF, and type I collagen production was estimated by Western blot analysis. As shown in Fig. 1, total type I collagen production was reduced by MMF in a time- and dose-dependent manner. A decrease of approximately 60 and 80% with a concentration of 10 μM and a decrease of 50 and 72% with a concentration of 1 μM were observed after 48 and 72 h of treatment, respectively. Even the lowest concentration of MMF (0.1 μM) had a significant inhibitory activity after 48 h of incubation, suppressing collagen production by as much as 20%. No toxicity of MMF and no change in cell proliferation, as assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyльтetrazo-

Fig. 1. MMF decreases type I collagen production. Confluent fibroblast cultures were treated with various concentrations of MMF (10, 1, or 0.1 μM) for 24, 48, and 72 h, as indicated. After incubations, type I collagen production was detected by Western blot analysis of whole-cell lysates (top). Specificity of the modulation was confirmed with an anti-actin antibody. The ratio of type I collagen to actin is plotted from the values of one representative experiment of four experiments (bottom, 24 h; middle, 48 h; right, 72 h).

**Fig. 2.** MMF decreases COL1A1 and COL1A2 mRNA levels. Confluent fibroblast cultures were treated with MMF (10 μM) or not for various times (24, 48, or 72 h), following which total RNA was extracted, and COL1A1 (A) and COL1A2 (B) mRNA steady-state levels were determined by quantitative RT-PCR. Bars, mean ± S.D. of at least three independent experiments performed, each with duplicate samples.

**Fig. 3.** MMF decreases COL1A1 and COL1A2 transcription. Fibroblast cultures were transfected with −2300COL1A1/CAT (A) and −3500COL1A2/CAT (B) collagen promoter/reporter constructs. Twelve hours after transfections, MMF (10 μM) was added, and incubations continued for another 48 h. Autoradiograms from CAT assays are from a representative experiment, each for the three experiments being performed with duplicate samples (top). Quantitation of CAT activity in each sample using a PhosphorImager is plotted as percentage of control for each promoter. Values are mean ± S.D. of three experiments performed with duplicate samples (bottom).

**Fig. 4.** MMF increases MMP-1 both at mRNA and protein levels. A, subconfluent fibroblasts were treated with MMF (10 μM) or not for various times (24, 48, and 72 h). After incubation, cell culture supernates were collected, and MMP-1 protein levels were estimated by immunoas-

**Bar graphs** show the levels of MMP-1 protein and mRNA in fibroblast cultures treated with MMF for the indicated times. Bars, mean ± S.D. of two independent experiments performed, each with six replicate samples.
rium test, was observed during the course of these experiments at any of the concentrations tested (data not shown).

To determine whether the reduction of type I collagen production in response to MMF occurred via modulation of the corresponding genes, COL1A1 and COL1A2 mRNA steady-state levels were estimated by quantitative RT-PCR. As shown in Fig. 2, MMF (10 μM) decreased COL1A1 (Fig. 2A) and COL1A2 (Fig. 2B) mRNA levels in a time-dependent manner, the extent of inhibition reaching 60 and 75%, respectively, after 48 h, and reaching 80 to 85% after 72 h of treatment. Similar results were obtained in response to MPA, the bioactive compound of MMF (data not shown).

Next, to determine whether MMF repressed type I collagen gene expression at the transcriptional level, transient cell transfections were performed with the collagen promoter/gene reporter constructs −2300COL1A1/CAT and −3500COL1A2/CAT. As shown in Fig. 3, COL1A1 (Fig. 3A) and COL1A2 (Fig. 3B) gene promoter activities were, respectively, reduced by 63 and 50% in the presence of MMF (10 μM) after 48 h of treatment, suggesting that the effect of MMF observed at the mRNA levels occurs via transcriptional down-regulation of the corresponding genes.

MMF Increases MMP-1 Gene Expression and Protein Synthesis. We first wanted to determine whether MMF had a direct effect on MMP-1 synthesis. To address this point, MMP-1 protein production was measured using MMP-1 immunoassay approach. As shown in Fig. 4A, MMF treatment of WI-26 fibroblasts led to increase in MMP-1 protein production up to 2.6- and 4.6-fold, respectively, after 48 and 72 h at the concentration of 10 μM. We next determined whether MMF modulates MMP-1 and/or TIMP-1 gene expression. To address this point, the effects of MMF on MMP-1 and TIMP-1 mRNA steady-state levels were studied by quantitative RT-PCR. MMF treatment of WI-26 fibroblasts led to a time-dependent increase in MMP-1 mRNA levels, up to 5.3-fold after 72 h at the concentration of 10 μM (Fig. 4B). On the other hand, TIMP-1 gene expression remained unaltered over the same incubation period (data not shown).

MMF Reduces Fibroblast Capacity to Contract Free-Floating Collagen Lattices. We next wanted to determine whether MMF alters ECM contraction by fibroblasts, a phenomenon that mechanistically involves both ECM remodeling and cell motility (Grinnell, 1994). To this end, WI-26 were

![Image](image-url)
seeded in free-floating, mechanically unloaded collagen gels and incubated in the presence or absence of MMF. The kinetics of collagen lattice contraction was then recorded over a 5-day period. Results shown in Fig. 5 indicate that MMF had a potent dose-dependent inhibitory effect on collagen lattice contraction by WI-26 fibroblasts. At day 5, MMF treatment at concentrations ranging from 0.1 μM to 10 μM resulted in significant greater lattice diameter relative to control, indicating inhibition of fibroblast contractile activity; up to a 50% increase in lattice diameter relative to control was observed at 5 days following treatment with 10 μM MMF. In addition, MPA (10 μM) mimics the effect of MMF (data not shown).

**MMF Reduces α-Smooth Muscle Actin Gene Expression.** α-SMA expression is the hallmark of the myofibroblast phenotype and is thought to contribute to their contractile activity (Skalli et al., 1986; Desmouliere et al., 2005). To determine whether MMF had a direct effect on α-SMA expression, WI-26 fibroblast cultures were incubated with MMF for various time periods, following which α-SMA production was measured by Western blot analysis. As shown in
Fig. 6A, α-SMA production was reduced by MMF in a time-dependent manner. A decrease of 22, 30, and 69% with a MMF concentration of 10 μM was observed after 2, 3, and 5 days, respectively, of treatment. To determine whether the reduction of α-SMA production by MMF occurred via modulation of the corresponding gene, we measured α-SMA mRNA steady-state levels following MMF treatment by quantitative RT-PCR. As shown in Fig. 6B, MMF at a concentration of 10 μM decreased α-SMA mRNA levels in a time-dependent manner. A decrease of 34, 60, and 83% with a MMF concentration of 10 μM was observed after 2, 3, and 5 days of treatment, respectively.

**MMF Inhibits Fibroblast Motility.** The effects of MMF on WI-26 fibroblasts migration were assessed firstly in a scrape wounding assay. As shown in Fig. 7A, photomicrographs taken 3, 8, and 24 h after wounding showed delayed wound closure by WI-26 fibroblast cultures treated with MMF at concentrations ranging from 0.1 to 10 μM compared with untreated control cultures. Quantitation of the wound closure over time revealed a significant inhibitory effect of MMF on fibroblast motility (Fig. 7B), even the lowest concentration of MMF (0.1 μM) after 24 h of incubation. Secondly, we utilized another motility assay based on the Transwell system, which is able to measure the cell migratory potential through nylon membrane pores. As shown in Fig. 7C, in the presence of 10 μM MMF, WI-26 fibroblasts exhibited a much lower capacity to migrate through the Transwell membrane than in the absence of MMF. These data, in full compliance with those obtained using the in vitro wound closure assay, allow us to conclude that MMFs alter fibroblast motility.

**Discussion**

In this study, we unveil direct inhibitory activity of the immunosuppressive drugs MMF on several fibroblast functions that are exacerbated during fibrotic processes. Specifically, we show that MMF transcriptionally inhibits type I collagen expression, enhanced the expression of MMP-1, and alters both the migratory and contractile functions of fibroblasts. These results suggest that MMF may have direct antifibrotic properties in addition to its well defined immunosuppressive effects.

MMF is a potent suppressor of T- and B-cell proliferation but may also be cytostatic for monocytes, vascular smooth muscle cells, and fibroblasts (Gregory et al., 1993; Allison and Eugui, 2000). It should be noted that under our experimental conditions (e.g., at confluence in medium containing 1% FCS), fibroblasts are contact-inhibited and do not proliferate, and MMF has no cytotoxic effect while exerting significant down-regulation of collagen gene expression and contraction. Most importantly, the MMF concentrations used for this in vitro study were calculated to achieve similar concentrations as those currently recommended for plasma levels in clinical practice among patients treated by MMF. The daily dose recommended for these patients is 2 g orally to reach plasma levels varying from 1 mg/l (trough levels) to 5 mg/l at 3 h, which corresponded to concentrations of 0.1 to 10 μM (Manzia et al., 2005).

During wound healing, fibroblasts undergo a transition to the myofibroblast phenotype that corresponds to the commencement of connective-tissue compaction and the contraction of the wound (Singer and Clark, 1999; Desmouliere et al., 2005). Such a phenotype is also found in pathological fibrotic situations because they occur for example during complications of organ transplantation, when excessive matrix deposition and excessive scarring takes place within the graft, leading to organ failure (Uitto and Kouba, 2000).

In our study, MMF was shown to reduce fibroblast capacity to contract free-floating collagen lattices, accompanied with a reduction in α-SMA expression. These results are in accordance with a previous report showing that MMFs reduce α-SMA production by fibroblasts in nephrectomized rats, thus preventing fibrosis (Badid et al., 2005). α-SMA participates importantly in force production by myofibroblasts both in vitro (Hinz et al., 2001a) and in vivo (Hinz et al., 2001b), contributing to scar formation, tissue contraction, scar hypertrophy, and, potentially, fibrosis. Furthermore, myofibroblasts not only promote ECM contraction but also synthesize elevated levels of ECM components (Desmouliere et al., 2005). Thus, the persistence of myofibroblasts within a fibrotic lesion leads to excessive scarring with the functional impairment of the affected organ(s). The ability of MMF to decrease α-SMA expression and subsequent matrix contraction by fibroblast thus is likely to confer direct antifibrotic activities to this immunosuppressant drug. Recently, we demonstrated that rapamycin, another immunosuppressive drug, post-transcriptionally alters fibrillar collagen synthesis by repressing its mRNA stability (Poulalhon et al., 2006), therefore utilizing mechanisms that are clearly distinct from those of MMF. In addition, rapamycin did not affect the migratory potential of fibroblasts or their capacity to contract unloaded collagen lattices (F. Verrecchia, N. Poulalhon, and D. Farge, unpublished data). Thus, our studies suggest that both immunosuppressive drugs directly affect fibroblast functions but with distinct molecular mechanisms that are independent from their immunosuppressive activity.

In conclusion, we have identified MMF as a molecule that may affect fibroblast functions important for wound healing and implicated in tissue scarring and fibrosis. These results suggest that MMF represents a novel therapeutic option for the treatment of fibrotic complications of autoimmune diseases and graft rejection.

**References**


Address correspondence to: Dr. Franck Verrecchia, Institut National de la Sante´ et de la Recherche Me ´dicale U697, Ho ˆpital Saint-Louis, Pavillon Bazin, 1 Avenue Claude Vellefaux, 75010 Paris, France. E-mail: franck.verrecchia@stlouis.inserm.fr