The Journal *of* Immunology

Hepatocyte Growth Factor Significantly Suppresses Collagen-Induced Arthritis in Mice

This information is current as of November 12, 2019.

Katsuhide Okunishi, Makoto Dohi, Keishi Fujio, Kazuyuki Nakagome, Yasuhiko Tabata, Takahiro Okasora, Makoto Seki, Mihoko Shibuya, Mitsuru Imamura, Hiroaki Harada, Ryoichi Tanaka and Kazuhiko Yamamoto

References   This article **cites 64 articles**, 20 of which you can access for free at:
http://www.jimmunol.org/content/179/8/5504.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Subscription   Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions   Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts   Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

Hepatocyte Growth Factor Significantly Suppresses Collagen-Induced Arthritis in Mice

Katsuhide Okunishi,* Makoto Dohi,[1]* Keishi Fujio,* Kazuyuki Nakagome,* Yasuhiko Tabata,[†] Takahiro Okasora,[†] Makoto Seki,[‡] Mihoko Shibuya,* Mitsuru Imamura,* Hiroaki Harada,* Ryoichi Tanaka,* and Kazuhiko Yamamoto*

Hepatocyte growth factor (HGF) plays an important role in angiogenesis, cell proliferation, antifibrosis, and antiapoptosis. Moreover, recent studies have highlighted the immunosuppressive effect of HGF in animal models of allogenic heart transplantation and autoimmune myocarditis and in studies in vitro as well. We also reported that HGF significantly suppresses dendritic cell function, thus down-regulating Ag-induced Th1-type and Th2-type immune responses in allergic airway inflammation. However, the immunosuppressive effect of HGF in many other situations has not been fully clarified. In the present study, using a mouse model of collagen-induced arthritis (CIA) and experiments in vitro, we examined the effect of HGF on autoimmune arthritis and then elucidated the mechanisms of action of HGF. To achieve sufficient delivery of HGF, we used biodegradable gelatin hydrogels as a carrier. HGF suppressed Ag-induced T cell priming by regulating the functions of dendritic cells in the Ag-sensitization phase with down-regulation of IL-10. In contrast, under continuous Ag stimulation HGF induced IL-10-producing immunocytes both in vivo and in vitro. Moreover, HGF potently inhibited the development of CIA with enhancing the Th2-type immune response. We also confirmed that HGF significantly suppressed the production of IL-17 by immunocytes. These results indicate that HGF suppresses the development of CIA through different ways at different phases. They also suggest that HGF could be an attractive tool for treating patients with rheumatoid arthritis. *The Journal of Immunology,* **2007, 179: 5504–5513.**

H epatocyte growth factor (HGF),[2] originally identified and cloned as a potent mitogen for hepatocytes (1–3) and a scatter factor (4), targets various cell types (5). HGF has many functions such as induction of angiogenesis, promotion of cell proliferation and migration (5), and inhibition of apoptosis (6, 7). HGF exhibits these functions through its receptor c-Met (5). It is well established that HGF promotes tumor progression (8–12) and suppresses the development of fibrosis after injury (13–15).

The role of HGF in immune-mediated disorders has not been fully studied. HGF promotes adhesion and migration of B (16, 17) and T cells (18) and enhances dendritic cell (DC) migration (19, 20). HGF frequently counteracts TGF-β, a potent immunosuppressive cytokine (13, 14, 21). These results indicate that HGF might accelerate immune responses. In contrast, recent studies clarified an immunosuppressive effect of HGF. In a mouse model of allogenic heart transplantation, HGF reduced acute and chronic rejection of the allograft with increased expression of TGF-β and IL-10,

indicating that HGF might induce allograft tolerance (22). HGF ameliorates the progression of experimental autoimmune myocarditis, a Th1-type dominant immune response, inducing production of Th2 cytokines (23). In addition, other articles reported that HGF suppresses the development of Th2-type responses as well (24–26). HGF attenuates allergic airway inflammation (24, 25), and one article recently reported that HGF prevents lupus nephritis in a murine lupus model of chronic graft-vs-host disease through suppression of Th2-type immune responses (26). These results indicate that HGF could suppress both Th1-type and Th2-type immune responses. As to the mechanisms of immune suppression by HGF, two major possibilities have been reported. One is the down-regulation of functions of DCs, a mechanism elucidated in the case of allergic airway inflammation that was reported by us previously (24). Another mechanism is to induce the regulatory phenotype of $CD4^+$ T cells that produce IL-10 or TGF-β, which was studied in an experimental system of allogenic heart transplantation (22) and in vitro (23).

Rheumatoid arthritis (RA) is an autoimmune disorder and a systemic chronic inflammatory disease characterized by persistent synovial cell proliferation with inflammatory cell infiltration and destruction of joints (27). The mechanism and pathogenesis of RA have not been fully clarified. RA has traditionally been assumed to be a Th1-type disease (28, 29). However, recent studies revealed a new lineage of effector $CD4^+$ T cells characterized by the production of IL-17, and this Th17 lineage plays an essential role in both the development of autoimmune arthritis (30, 31) and bone destruction (32). In addition to the T cell-mediated immune responses, angiogenesis plays a very important role in maintaining and promoting RA (33).

The role of HGF in RA has been reported in a few cases. HGF and its receptor c-Met were found in the synovial tissue of patients with RA (34). HGF levels in synovial fluids were significantly higher in patients with RA than in those with arthritis of other

*Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; [†]Institute of Frontier Medical Sciences, Kyoto University, Kyoto, Japan; and [‡]Research Laboratory III, Pharmaceutical Research Division, Mitsubishi Pharma Corporation, Yokohama, Japan

[1] Address correspondence and reprint requests to Dr. Makoto Dohi, Department of Allergy and Rheumatology, Graduate School of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan. E-mail address: mdohi-tky@umin.ac.jp

[2] Abbreviations used in this paper: HGF, hepatocyte growth factor; CIA, collagen-induced arthritis; CII, type II collagen; DC, dendritic cell; EU, ELISA unit; LN, lymph node; RA, rheumatoid arthritis; rhHGF, recombinant human HGF; Treg, regulatory T.

causes such as osteoarthritis (34–36). Moreover, RA synovial fluids induced a greater scattering of cells than did osteoarthritis synovial fluids (34). These reports indicate that HGF may play some role in RA. Because HGF is an angiogenesis factor, it might promote joint inflammation. In contrast, considering its immunosuppressive effect, HGF might suppress the development of Ag-induced arthritis. To date, it has not been studied whether HGF would suppress immune-mediated arthritis.

To determine the effect of HGF on autoimmune arthritis, we delivered HGF to mice and examined the effect on collagen-induced arthritis (CIA). We immunized mice with type II collagen (CII) and induced experimental arthritis. HGF was applied s.c. and delivered by gelatin-coupled controlled release to achieve a sustained and effective delivery. The T cell response to CII was analyzed in vitro, and arthritis was examined in vivo. HGF suppressed CII-induced T cell priming in the spleen and diminished the severity and incidence of arthritis with up-regulation of IL-10 and suppression of IL-17.

Materials and Methods

Mice

Male BALB/c mice (aged 6 wk) and DBA/1 mice (aged 7 wk) were obtained from Charles River Laboratories Japan. They were maintained under conventional animal housing conditions in a specific pathogen-free setting. All of the animal experiments conducted in this study were approved by the Animal Research Ethics Board of the Department of Allergy and Rheumatology, University of Tokyo, Tokyo, Japan.

ELISA

Concentrations of mouse IL-4, IL-10, IL-12p70, IFN-γ (BD Pharmingen), IL-23 (eBioscience), and CII-specific IgG (Chondrex) were measured using an ELISA kit following the manufacturer's protocol. Concentrations of human HGF in the sera were measured using an IMMUNIS HGF enzyme immunoassay kit (Institute of Immunology, Tokyo, Japan). CII-specific IgG2a was measured with ELISA grade type II collagen (Chondrex) for capture and HRP-conjugated anti-mouse IgG2a Ab (BD Pharmingen) for detection. The average concentration of the sera from the control mice on day 40 was defined as 1000 ELISA unit (EU). Mouse IL-17 was measured by ELISA using purified rat anti-mouse IL-17 mAb for capture and biotinylated rat anti-mouse IL-17 mAb for detection (BD Pharmingen). The titers of samples for IL-17 were calculated by comparison with internal standards. On day 10 after sensitization, lymph node (LN) cells were obtained from mice sensitized with CII/CFA and restimulated in vitro with CII (10 μg/ml) for 4 days. The average concentration in the supernatants was defined as 1000 EU. Cell proliferation was measured by BrdU incorporation using a BrdU cell proliferation ELISA kit (Roche). The data were analyzed with Microplate Manager III, version 1.45 (Bio-Rad).

Preparation of gelatin microspheres incorporating HGF

Acidic gelatin hydrogel microspheres were prepared from gelatin with an isoelectric point of 5.0 (Nitta Gelatin) as reported previously (37, 38). The solution (5 mg/ml) of recombinant human HGF (rhHGF) (1, 2) was dropped onto 2 mg of gelatin microspheres and left at 37°C for 1 h so that the HGF could impregnate the microspheres. In a previous study, we confirmed that when this gelatin/rhHGF complex was s.c. injected into mice a controlled release of HGF was achieved based on hydrogel degradation and that the degradation occurred over 10 days (37). In the present study, gelatin or gelatin/rhHGF were diluted in 100 μl of PBS and then injected into mice.

Conditions for cell culture

Throughout the present study complete DMEM was used as the medium for cell incubation as we previously reported (24, 39). Cells were incubated in a 96-well, flat-bottom, microtiter assay plate in an incubator (37°C with 5% CO2 and 90% humidity) for given periods.

Preparation of single cell suspensions of spleen and lymph node cells

Single cell suspensions of spleens and femoral lymph nodes were prepared as in previous reports (39).

Purification of mouse splenic CD4$^+$ T cells and DCs

Mouse splenic CD4$^+$ T cells were negatively selected using an anti-mouse CD4$^+$ T cell isolation kit (Miltenyi Biotec). Mouse splenic DCs were positively selected using anti-mouse CD11c colloidal superparamagnetic microbeads (Miltenyi Biotec) as reported previously (24, 39–41). The purity of CD4$^+$ and CD11c$^+$ cells, confirmed by flow cytometry, was >95% and >85%, respectively.

Protocol for OVA/alum-induced immune responses

BALB/c mice were sensitized with 2 μg of OVA (Sigma-Aldrich) in 2 mg of alum (Serva) on day 0 as reported previously (24, 39). Then, a few hours after the OVA/alum injection mice received a single s.c. injection of gelatin (2 mg) or a gelatin/rhHGF complex (2 mg and 100 μg, respectively) in the dorsal skin. On day 10, spleen cells from each group of mice were collected and then restimulated in vitro with OVA. After 3 days of incubation with OVA at several concentrations, spleen cell proliferation was measured based on BrdU incorporation. After 4 days of incubation with OVA (100 μg/ml), cytokine concentrations in the supernatants were measured. CD4$^+$ T cells (1 × 10^6 cells/ml) were also negatively selected and then stimulated with PMA (1 ng/ml; Sigma-Aldrich) and ionomycin (0.1 μg/ml). After 2 days of incubation, IL-10 concentrations in the supernatants were measured.

Induction of CIA

CIA was induced as reported previously (42). In brief, CII (2 mg/ml in 0.05 M acetic acid) was emulsified with an equal volume of CFA (4 mg/ml; Chondrex). Mice were injected s.c. ~1–2 cm from the base of the tail with 100 μl of the emulsion on day 0. On day 21, the mice received a booster injection of the CII/IFA emulsion s.c. around the base of the tail. Mice also received s.c. injections of gelatin (2 mg) or gelatin/rhHGF (100 μg) complex diluted in 100 μl of PBS on day 0 and every 10 days thereafter. The development of arthritis was assessed by inspection on day 25 and then every 2 to 3 days. The clinical severity of arthritis in each paw was quantified according to a graded scale from 0 to 4 as follows: 0, no swelling; 1, swelling in one digit or mild edema; 2, moderate swelling affecting several digits; 3, severe swelling affecting most digits; and 4, the most severe swelling and/or ankylosis (42). A mean arthritis score was determined by summing the scores of all joints of all mice and dividing the result by the total number of mice in the group.

Histologic examination and ex vivo examination

Mice were killed on day 40 and the joints of the more severely swollen hind paw were obtained. Histologic examination of the joints was performed as reported previously (42). The pathologic condition was scored by two blinded examiners from the Sapporo General Pathology Institute (Sapporo, Japan) in four categories: cartilage, cellularity, pannus, and bone erosion. Each category was graded from 0 to 4 as follows: 0, normal; 1, minimal; 2, mild; 3, moderate; and 4, marked.

Protocol for ex vivo experiments in the CII-induced immune responses

CIA was induced as described above. To examine the effect of HGF on immunocytes, a single cell suspension of spleen or femoral LN was prepared and cell responses (5 × 10^6 cells/ml) to in vitro CII restimulation (10 μg/ml) were examined on days 10, 20, and 40. To examine the effect of HGF on DCs, splenic DCs were also positively selected from each group of mice on days 10, 20, and 40, and the production of cytokines by DCs (1 × 10^6 cells/ml) after LPS (1 μg/ml) stimulation for 2 days was examined. To examine the effect of DCs on CD4$^+$ T cells, in some experiments, DCs were cocultured with CD4$^+$ T cells with CII in the medium. For analysis of the Ag-presenting capacity of DCs after mitomycin C treatment (10 μg/ml for 35 min at 37°C) to inhibit cell proliferation of DCs themselves, DCs (1 × 10^5 cells/ml) and splenic CD4$^+$ T cells (1 × 10^6 cells/ml) from CII/CFA-sensitized control mice on day 10 were cocultured in the presence of CII (3 μg/ml). After 3 days of coculture, cell proliferation was measured by BrdU incorporation. For analysis of the effect of DCs on cytokine production by CD4$^+$ T cells, DCs from each group of mice and splenic CD4$^+$ T cells were cocultured with CII (10 μg/ml) in the medium. After 4 days of coculture, we examined cytokine production by CD4$^+$ T cells. We also examined the effect of HGF on the cytokine profile of CD4$^+$ T cells. CD4$^+$ T cells purified from each group of mice on days 10, 20, and 40 were stimulated with PMA and ionomycin as described above. Cytokine concentrations in the supernatants were measured after the indicated duration of incubation. To examine the effect of HGF in the presence of Ag on Ag-induced T cell activation, spleen cells (5 × 10^6 cells/ml) obtained

Table I. *Time course of HGF concentration in the sera (pg/ml)[a]*

	Hours or Days after Injection			
	4 h	Day 1	Day 2	Day 4
HGF protein				
HGF (10 μg)	ND[b]	ND	ND	ND
HGF (100 μg)	1658 ± 447	216 ± 71	ND	ND
Gelatin/rhHGF complex				
HGF (0 μg)	ND	ND	ND	ND
HGF (100 μg)	801 ± 117	188 ± 34	174 ± 174	ND

[a] Data are the mean ± SEM from three to four animals per group.
[b] Not detected.

FIGURE 1. Controlled release of HGF in vivo potently suppresses T cell priming with OVA/alum. BALB/c male mice were sensitized with OVA/alum and a few hours later received a s.c. injection of gelatin (2 mg) (control mice) or gelatin/rhHGF (100 μg) complex (HGF) on day 0. On day 10, spleen cells were obtained from each group of mice. *A–E*, Spleen cell responses (2.5×10^6 cells/ml) to OVA restimulation in vitro were examined. *A*, Cell proliferation was measured after 3 days of incubation with the indicated concentrations of OVA. Data are expressed as a percentage of the response compared with that of spleen cells from control mice at OVA (1000 μg/ml). *B–D*, Production of IL-4 (*B*) and IFN-γ (*C*) as well as IL-10 (*D*) was measured by ELISA after 4 days of incubation with OVA (100 μg/ml). *E*, IL-10 production by CD4$^+$ T cells after nonspecific stimulation. CD4$^+$ T cells were negatively selected and then stimulated in vitro with PMA (1 ng/ml) and ionomycin (0.1 μg/ml) for 2 days. IL-10 concentrations in the supernatants were measured. Data were obtained from four wells per group of mice. ##, $p < 0.01$; and ###, $p < 0.001$ (vs control mice).

from CII/CFA-sensitized mice on day 10 were restimulated with CII (10 μg/ml) in the presence or absence of rhHGF at several concentrations. After 3 to 4 days of incubation, cytokine production was measured.

Flow cytometry

Expression of surface molecule on DCs obtained from each group of mice on day 10 was examined as reported previously (43) by flow cytometry (EPICS XL System II; Beckman Coulter). We also examined the expression of CD25 and Foxp3 in CD4$^+$ T cells on days 10, 20, and 40. Staining of spleen or LN cells with anti-mouse CD4, CD25, and Foxp3 Abs was conducted following the manufacturer's protocol. In brief, first the cells were stained with allophycocyanin-conjugated anti-mouse CD4 Ab and FITC anti-mouse CD25 Ab (BD PharMingen). Then, intracellular Foxp3 staining was conducted using anti-mouse Foxp3 Ab and fixation/permeabilization solution and permeabilization buffer contained in a mouse regulatory T cell staining kit (eBioscience). Then stained cells were analyzed by flow cytometry (EPICS Elite; Beckman Coulter).

RT-PCR

mRNA was extracted from CD4$^+$ T cells by the acid-guanidium phenol chloroform method using Isogen (Nippon Gene). Then, RT-PCR was conducted as reported previously (39). PCR for GATA-3 consisted of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C for 26 cycles. PCR for β-actin consisted of 1 min of denaturation at 94°C, 1 min of annealing at 61°C, and 1 min of extension at 72°C for 18 cycles. The sense primer for the transcription factor GATA-3 was 5′-TCT GGAGGAGGAAACGCTAATGG-3′ and the antisense primer was 5′-GAACTCTTCGCACACTTGGAGACTC-3′. The sense primer for β-actin was 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and the antisense primer was 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′. PCR products were electrophoresed in a 3% agarose gel, and the results were visualized by ethidium bromide staining.
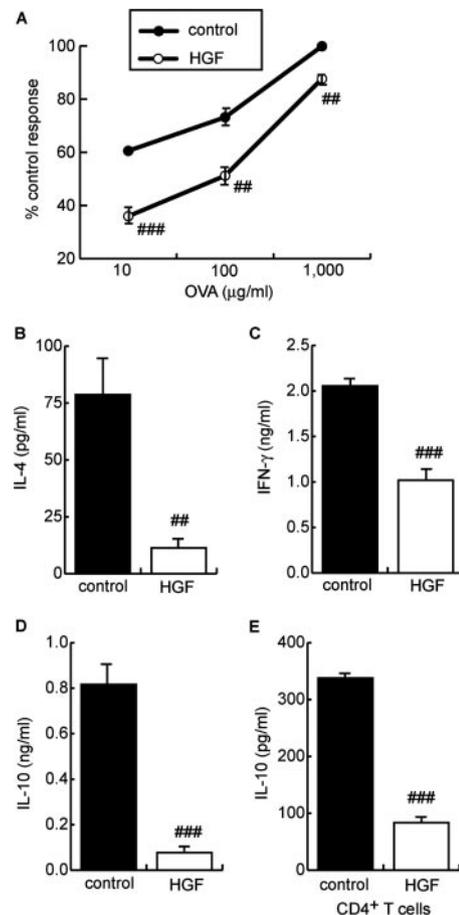
Statistical analysis

Values are expressed as the mean ± SEM. The Mann-Whitney *U* test was used to analyze the clinical scores and histologic findings. The unpaired *t* test was used to analyze the other results. Values of $p < 0.05$ were considered to be significant.

Results

HGF significantly suppresses T cell priming induced by OVA/alum

Generally, exogenously administered HGF protein delivered by i.v. injection vanishes from organs within several hours (44). So, to achieve efficient delivery of HGF we adopted biodegradable gelatin hydrogels as a carrier for the CIA model and delivered the HGF/gelatin complex by s.c. injection (37). First, we examined the time course of HGF concentration in sera after s.c. injection of HGF protein, gelatin, or gelatin/rhHGF complex. We confirmed that the more sustained release of HGF was achieved by s.c. injection of gelatin/rhHGF complex compared with the injection of HGF protein alone (Table I). Then, we examined the effect of this gelatin/rhHGF complex (designated HGF in figures) on OVA-induced immune responses. Spleen cells obtained from the mice

treated with HGF demonstrated significantly reduced cell proliferation (Fig. 1*A*) and the production of IL-4 (Fig. 1*B*), IFN-γ (Fig. 1*C*), and IL-10 (Fig. 1*D*) upon stimulation with OVA-Ag. Then, we also confirmed that treatment with HGF in vivo significantly suppressed IL-10 production by CD4$^+$ T cells in response to nonspecific stimulation with PMA and ionomycin (Fig. 1*E*). These results indicated that HGF potently suppressed Ag-induced T cell priming with a down-regulation of IL-10 production.

HGF significantly suppresses T cell priming induced by CII/CFA

Then, we examined the immunosuppressive effect of HGF in the CIA model. DBA/1 mice were sensitized with CII/CFA and received a s.c. injection of gelatin or gelatin/rhHGF complex once on day 0. On day 10, spleen cells were obtained and then restimulated
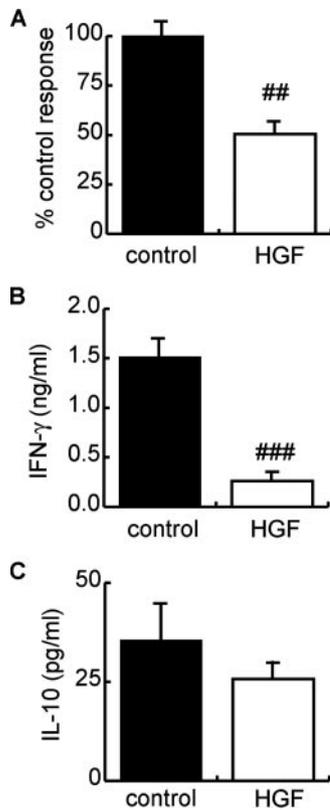
FIGURE 2. Controlled release of HGF in vivo potently suppresses T cell priming by CII/CFA. DBA/1 male mice were sensitized with CII/CFA and a few hours later, received a s.c. injection of gelatin (control) or gelatin/rhHGF (HGF) complex on day 0. On day 10, spleen cells were obtained from each group of mice and spleen cells (5×10^6 cells/ml) were restimulated with CII (10 μg/ml) in vitro. *A*, Cell proliferation after 3 days of incubation was measured by BrdU incorporation. Data are expressed as a percentage of the response compared with that of spleen cells from control mice. *B* and *C*, Production of IFN-γ after 3 days of incubation (*B*) and IL-10 after 4 days of incubation (*C*) was measured by ELISA. Data were obtained from four wells per group of mice. ##, $p < 0.01$ (vs control mice).

in vitro with CII. Spleen cells obtained from the mice treated with HGF demonstrated significantly reduced cell proliferation (Fig. 2*A*) and IFN-γ production (Fig. 2*B*). The production of IL-10 by spleen cells from mice treated with HGF also tended to decrease compared with that by cells from control mice (Fig. 2*C*). At this time point, IL-4 production was very low. We obtained almost the same results using femoral LN cells instead of spleen cells (data not shown). In preliminary experiments, we confirmed that the s.c. injection of HGF protein (10 μg/mouse/day) once daily on days 0–9 had no effect on CII/CFA-induced T cell priming (data not shown). These results indicated that the controlled release of HGF using the gelatin/rhHGF complex could suppress Ag-induced T cell priming independently of the kind of Ag and mouse strain and that this immunosuppressive effect might be exhibited without up-regulation of IL-10 production.

HGF significantly suppresses Ag-induced DC activation

We previously reported that HGF significantly suppressed DC functions such as Ag presentation and cytokine production, thus inhibiting OVA-induced not only Th2-type immune responses but also Th1-type immune responses (24). In the present study, we examined the mechanism of immunosuppression by HGF in CII/CFA-induced sensitization. DBA/1 mice were sensitized and treated as described above, and on day 10 DCs were purified from each group of mice. Then cytokine production by DCs after in vitro LPS stimulation was examined. Treatment with the HGF complex in vivo significantly suppressed the production of IL-10 (Fig. 3*A*), IL-12p70 (Fig. 3*B*), and IL-23 (Fig. 3*C*) by DCs after LPS stimulation. Moreover, compared with DCs from control mice, DCs from HGF-treated mice demonstrated a significantly decreased capacity to induce the proliferation of CD4$^+$ T cells (Fig. 3*D*) and the production of IL-10 (Fig. 3*E*) and IFN-γ (Fig. 3*F*) from CD4$^+$ T cells obtained from the CII/CFA-sensitized mice in the presence of CII in the medium. Moreover, we also confirmed that CD40 expression was reduced in DCs obtained from HGF-treated mice compared with that in DCs from control mice (Fig. 3*G*). These results suggested that HGF significantly suppressed DC function in the early stages of the Ag-induced immune response, thus suppressing Ag-induced CD4$^+$ T cell activation.

### HGF up-regulates IL-10 production by immunocytes under continuous Ag stimulation

Next, we examined the effect of HGF on Ag-primed T cells using ex vivo and in vitro experiments. In ex vivo experiments, mice were sensitized with CII/CFA on day 0, received gelatin or gelatin/rhHGF complex on days 0 and 10, and spleen cells were collected on day 20 from each group of mice. Then the spleen cells were restimulated in vitro with CII. Spleen cells obtained from the mice treated with HGF demonstrated significantly increased IL-10 production (Fig. 4*A*). The production of IFN-γ by spleen cells from mice treated with HGF tended to decrease compared with that of cells from control mice (Fig. 4*B*). IL-4 production by spleen cells from each group of mice was very low and did not differ between each group at this time point (data not shown). We also confirmed that CD4$^+$ T cells obtained on day 20 from the mice treated with HGF demonstrated significantly increased IL-10 production after nonspecific PMA and ionomycin stimulation (Fig. 4*C*). Moreover, we examined the cytokine profile of splenic DCs purified on day 20 and found that IL-10 production by DCs from mice treated with HGF tended to increase compared with that of DCs from control mice (Fig. 4*D*), while IL-12p70 production by DCs was as significantly suppressed by HGF as it was on day 10 (Fig. 4*E*). These results indicated that, under continuous Ag-stimulation, HGF could induce IL-10-producing immunocytes including T cells and DCs. To confirm this possibility, we then conducted in vitro studies. Spleen cells obtained on day 10 from CII/CFA-sensitized mice were restimulated in vitro with CII in the presence or absence of HGF in the medium. Like the treatment with HGF in vivo, HGF in vitro significantly up-regulated IL-10 (Fig. 4*F*) production by splenocytes without affecting IFN-γ and IL-4 production (Fig. 4*G*).

### HGF significantly reduces IL-17 production by T cells

We also examined the effect of HGF on the production of IL-17 by T cells. The femoral LN cells from HGF-treated mice produced significantly less IL-17 than those from control mice on days 10 (Fig. 5*A*) and 20 (Fig. 5*B*), although no significant difference was detected in spleens (data not shown).

Controlled release of HGF significantly suppresses development of CIA in mice

Then, we examined the effect of HGF on the development of experimental arthritis. DBA/1 mice were sensitized with CII/CFA on day 0 and received a booster injection of CII/IFA on day 21. Mice received s.c. injections of gelatin or gelatin/rhHGF complex on day 0 and every 10 days. The severity of the arthritis in the mice was scored on a scale of 0–4 for each limb. Progression of the
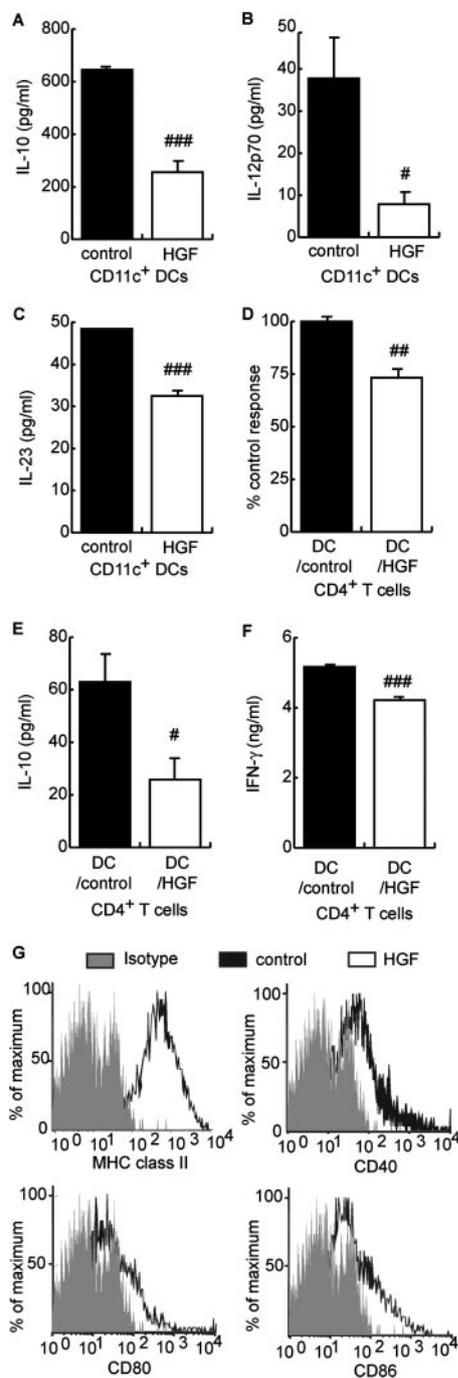
FIGURE 3. Controlled release of HGF in vivo potently suppresses DC functions, thus down-regulating Ag-induced $CD4^+$ T cell activation. Mice were treated as described in Fig. 2. On day 10, $CD11c^+$ DCs and $CD4^+$ T cells were purified from spleen cells as described in *Materials and Methods*. Then, the functions of DCs from each group of mice were examined. *A–C*, Cytokine production by DCs after LPS stimulation in vitro. DCs (1×10^6 cells/ml) from each group of mice were stimulated with LPS ($1 \mu g/ml$) in vitro. After 2 days, IL-10 (*A*), IL-12p70 (*B*), and IL-23 (*C*) in the supernatants were measured. *D–F*, Effects of DCs from each group of mice on the cell proliferation of and cytokine production by primed $CD4^+$ T cells. $CD4^+$ T cells (1×10^6 cells/ml) were obtained from control mice and cocultured with DCs (1×10^5 cells/ml) from each group of mice in the presence of CII ($3 \mu g/ml$ for *D* and $10 \mu g/ml$ for *E* and *F*) in the medium. After 3 days (*D*), the cell proliferation of $CD4^+$ T cells was measured. After 4 days of incubation, the production by $CD4^+$ T cells of IL-10 (*E*) and IFN-γ (*F*) was measured. Data were obtained from three to four wells per group of mice. #, $p < 0.05$; ##, $p < 0.01$; ###, $p < 0.001$ (vs DCs from control mice). *G*, Effect of HGF on surface molecule expression on
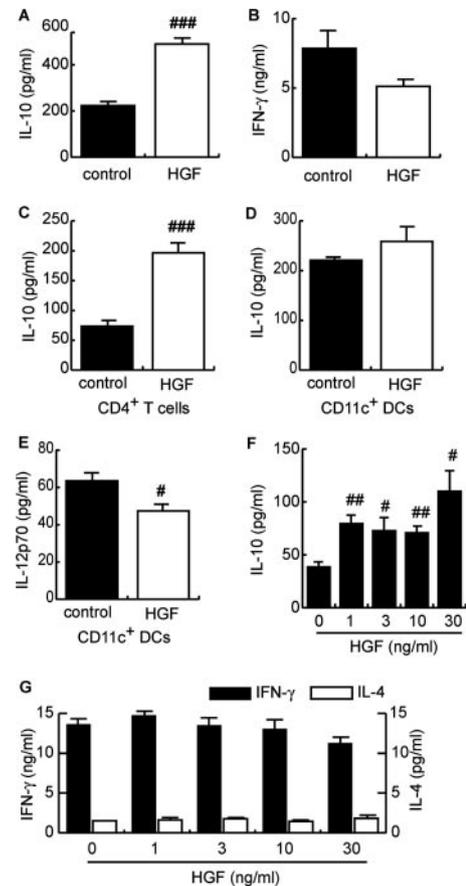
FIGURE 4. HGF significantly increased IL-10 production by Ag-primed immunocytes. *A–E*, Effect of treatment with HGF in vivo after Ag priming on cytokine production by spleen cells, $CD4^+$ T cells, or DCs. Mice were sensitized with CII/CFA on day 0. Mice also received gelatin (control) or gelatin/HGF complex (HGF) on days 0 and 10. On day 20, whole spleen cells, splenic $CD4^+$ T cells, or DCs were obtained from each group of mice. Then, spleen cells (5×10^6 cells/ml) were restimulated with CII ($10 \mu g/ml$) in vitro. Production of IL-10 (*A*) and IFN-γ (*B*) after 4 days of incubation was measured. $CD4^+$ T (1×10^6 cells/ml) cells were stimulated in vitro with PMA ($1 ng/ml$) and ionomycin ($0.1 \mu g/ml$) for 2 days, and IL-10 concentrations in the supernatants were measured (*C*). DCs (1×10^6 cells/ml) were stimulated with LPS ($1 \mu g/ml$) for 2 days, and IL-10 (*D*) and IL-12p70 (*E*) concentrations in the supernatants were measured. Data were obtained from four wells per group of mice. *F* and *G*, Effect of in vitro treatment with HGF on cytokine production by spleen cells induced by Ag restimulation. Mice were sensitized with CII/CFA on day 0, and spleen cells were obtained on day 10. Spleen cells (5×10^6 cells/ml) were restimulated with CII ($10 \mu g/ml$) in vitro in the presence or absence of rhHGF at several concentrations for 4 days. Concentrations of IL-10 (*F*), IFN-γ (■), and IL-4 (□) (*G*) in the supernatant were measured. #, $p < 0.05$; ##, $p < 0.01$; and ###, $p < 0.001$ (vs spleen cells, $CD4^+$ T cells, or DCs from control mice, respectively).

arthritis was evaluated until day 39 after immunization. On day 40, the most severely swollen hind paw was obtained from each mouse, and a histologic examination was conducted. HGF treatment significantly suppressed the severity (Fig. 6*A*) and incidence (Fig. 6*B*) of CII-induced arthritis. Histologic examination demonstrated that HGF potently reduced articular destruction such as cartilage destruction, synovial hypertrophy, pannus formation, and

---

$CD11c^+$ DCs. The expression of MHC class II, CD40, CD80, and CD86 was examined by flow cytometry. Representative data from three independent experiments are shown.
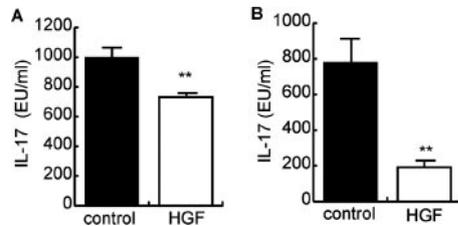
FIGURE 5. Treatment with gelatin/HGF complex in vivo potently suppresses IL-17 production. Mice were sensitized with CII/CFA and a few hours later received a s.c. injection of gelatin (control) or gelatin/rhHGF complex (HGF) on day 0. On day 10, femoral LN cells were obtained from each group of mice. Some mice also received additional treatment with gelatin (control) or gelatin/rhHGF complex on day 10 and femoral LN cells were obtained on day 20. Then the cells obtained on the indicated days were restimulated with CII (10 μg/ml) in vitro for 4 days and IL-17 concentrations in the supernatants were measured. IL-17 production by LN cells obtained from control mice on day 10 was defined as 1000 EU. *A*, IL-17 production by LN cells obtained on day 10. *B*, IL-17 production by LN cells obtained on day 20. ##, $p < 0.01$ (vs control mice).

Table II. *Impact of treatment with HGF in the murine CIA model[a]*

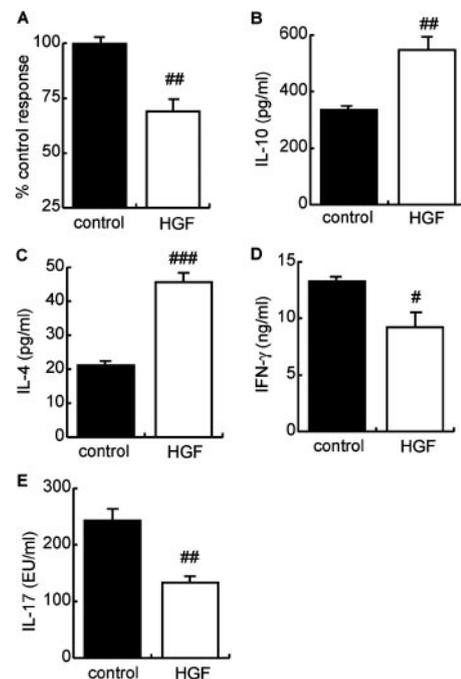| Pathologic Category	Control	HGF
Cartilage	1.33 ± 0.441	0.111 ± 0.111^{b}
Cellularity	1.22 ± 0.521	0.222 ± 0.222
Pannus	1.11 ± 0.455	0.111 ± 0.111
Bone erosion	1.11 ± 0.484	0.111 ± 0.111

[a] Data are the mean $\pm$ SEM pathologic score from nine animals per group (0, normal; 1, minimal; 2, mild; 3, moderate; and 4, marked).
[b] $p < 0.05$ vs control mice (Mann-Whitney U test).

HGF protein (10 μg/mouse/day) once daily on days 0–40 had no suppressive effect on the development of CII-induced arthritis (data not shown). These results indicated that controlled release of HGF could suppress Ag-induced arthritis.

Continuous treatment with HGF during Ag-induced chronic inflammation enhances Th2-type immune responses

Finally, we elucidated the mechanism of suppression by HGF in the chronic phase of arthritis. Mice were sensitized and then treated as described above. On day 40, spleen cells were obtained from each group of mice and restimulated in vitro with CII. Spleen cells obtained on day 40 from the mice treated with HGF demonstrated significantly reduced cell proliferation (Fig. 7*A*) and enhanced IL-10 production (Fig. 7*B*) in response to in vitro CII restimulation. Interestingly, in this chronic phase of Ag-induced

bone erosion (Fig. 6, *C–F* and Table II). HGF significantly reduced CII-specific total IgG (Fig. 6*G*) and IgG2a (Fig. 6*H*) production. In a preliminary experiment, we confirmed that the s.c. injection of
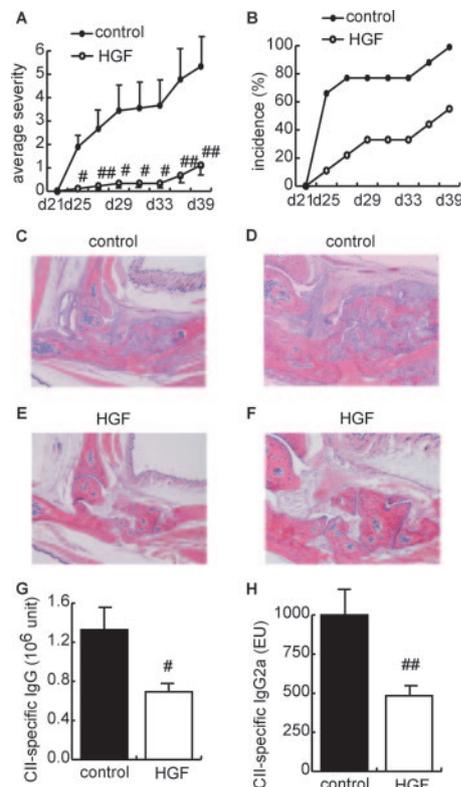
FIGURE 6. Treatment with gelatin/HGF complex in vivo significantly suppresses development of CIA. Arthritis was induced in DBA/1 mice by immunization with CII in Freund's complete adjuvant on day 0. On day 21, mice were injected s.c. with CII in Freund's incomplete adjuvant. Mice also received gelatin (control; $n = 9$) or gelatin/HGF complex (HGF; $n = 9$) on day 0 and every 10 days. *A*, Arthritis scores in the two groups. Clinical scores were determined as described in *Materials and Methods*. *B*, Incidence of arthritis in the two groups. *C–F*, H&E staining of representative hind paws from control mice (*C* and *D*) and mice treated with gelatin/HGF complex (*E* and *F*). Original magnification: ×16 for *C* and *D* and × 32 for *E* and *F*. *G* and *H*, CII-specific total IgG (*G*) and IgG2a (*H*) concentration in the sera obtained from each group of mice on day 40. Data were obtained from nine mice per group. #, $p < 0.05$; ##, $p < 0.01$ (vs control mice).

FIGURE 7. In vivo treatment with gelatin/HGF complex (HGF) in the presence of persistent Ag stimulation enhances Ag-specific Th2-type immune responses. Mice were treated as described in Fig. 6. On day 40, spleen cells were collected from each group of mice. *A–E*, Spleen cell responses to in vitro CII (10 μg/ml) stimulation were examined. *A*, Cell proliferation after 3 days of incubation. Data are expressed as a percentage of the response compared with that of spleen cells from control mice. *B–E*, Concentrations of IL-10 (*B*) and IL-4 (*C*) after 5 days of incubation, IFN-γ after 4 days of incubation (*D*), and IL-17 (*E*) after 3 days of incubation in the supernatants were measured. Data were obtained from four wells per group of mice. #, $p < 0.05$; ##, $p < 0.01$; and ###, $p < 0.001$ (vs spleen cells from control mice).
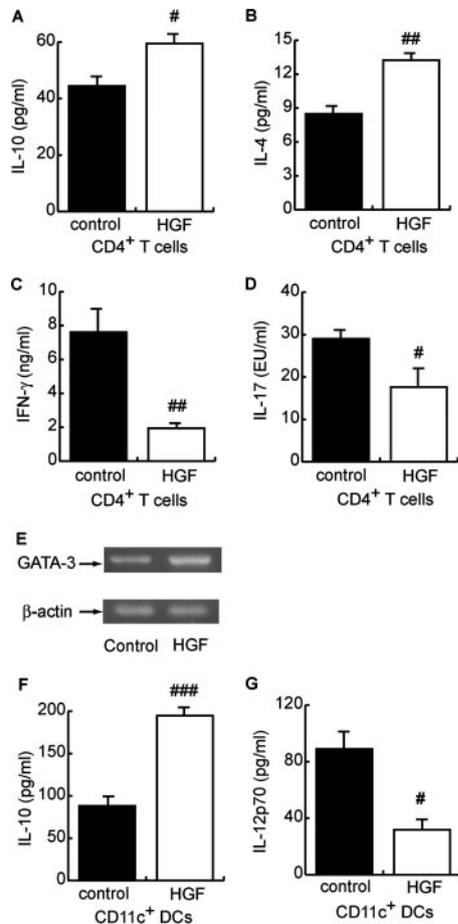
FIGURE 8. Effect of repeated treatment with gelatin/HGF complex (HGF) in vivo on cytokine production by CD4$^+$ T cells and DCs. Mice were treated as described in Fig. 6. On day 40, splenic CD4$^+$ T cells and DCs were purified from each group of mice. Then, CD4$^+$ T cells (1 × 10^6 cells/ml) were stimulated with PMA (1 ng/ml) and ionomycin (0.1 μg/ml) and IL-10 production after 1 day of incubation (*A*), IL-4 production after 20 h of incubation (*B*), and IFN-γ (*C*) and IL-17 (*D*) production after 2 days of incubation were measured. *E*, GATA-3 mRNA expression in CD4$^+$ T cells. RNA was extracted from splenic CD4$^+$ T cells and then RT-PCRs for GATA-3 and β-actin were conducted. *F* and *G*, DCs were stimulated with LPS (1 μg/ml) for 2 days, and IL-10 (*F*) and IL-12p70 (*G*) concentrations in the supernatants were measured. Data were obtained from three to four wells per group of mice. #, $p < 0.05$; ##, $p < 0.01$; and ###, $p < 0.001$ (vs CD4$^+$ T cells or DCs from control mice, respectively).

immune response, spleen cells obtained from control mice produced a significant amount of IL-4 in response to Ag restimulation, and spleen cells from HGF-treated mice demonstrated significantly enhanced production of IL-4 after Ag restimulation (Fig. 7*C*) with down-regulation of cytokine production for IFN-γ (Fig. 7*D*) and IL-17 (Fig. 7*E*). Further, the cytokine profiles of CD4$^+$ T cells from each group of mice after PMA and ionomycin stimulation (Fig. 8, *A–D*) were the same as those of spleen cells after CII restimulation (Fig. 7, *B–E*). We also confirmed that treatment with HGF enhanced mRNA expression of the transcription factor GATA-3, which is known as a master gene for Th2 cell development (45), in splenic CD4$^+$ T cells obtained on day 40 (Fig. 8*E*). Moreover, we found that continuous treatment with HGF in vivo significantly increased IL-10 production (Fig. 8*F*) and decreased IL-12p70 production (Fig. 8*G*) by DCs after LPS stimulation. These results indicated that repeated treatment with HGF in

chronic inflammation could induce Th2-type immune responses with up-regulation of IL-10 production by DCs.

Discussion

The results of the present study clearly demonstrated that HGF strongly suppresses collagen-induced immune responses, thus attenuating experimental arthritis. In the early phase, systemic delivery of HGF suppressed the activation of DCs in the spleen that was provoked by sensitization with CII, thus down-regulating CII-induced CD4$^+$ T cell activation. During continuous Ag stimulation, HGF up-regulated IL-10 production by immunocytes. Further, the delivery of HGF attenuated the severity and incidence of arthritis in the CIA model with down-regulation of IL-17 production. To our knowledge, this is the first report that clearly demonstrates the effect of HGF on immune-mediated arthritis.

The presentation of Ag by APCs to T cells initiates the differentiation of naive Th cells into the effector T cells. During the differentiation into each phenotype such as Th1, Th2, or regulatory T (Treg) cells, the expression of costimulatory molecules on APCs and the cytokine profile produced by APCs play a critical role (46). Among various APCs, DCs are most efficient and crucial (47).

Recent articles reported the effect of HGF on DC functions (24, 48). Rutella et al. (48) reported that, in in vitro experiments, HGF suppresses alloantigen-presenting capacity, modulates the costimulatory molecule expression and cytokine production of DCs, and generates DCs that induce Treg cells ("tolerogenic DCs"). In contrast, we reported that HGF potently suppresses Ag-presenting capacity and IL-12p70 production of DCs, thus inhibiting the development of both Th1- and Th2-type immune responses induced by OVA (24).

In the present study, we confirmed that treatment with HGF in vivo suppressed the production of both IL-10 and IL-12p70 by CII/CFA-induced DCs (Fig. 3, *A* and *B*). When the DCs and CD4$^+$ T cells were cocultured in the presence of CII, DCs from HGF-treated mice showed a reduced capacity to present Ag to CD4$^+$ T cells (Fig. 3*D*) and to induce IFN-γ and IL-10 production by CII/CFA-primed CD4$^+$ T cells compared with DCs obtained from CII/CFA-sensitized control mice (Fig. 3, *E* and *F*). Moreover, we also found that HGF decreased CD40 expression on DCs (Fig. 3*G*), which was consistent with our previous study (24). We also confirmed that HGF potently inhibited CII/CFA-induced T cell priming (Fig. 2). Based on these results, in a situation such as Ag-induced T cell priming in which DCs play an essential role, HGF would suppress immune responses through down-regulation of DC function.

Then, with continuous Ag stimulation, HGF up-regulated IL-10 production by immunocytes including T cells (Fig. 4, *A*, *C*, and *F*). IL-10 is an immunosuppressive and regulatory cytokine (49–51). This is consistent with a recent report that HGF reduced acute and chronic rejection of allografts with the increased expression of IL-10 in a mouse model of allogenic heart transplantation (22). The exact mechanism of induction of IL-10-producing T cells remains unclear. Generally, exogenous IL-10 itself plays an important role in the induction of IL-10-producing T cells (50, 51). In our study, HGF did not directly increase IL-10 production when added to cocultures of DCs and CD4$^+$ T cells obtained from CII/CFA-sensitized control mice on day 10 in the presence of CII (data not shown). HGF did not increase PMA and ionomycin-induced production of IL-10 by CD4$^+$ T cells obtained from CII/CFA-sensitized mice (data not shown). Moreover, to clarify whether IL-10 was produced by Foxp3$^+$ Treg cells, we also examined the percentage and the absolute number of CD4$^+$ (CD25$^+$) Foxp3$^+$ cells in the spleens or draining LNs of each group of mice on days 10, 20, and 40. We found that treatment with HGF in vivo did not

increase $CD4^+Foxp3^+$ Treg cells in spleens and LNs in the present study (data not shown). Treatment of splenocytes with HGF in vitro during CII restimulation did not increase $Foxp3^+$ Treg cells either. In contrast, repeated treatment with HGF in vivo gradually increased IL-10 production by DCs (Figs. 4D and 8F). These results indicated that the augmented IL-10 production by $CD4^+$ T cells was not mediated by $Foxp3^+$ Treg cells but, at least in vivo, by up-regulation of IL-10 production by DCs after repeated HGF treatment. The precise mechanism of induction of IL-10-producing $CD4^+$ T cells by HGF is not clear at present and should be further investigated.

IL-10 also enhances the formation of Th2 cells by down-regulating IL-12 production by DCs (52). Moreover, some reports also emphasize the importance of IL-10 in the induction of Th2 cells (53, 54). As described above, after T cells were primed with Ag, HGF in the presence of continuous Ag stimulation increased IL-10 production by immunocytes, including DCs, along with suppression of IL-12 production by DCs (Figs. 4, 7, and 8), indicating that under continuous Ag stimulation HGF could induce Th2-type immune responses in the chronic phase. In fact, in the chronic phase of CII-induced immune responses, repeated treatment with HGF up-regulated both IL-4 and IL-10 production in T cells (Figs. 7 and 8). These results were consistent with a recent report that HGF ameliorates the progression of experimental autoimmune myocarditis with the induction of Th2 cytokines (23). We also confirmed that HGF enhanced mRNA expression of GATA-3, which specifies Th2 cell development, in $CD4^+$ T cells in the chronic inflammatory phase (Fig. 8E). Th2-type immune responses suppress Th1-type immune responses (55), and a recent study reported that IL-4 significantly suppresses the development of Th17 cells, a new subset of effector $CD4^+$ T cells distinct from Th1 or Th2 cells (56). However, in the current study we found that neutralization of IL-4 in vitro did not increase IL-17 production by splenocytes after CII restimulation (data not shown). Collectively, HGF would enhance Th2-type immune responses in chronic inflammation, thus inhibiting both Th1- and Th17-type responses at least in vivo.

Recent studies clarified that IL-17 produced by Th17 cells has a crucial role in the induction of autoimmune tissue injury (30–32, 57, 58). Accumulating evidence indicates that IL-17 plays an essential role not only in the induction of autoimmune arthritis (30, 31) but also in the subsequent bone destruction (32). In the current study, HGF potently suppressed IL-17 production by draining LN cells after in vitro CII restimulation in the early stage of Ag-induced immune responses (Fig. 5). Further, in addition to the sensitization phase, even in the chronic inflammation phase with joint destruction HGF significantly suppressed IL-17 production by spleen cells (Fig. 7E). Moreover, HGF significantly suppressed DC production of IL-23 (Fig. 3C), which is now recognized as a very important cytokine for IL-17 secretion from activated $CD4^+$ T cells (57, 59). These results indicated that HGF would be beneficial in treating autoimmune arthritis.

TGF-β is an immunosuppressive growth factor. Some phenotypes of T cells function as Treg cells by producing TGF-β. In contrast, the role of TGF-β in the induction of the Th17 cell lineage to promote an autoimmune response has been recently highlighted (52, 60, 61). Generally, HGF counteracts the biological functions of TGF-β such as promoting fibrosis (13, 14). In the immune response, however, the relation between HGF and TGF-β differs among experimental systems. HGF suppresses acute and chronic rejection in a mouse model of cardiac allograft transplantation with unexpectedly enhanced expression of TGF-β mRNA (22). In contrast, in allergic airway inflammation HGF did not up-regulate TGF-β production in the lung (24). In the present study on arthritis, HGF reduced mRNA

expression of TGF-β in $CD4^+$ T cells at both early and chronic phases (data not shown).

Generally, exogenously administered HGF proteins vanish from organs within several hours (44). In a preliminary study, we confirmed that s.c. injection of HGF protein (10 μg per mouse) once daily failed to suppress the Ag-induced T cell priming and development of CII-induced arthritis (data not shown). Previously, we used a hydrodynamic-based transfer system to deliver HGF effectively and confirmed that a slight but continuous up-regulation of HGF protein in the sera potently suppressed OVA/alum-induced T cell priming and allergic airway inflammation (24). However, this delivery system could not be applied to an experimental model of arthritis due to an anatomical narrowing of the tail vein provoked by injection of CII/CFA into the subcutis of the tail. Thus, to achieve a controlled release of HGF, we adopted biodegradable gelatin hydrogels as carriers of HGF. We previously confirmed that when this gelatin/HGF complex was s.c. injected into mice, HGF was delivered under a controlled release based on hydrogel degradation and that the degradation occurred over 10 days (37). We reconfirmed that controlled release of HGF was achieved using a gelatin/rhHGF complex by examining the time course of concentration of HGF in the sera (Table I). In this study, the controlled release of HGF potently suppressed Ag-induced T cell priming and development of CII-induced arthritis. Thus, gelatin hydrogels would be an ideal carrier for HGF to exhibit its biological effects, and further application in various models can be expected.

Pulmonary fibrosis is often associated with RA and is one of the major causes of death in RA patients (62). To date, several articles, including our own, reported that HGF inhibits the progression of experimental pulmonary fibrosis (15, 63, 64). Considering the simultaneous effect on pulmonary fibrosis and arthritis, HGF could be an attractive tool in treating RA with pulmonary involvement in a clinical situation. In contrast, in the clinical use of HGF the possibility of promoting tumor progression should be considered. Therefore, for practical usage of HGF in clinical situations further studies should be performed.

In summary, our results in the present study indicated that HGF could exhibit its immunosuppressive effects in different manners at different stages of immune response. In the early phase of Ag-induced immune responses HGF potently suppressed DC function, thus inhibiting T cell priming by Ag. In contrast, during chronic inflammation HGF gradually increased IL-10 production by DCs, which subsequently induced IL-10 producing T cells and Th2-type immune responses. The precise mechanism should be further investigated in detail.

Acknowledgment

Disclosures

References

1. Gohda, E., H. Tsubouchi, H. Nakayama, S. Hirono, O. Sakiyama, K. Takahashi, H. Miyazaki, S. Hashimoto, and Y. Daikuhara. 1988. Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. *J. Clin. Invest.* 81: 414–419.
2. Miyazawa, K., H. Tsubouchi, D. Naka, K. Takahashi, M. Okigaki, N. Arakaki, H. Nakayama, S. Hirono, O. Sakiyama, K. Takahashi, et al. 1989. Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. *Biochem. Biophys. Res. Commun.* 163: 967–973.
3. Nakamura, T., T. Nishizawa, M. Hagiya, T. Seki, M. Shimonishi, A. Sugimura, K. Tashiro, and S. Shimizu. 1989. Molecular cloning and expression of human hepatocyte growth factor. *Nature* 342: 440–443.
4. Stoker, M., E. Gherardi, M. Perryman, and J. Gray. 1987. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* 327: 239–242.

5. Zarnegar, R., and G. K. Michalopoulos. 1995. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. *J. Cell Biol.* 129: 1177–1180.

6. Bardelli, A., P. Longati, D. Albero, S. Goruppi, C. Schneider, C. Ponzetto, and P. M. Comoglio. 1996. HGF receptor associates with the anti-apoptotic protein BAG-1 and prevents cell death. *EMBO J.* 15: 6205–6212.

7. Huh, C. G., V. M. Factor, A. Sanchez, K. Uchida, E. A. Conner, and S. S. Thorgeirsson. 2004. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc. Natl. Acad. Sci. USA* 101: 4477–4482.

8. Trusolino, L., and P. M. Comoglio. 2002. Scatter-factor and semaphoring receptors: cell signaling for invasive growth. *Nat. Rev. Cancer* 2: 289–300.

9. Imaizumi, Y., H. Murota, S. Kanda, Y. Hishikawa, T. Koji, T. Taguchi, Y. Tanaka, Y. Yamada, S. Ikeda, T. Kohno, et al. 2003. Expression of the c-met proto-oncogene and its possible involvement in liver invasion in adult T-cell leukemia. *Clin. Cancer Res.* 9: 181–187.

10. Siegfried, J. M., L. A. Weissfeld, P. Singh-Kaw, R. J. Weyant, J. R. Testa, and R. J. Landreneau. 1997. Association of immunoreactive hepatocyte growth factor with poor survival in resectable non-small cell lung cancer. *Cancer Res.* 57: 433–439.

11. To, Y., M. Dohi, K. Matsumoto, R. Tanaka, A. Sato, K. Nakagome, T. Nakamura, and K. Yamamoto. 2002. A two-way interaction between hepatocyte growth factor and interleukin-6 in tissue invasion of lung cancer cell line. *Am. J. Respir. Cell Mol. Biol.* 27: 220–226.

12. Wislez, M., N. Rabbe, J. Marchal, B. Milleron, B. Crestani, C. Mayaud, M. Antoine, P. Soler, and J. Cadranel. 2003. Hepatocyte growth factor production by neutrophils infiltrating bronchoalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. *Cancer Res.* 63: 1405–1412.

13. Mizuno, S., T. Kurosawa, K. Matsumoto, Y. Mizuno-Horikawa, M. Okamoto, and T. Nakamura. 1998. Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mouse model of chronic renal disease. *J. Clin. Invest.* 101: 1827–1834.

14. Ueki, T., Y. Kaneda, H. Tsutsui, K. Nakanishi, Y. Sawa, R. Morishita, K. Matsumoto, T. Nakamura, H. Takahashi, E. Okamoto, and J. Fujimoto. 1999. Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat. Med.* 5: 226–230.

15. Dohi, M., T. Hasegawa, K. Yamamoto, and B. C. Marshall. 2000. Hepatocyte growth factor attenuates collagen accumulation in a murine model of pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 162: 2302–2307.

16. van der Voort, R., T. E. I. Taher, R. M. J. Keehnen, L. Smit, M. Groenink, and S. T. Pals. 1997. Paracrine regulation of germinal center B cell adhesion through the c-met-hepatocyte growth factor/scatter factor pathway. *J. Exp. Med.* 185: 2121–2131.

17. Weimar, I. S., D. de Jong, E. J. Muller, T. Nakamura, J. M. van Gorp, G. C. de Gast, and W. R. Gerritsen. 1997. Hepatocyte growth factor/scatter factor promotes adhesion of lymphoma cells to extracellular matrix molecules via $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins. *Blood* 89: 990–1000.

18. Adams, D. H., L. Harvath, D. P. Bottaro, R. Interrante, G. Catalano, Y. Tanaka, A. Strain, S. G. Hubscher, and S. Shaw. 1994. Hepatocyte growth factor and macrophage inflammatory protein 1b: structurally distinct cytokines that induce rapid cytoskeletal changes and subset-preferential migration in T cells. *Proc. Natl. Acad. Sci. USA* 91: 7144–7148.

19. Kurz, S. M., S. S. Diebold, T. Hieronymus, T. C. Gust, P. Bartunek, M. Sachs, W. Birchmeier, and M. Zenke. 2002. The impact of c-met/scatter factor receptor on dendritic cell migration. *Eur. J. Immunol.* 32: 1832–1838.

20. Scarpino, S., A. Stoppacciaro, F. Ballerini, M. Marchesi, M. Prat, M. C. Stella, S. Sozzani, P. Allavena, A. Mantovani, and L. P. Ruco. 2000. Papillary carcinoma of the thyroid: hepatocyte growth factor (HGF) stimulates tumor cells to release chemokines active in recruiting dendritic cells. *Am. J. Pathol.* 156: 831–837.

21. Kretzschmar, M., J. Doody, and J. Massague. 1997. Opposing BMP and EGF signaling pathways converge on the TGF-β family mediator Smad1. *Nature* 389: 618–622.

22. Yamamura, K., K. Ito, K. Tsukioka, Y. Wada, A. Makiuchi, M. Sakaguchi, T. Akashima, M. Fujimori, Y. Sawa, R. Morishita, et al. 2004. Suppression of acute and chronic rejection by hepatocyte growth factor in a murine model of cardiac transplantation. *Circulation* 110: 1650–1657.

23. Futamatsu, H., J. Suzuki, S. Mizuno, N. Koga, S. Adachi, H. Kosuge, Y. Maejima, K. Hirao, T. Nakamura, and M. Isobe. 2005. Hepatocyte growth factor ameliorates the progression of experimental autoimmune myocarditis: a potential role for induction of T helper 2 cytokines. *Circ. Res.* 96: 823–830.

24. Okunishi, K., M. Dohi, K. Nakagome, R. Tanaka, S. Mizuno, K. Matsumoto, J. Miyazaki, T. Nakamura, and K. Yamamoto. 2005. A novel role of hepatocyte growth factor as an immune regulator through suppressing dendritic cell function. *J. Immunol.* 175: 4745–4753.

25. Ito, W., A. Kanehiro, K. Matsumoto, A. Hirano, K. Ono, H. Maruyama, M. Kataoka, T. Nakamura, E. W. Gelfand, and M. Tanimoto. 2005. Hepatocyte growth factor attenuates airway hyperresponsiveness, inflammation, and remodeling. *Am. J. Respir. Cell Mol. Biol.* 32: 268–280.

26. Kuroiwa, T., T. Iwasaki, T. Imado, M. Sekiguchi, J. Fujimoto, and H. Sano. 2006. Hepatocyte growth factor prevents lupus nephritis in a murine model of chronic graft-versus-host disease. *Arthritis. Res. Ther.* 8: R123.

27. Hale, L. P., and B. F. Haynes. 2001. Pathology of rheumatoid arthritis and associated disorders. In *Arthritis and Allied Conditions: A Textbook of Rheumatology*, 14th Ed., Vol. 1. W. J. Koopman, ed. Williams & Wilkins Press, Baltimore, pp. 1103–1127.

28. Dolhain, R. J., A. N. van der Heiden, N. T. ter Haar, F. C. Breedveld, and A. M. Miltenburg. 1996. Shift toward T lymphocytes with a T helper 1 cytokine-secretion profile in the joints of patients with rheumatoid arthritis. *Arthritis Rheum.* 39: 1961–1969.

29. Smolen, J. S., M. Tohidast-Arkad, A. Gal, M. Kunaver, G. Eberl, P. Zenz, A. Falus, and G. Steiner. 1996. The role of T-lymphocytes and cytokines in rheumatoid arthritis. *Scand. J. Rheumatol.* 25: 1–4.

30. Miossec, P. 2003. Interleukin-17 in rheumatoid arthritis. *Arthritis Rheum.* 46: 594–601.

31. Nakae, S., S. Saijo, R. Horai, K. Sudo, S. Mori, and Y. Iwakura. 2003. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc. Natl. Acad. Sci. USA* 100: 5986–5990.

32. Sato, K., A. Suematsu, K. Okamoto, A. Yamaguchi, Y. Morishita, Y. Kadono, S. Tanaka, T. Kodama, S. Akira, Y. Iwakura, et al. 2006. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J. Exp. Med.* 203: 2673–2682.

33. Maruotti, N., F. P. Cantatore, E. Crivellato, A. Vacca, and D. Ribatti. 2006. Angiogenesis in rheumatoid arthritis. *Histol. Histopathol.* 21: 557–566.

34. Koch, A. E., M. M. Halloran, S. Hosaka, M. R. Shah, C. J. Haskell, S. K. Baker, R. J. Panos, G. K. Haines, G. L. Bennett, R. M. Pope, and N. Ferrara. 1996. Hepatocyte growth factor. A cytokine mediating endothelial migration in inflammatory arthritis. *Arthritis Rheum.* 39: 1566–1575.

35. Yukioka, K., M. Inaba, Y. Furumitsu, M. Yukioka, T. Nishino, H. Goto, Y. Nishizawa, and H. Morii. 1994. Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. *J. Rheumatol.* 21: 2184–2189.

36. Feuerherm, A. J., M. Borset, C. Seidel, A. Sundan, L. Leistad, M. Ostensen, and A. Faxvaag. 2001. Elevated levels of osteoprotegerin (OPG) and hepatocyte growth factor (HGF) in rheumatoid arthritis. *Scand. J. Rheumatol.* 30: 229–234.

37. Ozeki, M., T. Ishii, Y. Hirano, and Y. Tabata. 2001. Controlled release of hepatocyte growth factor from gelatin hydrogels based on hydrogel degradation. *J. Drug. Target.* 9: 461–471.

38. Oe, S., Y. Fukunaka, T. Hirose, Y. Yamaoka, and Y. Tabata. 2003. A trial on regeneration therapy of rat liver cirrhosis by controlled release of hepatocyte growth factor. *J. Control. Release* 88: 193–200.

39. Okunishi, K., M. Dohi, K. Nakagome, R. Tanaka, and K. Yamamoto. 2004. A novel role of cysteinyl leukotrienes to promote dendritic cell activation in the antigen-induced immune responses in the lung. *J. Immunol.* 173: 6393–6402.

40. Kuroda, E., and U. Yamashita. 2003. Mechanisms of enhanced macrophage-mediated prostaglandin E_2 production and its suppressive role in Th1 activation in Th2-dominant BALB/c mice. *J. Immunol.* 170: 757–764.

41. Dabbagh, K., M. E. Dahl, P. Stepick-Biek, and D. B. Lewis. 2002. Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J. Immunol.* 168: 4524–4530.

42. Sagawa, K., K. Nagatani, Y. Komagata, and K. Yamamoto. 2005. Angiotensin receptor blockers suppress antigen-specific T cell responses and ameliorate collagen-induced arthritis in mice. *Arthritis Rheum.* 52: 1920–1928.

43. Nakagome, K., M. Dohi, K. Okunishi, Y. Komagata, K. Nagatani, R. Tanaka, J. Miyazaki, and K. Yamamoto. 2005. In vivo IL-10 gene delivery suppresses airway eosinophilia and hyperreactivity by down-regulating APC functions and migration without impairing the antigen-specific systemic immune response in a mouse model of allergic airway inflammation. *J. Immunol.* 174: 6955–6966.

44. Michalopoulos, G. K., and R. Appasamy. 1993. Metabolis of HGF-SF and its role in liver regeneration. *EXS* 65: 275–283.

45. Zheng, W., and R. A. Flavell. 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89: 587–596.

46. Kapsenberg, M. L. 2003. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol.* 3: 984–993.

47. Bancherau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392: 245–252.

48. Rutella, S., G. Bonanno, A. Procoli, A. Mariotti, D. G. de Ritis, A. Curti, S. Danese, G. Pessina, S. Pandolfi, F. Natoni, et al. 2006. Hepatocyte growth factor favors monocyte differentiation into regulatory interleukin (IL)-$10^{+/+}$IL-$12^{low/neg}$ accessory cells with dendritic-cell features. *Blood* 108: 218–227.

49. Fehérvari, Z., and S. Sakaguchi. 2004. CD4$^+$ Tregs and immune control. *J. Clin. Invest.* 114: 1209–1217.

50. Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J. E. de Vries, and M. G. Roncarolo. 1997. A CD4$^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389: 737–742.

51. Hawrylowicz, C. M., and A. O'Garra. 2005. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat. Rev. Immunol.* 5: 271–283.

52. De Smedt, T., M. Van Mechelen, G. De Becker, J. Urbain, O. Leo, and M. Moser. 1997. Effect of interleukin-10 on dendritic cell maturation and function. *Eur. J. Immunol.* 27: 1229–1235.

53. Liu, L., B. E. Rich, J. Inobe, W. Chen, and H. L. Weiner. 1998. Induction of Th2 cell differentiation in the primary immune response: dendritic cells isolated from adherent cell culture treated with IL-10 prime naive CD4$^+$ T cells to secret IL-4. *Int. Immunol.* 10: 1017–1026.

54. Ballic, A., Y. M. Harcus, M. D. Taylor, F. Brombacher, and R. M. Maizels. 2006. IL-4R signaling is required to induce IL-10 for the establishment of Th2 dominance. *Int. Immunol.* 18: 1421–1431.

55. Boehm, U., T. Klamp, M. Groot, and J. C. Howard. 1997. Cellular responses to interferon-γ. *Annu. Rev. Immunol.* 15: 749–795.

56. Mangan, P. R., L. E. Harrington, D. B. O'Quinn, W. S. Helms, D. C. Bullard, C. O. Elsom, R. D. Hatton, S. M. Wahl, T. R. Schoeb, and C. T. Weaver. 2006. Transforming growth factor-β induces development of the Th17 lineage. *Nature* 441: 231–234.

57. Langrish, C. L., Y. Chen, W. M. Blumenschein, J. Mattson, B. Basham, S. D. Sedgwick, T. McClanahan, R. A. Kastelein, and D. J. Cua. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* 201: 233–240.

58. Park, H., Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4$^+$ T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6: 1133–1141.

59. Aggarwal, S., N. Ghilardi, M. H. Xie, F. J. de Sauvage, and A. L. Gurney. 2003. Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* 278: 1910–1914.

60. Veldhoen, M., R. J. Hocking, C. J. Atkins, R. M. Locksley, and B. Stockinger. 2006. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24: 179–189.

61. Bettelli, E., Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, and V. K. Kuchroo. 2006. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cell. *Nature* 441: 235–238.

62. Jindal, S. K., and R. Agarwal. 2005. Autoimmunity and interstitial lung disease. *Curr. Opin. Pulm. Med.* 11: 438–446.

63. Yaekashiwa, M., S. Nakayama, K. Ohnuma, T. Sakai, T. Abe, K. Satoh, K. Matsumoto, T. Nakamura, T. Takahashi, and T. Nukiwa. 1997. Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. *Am. J. Respir. Crit. Care Med.* 156: 1937–1944.

64. Watanabe, M., M. Ebina, F. M. Orson, A. Nakamura, K. Kubo, K. Kubota, D. Koinuma, K. Akiyama, M. Maemondo, S. Okouchi, et al. 2005. Hepatocyte growth factor gene transfer to alveolar septa for effective suppression of lung fibrosis. *Mol. Ther.* 12: 58–67.