Depletion of Collagen II-Reactive T Cells and Blocking of B Cell Activation Prevents Collagen II-Induced Arthritis in DBA/1j Mice

Huang-Ge Zhang, PingAr Yang, Jinfu Xie, Zhongyu Liu, Di Liu, Liang Xiu, Tong Zhou, Yongming Wang, Hui-Chen Hsu and John D. Mountz

*J Immunol* 2002; 168:4164-4172; doi: 10.4049/jimmunol.168.8.4164

http://www.jimmunol.org/content/168/8/4164

**References**

This article cites 45 articles, 19 of which you can access for free at:
http://www.jimmunol.org/content/168/8/4164.full#ref-list-1

**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
Depletion of Collagen II-Reactive T Cells and Blocking of B Cell Activation Prevents Collagen II-Induced Arthritis in DBA/1j Mice

Huang-Ge Zhang,1* PingAr Yang,* Jinfu Xie,* Zhongyu Liu,* Di Liu,* Liang Xiu,* Tong Zhou,* Yongming Wang,* Hui-Chen Hsu,* and John D. Mountz*†

Collagen II (CII)-induced arthritis in DBA/1j mice is mediated by both CII-reactive T cells and anti-CII Ab-producing B cells. To determine the relative role of these processes in the development of arthritis, we specifically eliminated CII-reactive T cells by treating the mice with CII-pulsed syngeneic macrophages that had been transfected with a binary adenovirus system. These macrophages express murine Fas ligand in a doxycycline-inducible manner with autocrine suicide inhibited by concomitant expression of p35. The mice were treated i.v. with four doses of CII-APC-AdFasLp35Tet or a single dose of AdCMVsTACI (5 × 10⁹ PFU), or both simultaneously, beginning 2 wk after priming with CII in CFA. Treatment with CII-APC-AdFasLp35Tet alone or in combination with a single dose of AdCMVsTACI prevented the development of CII-induced arthritis and T cell infiltration in the joint. The elimination of T cells was specific in that a normal T cell response was observed on stimulation with OVA after treatment with CII-APC-AdFasLp35Tet. Treatment with AdCMVsTACI alone prevented production of detectable levels of circulating anti-CII autoantibodies and reduced the severity of arthritis but did not prevent its development. These results indicate that the CII-reactive T cells play a crucial role in the development of CII-induced arthritis and that the anti-CII Abs act to enhance the development of CII-induced arthritis. The Journal of Immunology, 2002, 168: 4164–4172.

Rheumatoid arthritis is a chronic, progressive joint disease that is characterized by lymphocytic invasion of the synovial lining and hyperplasia of the resident synoviocytes (1). The overproduction of cytokines along with other factors results in cartilage destruction, bone erosion, and remodeling of joint structures (2–5). Typically, patients suffering from rheumatoid arthritis exhibit systemic manifestations, most notably the production of autoantibodies (6, 7). An understanding of the relative contributions of the T and B cell compartments of the immune system in the initiation and perpetuation of the disease process in rheumatoid arthritis is necessary for the design of effective therapeutic strategies.

Collagen II (CII)-induced arthritis in DBA/1j mice is an autoimmune model of human rheumatoid arthritis (8). During the initial stages of this disease, severe joint inflammation is associated with infiltration of leukocytes and production of proinflammatory cytokines. A chronic disease state, characterized by synovial cell hyperplasia and destruction of cartilage and bone, is then established. CII arthritis is associated with production of murine CII autoantibodies that have been proposed to contribute to disease progression (9–12). CII-induced arthritis can be transferred using CD4-positive T cells, indicating that T cells can initiate the disease and play an important role in the early stages of development of this disease (13, 14). CII arthritis is associated with generation of T cells specific for CII epitopes presented by APCs since it has been possible to transfer disease using a CII-specific T cell clone in SCID mice after boosting with CII (15–19).

A central objective in the development of the new generation of therapies for the treatment of rheumatoid arthritis is specific inhibition of the inflammatory disease processes in the absence of generalized immunosuppression (1–4). As APCs play a central role in defining Ag specificity, they provide an access point for specific manipulation of the immune system. It is well established that APCs, such as macrophages, express processed Ags that specifically stimulate those T cells that recognize processed Ag in the context of the MHC. We have developed techniques for modifying APCs such that they express specific Ags along with Fas ligand (FasL) and have demonstrated that these modified APCs delete only those T cells that recognize the specific Ag and do not induce general immunosuppression or decreased B cell function (20–25). The interaction of APCs, including macrophages, with B cells is mediated by the expression of the APCs of B lymphocyte stimulator (BlyS), A proliferation-inducing ligand, and B cell-activating factor of the TNF family that interact with the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), B cell maturation Ag, and BAFF receptor on the B cells (26–29). The interaction of the APCs and the B cells can be blocked by either soluble TACI (sTACI) or soluble B cell maturation Ag (29). CII arthritis was prevented by administration of high-dose sTACI (100 μg i.v.) administered 3 days a week for 6 wk, which at this level blocks both T cell and B cell activation (30).

In this study, we examined the relative contribution of Ag-specific T cells and autoantibodies by eliminating the Ag-specific T cells and blocking autoantibody production. The results show that treatment with CII-APC-AdFasLp35Tet eliminates CII-reactive T
cells and inhibits development of CII-induced arthritis but does not significantly diminish the production of anti-CII Abs. Treatment with AdCMVstTACI alone abrogates the production of anti-CII Abs, but at the levels used here, it did not decrease CII Ag-specific T cell responses and did not greatly diminish the development of CII-induced arthritis. Combined treatment with CII-APC-AdFasLp35Tet plus AdCMVstTACI prevents both the development of CII-reactive T cells and the production of anti-CII Abs, and effectively blocks the development of CII-induced arthritis.

Materials and Methods

Mice

Female homozygous DBA/1j mice (7 wk old) were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were kept in a room equipped with an air filtering system. The cages, bedding, water, and food were sterilized, and the mice were handled with sterile gloves.

Induction of arthritis

DBA/1j female mice were immunized at 7 wk of age at the base of the tail with 200 μg of bovine CII dissolved in 100 μl of 0.05 M acetic acid and mixed with an equal volume (100 μl) of CFA (Chondrex, Redmond, WA). Four weeks later, the animals were reimmunized with 200 μg of bovine CII in IFA.

Treatment protocols using CII-APC-AdFasLp35Tet and AdCMVstTACI

To differentiate the roles of T cells and B cells in CII-induced arthritis, the effects of five treatment protocols were compared as follows: 1) CII-APC-AdFasLp35Tet alone with or without a doxycycline (Dox) inducer; 2) Ad CMVstTACI alone; 3) CII-APC-AdFasLp35Tet in combination with AdCMV stTACI; 4) AdGFP; and 5) CII-APC-AdGFP. To delete the CII-reactive T cells, peritoneal macrophages from DBA/1j mice were pulsed with T cell proliferation grade Arthusen-CIA type II collagen (Chondrex) as described by the manufacturer, then transfected with AdTREfasLp35 plus AdCMVrtTA (CII-APC-AdFasLp35Tet) as described above. Treatment of the DBA/1j mice with these APCs was commenced 2 wk after the mice had been immunized with CII in CFA, with 106 CII-APC-AdFasLp35Tet administered twice per week for 2 wk. As a control, other groups of mice were administered CII-pulsed APCs that had been transfected with AdGFP (CII-APC-AdGFP) i.p. Induction of FasL on these APCs was accomplished by addition of Dox to the drinking water (from 1 to 8 mg/ml in 2-fold increments) with 4% sucrose for 6 wk starting at the time of administration of CII-APC-AdFasLp35Tet therapy. To test whether the treatment of CII-APC-AdFasLp35Tet resulted in CII-specific T cell deletion without impairing host immune response to an irrelevant Ag, all CII-primed mice were coadministered with OVA simultaneously. In brief, all five groups of mice were s.c. immunized with 10 μg of OVA in CFA at the age of 7 wk old and boosted at the same dose in IFA 4 wk later.

To block the CII-reactive B cell activation, a single dose (5 × 109 PFU) of recombinant adenovirus expressing soluble stTACI (AdCMVstTACI) was injected i.v. 2 wk after immunization of the mice with CII in CFA either alone or simultaneously with the initial CII-APC-AdFasLp35Tet treatment. Control mice were injected i.v. with CII-APC-AdGFP (5 × 109 PFU) or both.

Construction of inducible FasL adenovirus expression vector

An adenovirus coexpressing inducible FasL and p35 was constructed as described previously (23–25). Briefly, the genes encoding FasL (generously supplied by Dr. Jiang Ling, University of Chicago, IL) was directionally cloned into the BamHI and XhoI site of the pCA14 vector (Microbix, Ontario, Canada). The p35 expression cassette, including a CMV promoter and the SV40 poly(A) tail, was then excised with BgIII and subsequently inserted into the BgIII site of the pShuttle vector as described by He et al. (31), resulting in the production of a pShuttleCMVP35. The full-length FasL was first cloned into the BamHI polynucleotide site of a pTRE vector (Clontech Laboratories, Palo Alto, CA) site. Then, the TRE-regulated fas ligand fragment, including the bovine growth hormone poly(A) tail, was excised with XhoI and HindIII, followed by insertion into the Klenow-filled NotI site of the pShuttleCMVP35, leading to the production of pShuttlep35TREfasL. The recombinant adenovirus AdTREfasLp35 was produced by in vitro recombination of pShuttlep35TREfasL with pAdEase1 as described previously (31). AdTREfasLp35 was produced in 293 cells as described elsewhere (32). Recombinant AdCMVrtTA was constructed as previously described (33) using the rtTA construct generously provided by Dr. J. B. Uney (University of Bristol, Bristol, U.K.) (34) to allow expression of the reverse tetracycline transactivator (rtTA), thereby enabling Dox-inducible expression of FasL.

Regulation of FasL expression by Dox

Expression of functional FasL on the APC-AdFasLp35Tet or APC-AdGFP cells was evaluated by coinoculation with FasL-sensitive A20 cells. Peritoneal macrophages derived from DBA/1j mice were used as the APC. The macrophages were transfected with AdTREfasLp35 plus AdCMVrtTA (35, 36) at 50 PFU/cell of each virus for 1 h, followed by washing, and incubation for an additional 18 h. The transfected macrophages were then incubated with different concentrations of Dox (Sigma-Aldrich, St. Louis, MO) for 18 h. The in vitro activity of the macrophages was estimated using a cytotoxicity assay in which they were mixed with 3H-labeled A20 target cells at different E:T ratios. The cell cytotoxicity was determined by measuring the radioactivity in the cell culture supernatants at 8 h after coculture [(cytotoxicity = (cpmspontaneous − cpmmaximal) / cpmmaximal × 100)].

Prevention of autocrine apoptosis of Fas-positive engineered APC derived from macrophages obtained from DBA/1j mice

The ability of p35 to inhibit apoptosis of the Fas-positive peritoneal macrophages from DBA/1j mice was determined after transfection with either AdCMVpt35 with pAdEaseL (21, 25) or AdTREfasLp35 with AdCMVrtTA (AdTREfasLp35Tet) as described above and incubated with Dox at different concentrations for 24 h. The percentage of apoptotic cells after Dox induction of the FasL on the DBA/1j macrophages was evaluated using the ATP-lite assay (25). ATP is determined as light units measured as counts per second (cps) in a luminescence counter (Packard Instrument, Meriden, CT). The percentage of apoptotic cells was expressed as (1 − cps sample/cps control) × 100.

Construction of adenosviruses that produce stTACI and BlyS

An adenovirus expressing stTACI and murine BlyS was constructed according to a standard protocol (31). Briefly, the extracellular portion of TACI (aa 1–165) was PCR amplified using cDNA synthesized from total RNAs extracted from the Raji cell line purchased from American Type Culture Collection (Manassas, VA). After sequence confirmation, the stTACI was ligated directionally into the BglII and SalI sites of the pAdTrackCMV vector (31) and the human IgG Fc was fused into the downstream SalI site of stTACI in the pAdTrackCMV as described by Cheng et al. (37). Full-length murine BlyS was PCR amplified using cDNA synthesized from total RNAs extracted from the DBA/1j macrophages. The sequence of BlyS was confirmed, and BlyS was directionally ligated into the BglII and EcoRV sites of the pAdTrackCMV vector (31). The production of recombinant AdCMVBlyS was accomplished as described elsewhere (33). The production of recombinant AdCMV-stTACI was accomplished as described in the protocol above. stTACI protein was produced in 293 cells, purified using a protein G column, and stored at −20°C in 100-μl aliquots until used.

Evaluation of development of arthritis

A loop calibrator was used to determine the diameter of each paw of each mouse every day. Paw swelling was determined as the increase in diameter compared with the diameter at the initiation of the experiment. The severity of arthritis was graded according to the following scale, 0, normal with no swelling and erythema and no increase in joint diameter; 1, slight swelling and erythema with 0.1–0.3 mm increase in joint diameter; 2, swelling and erythema and 0.3–0.6 mm increase in joint diameter; 3, extensive swelling and erythema with 0.6–0.9 mm in joint diameter; and 4, pronounced swelling and erythema with joint thickness of 0.9–1.2 mm increase or obvious joint destruction associated with visible joint deformity or ankylosis. Each limb was graded, resulting in a maximum clinical score of 16 per animal and expressed as the mean score on a given day.

After sacrifice, the joints (knee, elbow, ankle, and wrist) were harvested, fixed in 10% formaldehyde/PBS for at least 24 h, decalcified using EDTA for 3 wk, sectioned at 4-μm thickness, deparaffinized, and stained with H&E (CMS, Houston, TX).

Evaluation of T cell or B cell infiltration in synovial joints

The phenotype of the infiltrating cells in the joints was determined by immunoperoxidase staining of the tissue sections with an anti-CD3 Ab to identify T cells or an anti-B220 Ab to identify B cells. Quenching of endogenous peroxidase was accomplished by incubating tissue sections with 3% H2O2 for 10 min at room temperature in a humidified chamber. After

Downloaded from http://www.jimmunol.org/ by guest on April 12, 2017
washing with PBS, tissue sections were incubated with 0.1% trypsin at 37°C for 10 min to reveal fixed Ag epitopes. Tissue sections were treated with the denaturing solution for 30 min at room temperature and blocking solution for 10 min at room temperature. They were then incubated with HRP-conjugated anti-CD3 or B220 (DAKO, Carpinteria, CA). A DAB staining kit (DAKO) was used for visualization of Ab binding and the slides were counterstained with methyl green. At least five areas were chosen randomly for assessment of the percentage of CD3-positive cells or B220-positive cells in each specimen.

Analysis of TACI-BlyS interactions in the presence of AdCMVtTACI

DBA/1j macrophages were transfected with AdCMVblyS and gamma irradiated before use as stimulator cells. These stimulator cells were cultured at different ratios with A20 B cells, which express TACI in the presence of different concentrations of sTACI. Proliferation was evaluated using the [3H]thymidine uptake method.

Analysis of Ag-specific T cell response after CII-APC-AdFasL treatment.

The CII-specific proliferative response of draining lymph node T cells was evaluated by measuring the [3H]thymidine uptake of T cells cocultured with gamma irradiated syngeneic APCs pulsed with or without bovine CII and quantification of IL-2 production as described previously (24). In brief, 1 μCi of [3H]thymidine was added daily after coculture, the cells were harvested 16 h later, and the incorporation of [3H]thymidine was determined using a scintillation counter.

To determine whether the CII-APC-AdFasL treatment is Ag specific, the gamma-irradiated syngeneic APCs were also pulsed with OVA and the incorporation of [3H]thymidine was determined using a scintillation counter.

ELISA quantification of sTACI induction and autoantibody production

The concentration of sTACI in the circulating blood was quantified using an ELISA at different time points up to 50 days after administration of AdCMVtTACI. In brief, the concentrations of the sTACI-Fc protein were determined using a rabbit anti-human Fc polyclonal Ab-coated plate to capture the human Fc component of the recombinant protein, and the captured protein was quantified using a rabbit anti-human Fc polyclonal Ab conjugated to HRP. Sample dilutions were compared with standard curves of human Fc (Sigma-Aldrich) to determine the concentrations of sTACI-Fc. The serum levels of anti-mouse CII IgG were assayed using a CII ELISA kit (Chondrex) before treatment and on day 50 after induction of CII arthritis. The standard curve was produced using an anti-CII Ab provided with the ELISA kit.

Statistical analysis

The results are expressed as the mean ± SEM. The two-tailed Student’s t test was used for statistical analysis. A p < 0.05 was considered to be statistically significant.

Results

AdTREFasLp35 plus AdCMVrTAT confers Dox-inducible expression of FasL in the absence of autocrine apoptosis

To develop a generally applicable strategy in which autocrine apoptosis of APC is inhibited and FasL is inducibly expressed, an improved binary adenovirus system was constructed (Fig. 1A). One of the adenoviruses contains the fas ligand gene under the regulation of the tetracycline response element (TRE) (35, 36, 38) and the apoptosis inhibitory p35 gene (39, 40) under the regulation of the CMV promoter (Fig. 1A). The other adenovirus contains the reverse tetracycline transactivator (rtTA) under the regulation of the CMV promoter leading to high expression of rtTA (34, 41). This binary adenovirus system will be referred to as AdFasLp35Tet. Macrophages transfected with AdFasLp35Tet will be referred to as APC-AdFasLp35Tet. In combination, these adenoviruses enable Dox-inducible expression of FasL with concomitant expression of the p35 anti-apoptosis gene product to prevent autocrine apoptosis of the transfected macrophages.

To confirm the inducibility of biologically active FasL in this system, peritoneal macrophages (1 × 10^6) from DBA/1j mice were transfected with 50 PFU/cell AdFasLp35Tet and then incubated with various concentrations of Dox for 18 h. The expression of functional FasL on the surface of the transfected cells was then evaluated by coculture at different E:T ratios with 51Cr-labeled A20 target cells. Only low levels of cytotoxicity of the A20 target cells were observed when macrophages were treated with Dox alone, but high levels of cytotoxicity were observed when macrophages were treated with Dox in the presence of AdFasLp35Tet. These results are consistent with the finding that high levels of cytotoxicity are observed in vivo after administration of Dox plus AdCMVtTACI.
cells were observed on incubation with the transfected macrophages in the absence of Dox (Fig. 1B). The addition of Dox resulted in a Dox dose-dependent increase in the death of the A20 target cells at 18 h, which was evident at doses of Dox that ranged from 1 to 10 μg/ml (Fig. 1B).

To test whether the AdFasLp35Tet construct effectively prevents autocrine apoptosis, the viability of the DBA/1j macrophages transfected with AdFasLp35Tet was evaluated after incubation with 0.0 to 8.0 μg/ml Dox for 18 h, as determined in an ATP-lite assay. As a control, DBA/1j macrophages were transfected with the AdLoxP/FasL plus AxCANCRe, which results in expression of FasL independently of Dox induction and in the absence of the anti-apoptosis gene p35 (39, 40). As anticipated, the control macrophages transfected with AdLoxP/FasL plus AxCANCRe underwent 80% of the apoptosis (Fig. 1C, column 1). In contrast, the macrophages transfected with AdFasLp35Tet exhibited only low levels of apoptosis even in the presence of high levels of Dox (Fig. 1C).

**Decreased CII arthritis after treatment with CII-APC-AdFasLp35Tet in vivo**

To demonstrate whether the treatment of CII-APC-AdFasLp35Tet can prevent CII-induced arthritis, the macrophages were pulsed with bovine CII and then transfected with AdFasLp35Tet. These macrophages were then used to treat DBA/1j mice commencing 2 wk after the mice had been immunized with CII in CFA at 9 wk of age, as shown in Fig. 2A. Mice received a total of four doses of macrophages (10^6 cells/dose) over a 2-wk time period. At the same time, the mice (10 mice/group) received 1.5 mg/ml Dox administered in the drinking water with 4% sucrose or 0.3% ethanol water with 4% sucrose as a control. The mice were next immunized with CII in IFA 4 wk after the first immunization at 11 wk of age, and the development of arthritis was assessed weekly up to 20 wk of age (Fig. 2A). The administration of Dox alone in the range of 1.0–8.0 mg/ml confirmed that the administration of Dox alone had no effect on the development of arthritis (data not shown). As anticipated, the control groups of mice treated with either CII-APC or CII-APC-AdGFP developed severe arthritis 9 wk after the second immunization with CII in IFA. Histological examination of the joints of the mice sacrificed at 9 wk after CII-APC-AdFasLp35Tet or control treatment confirmed that the control groups of mice that were treated with either CII-APC-AdGFP or CII-APC-AdFasLp35Tet without Dox exhibited histological changes indicative of severe arthritis, with nearly all of the joints showing pronounced synovial hyperplasia, cartilage erosion, and ankylosis (Fig. 2B).

These histological features were significantly less apparent in the group of mice treated with CII-APC-AdFasLp35Tet plus Dox (Fig. 2B). The severity of arthritis in the groups of mice treated with CII-APC-AdFasLp35Tet plus Dox was significantly lower than that of the control groups (p < 0.01; Fig. 2C). Interestingly, mice were treated with CII-APC-AdFasLp35Tet without Dox inducer also showed less severe arthritis at the first 3 wk after the treatment but not afterward (Fig. 2C), indicating that low-level expression of FasL may occur in the absence of Dox and is sufficient to decrease the initial severity of CII-induced arthritis.

**CII pulse is required for elimination of CII responsive T cells using APC-AdFasLp35Tet treatment**

To test whether loading of APC-AdFasLp35Tet with CII is necessary for inhibition of arthritis and deletion of CII-specific T cells, APC-AdFasLp35Tet treatment with or without CII was conducted. CD3 T cell staining showed that there was extensive T cell infiltration of the joints in the group of mice treated with APC-AdFasLp35Tet without CII (Fig. 3A). B220-positive B cell infiltration of the joints was minimal regardless of the treatments at 9 wk after the CII boost (data not shown). The requirement of CII-pulsed APC-AdFasLp35Tet treatment was further demonstrated by an in vitro T cell proliferation assay and IL-2 induction. T cell proliferation was determined at different times after stimulation by pulsing with [3H]thymidine 18 h before harvest of the supernatants at 48 and 72 h after stimulation (Fig. 3, B and C). There was a significant decrease in T cell proliferation as indicated by decreased [3H]thymidine uptake and a significant decrease in IL-2 production at 48 and 72 h in the group of mice treated with CII-APC-AdFasLp35Tet compared with APC-AdFasLp35Tet-treated mice. There was no T cell proliferation or IL-2 induction when the stimulator APC were not pulsed with CII, indicating that CII was required (Fig. 3B). Thus, the results indicated that CII-loaded APC-AdFasLp35Tet treatment is necessary to achieve high specificity deletion of CII-reactive T cells since both [3H]thymidine uptake and IL-2 induction were much higher in the group of mice treated with APC-AdFasLp35Tet (Fig. 3, B and C).
To determine whether the treatment of CII-APC-AdFasLp35Tet impairs an irrelevant T cell-dependent Ag response, DBA/1j mice were immunized with both CII and OVA at 7 and 11 wk of age and treated with CII-APC-AdFasLp35Tet plus Dox from 9 to 11 wk of age as described above. Draining lymph node T cells isolated from these mice were cocultured with OVA-pulsed APCs. Both T cell proliferation and induction of IL-2 in response to OVA were not impaired after treatment with CII-APC-AdFasLp35Tet, indicating that the decreased of these responses for CII is specific for CII (Fig. 3, D and E).

FIGURE 4. AdCMVsTACI produces functional sTACI. A, sTACI was produced by transfection of 293 cells with an AdCMVsTACI construct as described in Materials and Methods, and the sTACI was purified and concentrated to 1 μg/ml. DBA/1j macrophages were transfected (500 PFU/cell) with AdCMVBlyS followed by irradiation and culture with TACI-positive A20 B cells. After 18 h, the AdBlyS-transfected and gamma-irradiated APC were incubated with A20 B cells in the presence of media alone or with decreasing concentrations of sTACI. After 48 h, the proliferation was determined by an 18-h pulse of [3H]thymidine. The counts were determined using a scintillation counter. The data points represent the mean ± SEM of three experiments. B, Mice were immunized with CII as described in Fig. 2A and treated at 7 wk of age with a single dose of AdCMVsTACI (5 × 10^9 PFU/mouse). Serum levels of sTACI were evaluated up to 50 days after treatment with AdCMVsTACI by ELISA. The concentrations of the sTACI-Fc protein were determined using a rabbit anti-human Fc polyclonal Ab to capture the human Fc component of the recombinant protein, and the captured protein was quantified using a rabbit anti-human Fc polyclonal Ab conjugated to HRP. Sample dilutions were compared with standard curves of human Fc to determine the concentrations of sTACI-Fc. The data points represent the mean ± SEM of at least five mice at each time point.

To determine whether the treatment of CII-APC-AdFasLp35Tet impairs an irrelevant T cell-dependent Ag response, DBA/1j mice were immunized with both CII and OVA at 7 and 11 wk of age and treated with CII-APC-AdFasLp35Tet plus Dox from 9 to 11 wk of age as described above. Draining lymph node T cells isolated from these mice were cocultured with OVA-pulsed APCs. Both T cell proliferation and induction of IL-2 in response to OVA were not impaired after treatment with CII-APC-AdFasLp35Tet plus Dox, indicating that the decreased of these responses for CII is specific for CII (Fig. 3, D and E).

FIGURE 3. CII-pulsed APC-AdFasLp35Tet is required for induction of CII-specific deletion of T cells. DBA/1j mice were immunized with CII or OVA and treated as described in Fig. 2A. The mice were sacrificed at 20 wk of age. A, Anti-CD3 Ab staining of the joints of mice treated with APC-AdFasLp35Tet pulsed with or without CII. The photographs were taken using a DigiSpot camera system. Original magnification, ×20. B, Draining lymph node T cells were isolated from mice at the time of sacrifice (20 wk of age) and were stimulated with irradiated APCs from DBA/1j mice that were either pulsed with CII or control unpulsed APCs (stimulator:responder ratio, 1:10). The proliferation of the T cells from mice treated with CII-APC, CII-APC-AdGFP, or APC-AdFasLp35Tet pulsed with or without CII was determined at different times after an 18-h pulse of [3H]thymidine. The counts were determined using a scintillation counter. C, IL-2 was determined in the supernatant at 48 and 72 h after culture. The results represent the mean ± SEM of duplicate cultures of five mice per group analyzed separately. **, p < 0.01. Draining lymph node T cells proliferation (D) and IL-2 induction (E) after OVA stimulation in vitro were conducted as described in B and C.
AdCMVsTACI produces functional sTACI

To confirm first that sTACI can block the B cell proliferative response to APC expressing high levels of BlyS, DBA/1j macrophages were transfected with AdCMVBlyS followed by irradiation and culture with A20 B cells that express TACI (26, 28, 29). Proliferation of the A20 B cells was evaluated using the [3H]thymidine uptake method. The strong proliferative response of the TACI-positive A20 B cells to the AdCMVBlyS-transfected DBA/1j macrophages (Fig. 4A, column 1) was inhibited completely by the presence of sTACI at a concentration of 10 ng/ml (Fig. 4A, column 4). To confirm that transfection in vivo with AdCMVsTACI results in effective therapeutic levels of sTACI in the serum, AdCMVsTACI (5 × 10^9 PFU/mouse) was injected i.v. into DBA/1j mice and the sera were sampled at various time points. The levels of sTACI in the sera were then evaluated using an ELISA (26). The sera levels of sTACI reached a peak level of 330 ng/ml at day 7 postinjection, then gradually declined until day 50, at which time the levels of sTACI in the sera were 78 ng/ml (Fig. 4B).

CII-APC-AdFasLp35Tet plus AdCMVsTACI treatment blocks cartilage degradation and T cell infiltration

sTACI, at high dose, has recently been shown to inhibit development of CII arthritis (30). To determine the effect of sTACI delivered by gene therapy (AdCMVsTACI) either alone or in combination with treatment with CII-APCAdFasLp35Tet, mice were injected with AdCMVsTACI alone or AdCMVsTACI plus CII-APC-AdFasLp35Tet. As a control, mice were injected with either AdGFP or CII-APCAdGFP plus AdGFP. Control-treated mice exhibited extensive synovial hyperplasia, cartilage erosion, and bony ankylosis at 20 wk of age (Fig. 5). There was a significant decrease in the number of CD3-positive T cells infiltrating the synovium of mice treated with combined treatment of AdCMVsTACI plus CII-APC-AdFasLp35Tet (Fig. 5).

Discussion

In this study, we show that primary macrophages isolated from DBA/1j mice can be engineered successfully such that they express high levels of FasL in a Dox dose-dependent manner. These FasL-expressing macrophages (APC-AdFasLp35Tet) were capable of killing Fas-positive A20 cells. Treatment with CII-APC-AdTREFasLp35Tet effectively prevents CII-primed DBA/1j mice from developing arthritis (30). To determine whether enhancement of CII-APC-AdFasLp35Tet treatment combined with AdCMVsTACI is associated with reduction of CII Ab production, sera were collected at the time of sacrifice (20 wk of age) and analyzed for the anti-CII Ab by an ELISA. The anti-CII IgG Ab levels in the sera of mice treated with AdCMVsTACI alone were significantly lower than those in control mice (Fig. 6B). There was almost complete elimination of anti-CII Abs in the sera of mice treated with both CII-APC-AdFasLp35Tet plus AdCMVsTACI.

Decreased anti-CII Ab production after combined treatment with CII-APC-AdFasLp35Tet and AdCMVsTACI

There was a significant decrease in these parameters of arthritis in mice treated with AdCMVsTACI compared with treatment with AdGFP (p < 0.05, Fig. 6A). There was also a significant decrease in these parameters of arthritis in mice treated with AdCMVsTACI combined with CII-APC-AdFasLp35Tet compared with treatment with AdGFP plus CII-APC-AdGFP (p < 0.01). CII arthritis was significantly decreased using this combined therapy compared with treatment with CII-APC-AdFasLp35Tet alone (Fig. 6A, p < 0.05). These results indicate that using AdCMVsTACI gene therapy augments the efficacy of CII arthritis reduction compared with treatment with CII-APC-AdFasLp35Tet; but at gene therapy doses achieved here, it was unable to eliminate CII arthritis by treatment with AdCMVsTACI alone.

To determine whether enhancement of CII-APC-AdFasLp35Tet treatment combined with AdCMVsTACI is associated with reduction of CII Ab production, sera were collected at the time of sacrifice (20 wk of age) and analyzed for the anti-CII Ab by an ELISA. The anti-CII IgG Ab levels in the sera of mice treated with AdCMVsTACI alone were significantly lower than those in control mice (Fig. 6B). There was almost complete elimination of anti-CII Abs in the sera of mice treated with both CII-APC-AdFasLp35Tet plus AdCMVsTACI.

FIGURE 5. CII-APC-AdFasLp35Tet plus AdCMVsTACI treatment blocks cartilage degradation and T cell infiltration. DBA/1j mice were immunized with CII and treated with AdCMVsTACI or with CII-APC-AdFasLp35Tet plus AdCMVsTACI. Control DBA/1j mice were immunized with CII and treated with AdGFP or CII-APC-AdGFP plus AdGFP. The mice were sacrificed at 20 wk of age and the joints sectioned and stained with H&E and anti-CD3 Ab. The photographs were taken using a DigiSpot camera system. Original magnification, ×20.
from developing arthritis without impairing the host immune response to an irrelevant Ag OVA. Furthermore, we show that CII-APC-AdFasLp35Tet treatment in combination with blocking of the B cell costimulation TACI pathway is the most effective approach to blocking CII-induced arthritis in DBA/1j mice. Blocking of the TACI pathway alone resulted in decreased production of the anti-CII autoantibodies but failed to inhibit the development of the disease completely.

This is a significant advancement of our previous result using FasL-transfected macrophage cell lines capable of inducing Ag-specific T cell deletion, since this novel approach is not limited to the use of APCs with defective Fas expression (20, 21, 23–25). To expand the applicability of this system, we have coexpressed FasL with the anti-apoptosis molecule p35 to prevent autocrine cell death of the APCs (39, 40). In addition, the FasL has been placed under the regulation of an inducible Tet-On system (34–36, 38). Here, we have evaluated the use of this strategy in the treatment of an autoimmune disease, CII-induced arthritis, and to determine the role of T cells in the development and progression of the disease process. This strategy can be further applied to dissect the roles of other T cell-mediated diseases and, in particular, autoimmune diseases.

Both B cells (9–14) and T cells (15–19) are considered to play a pathogenic role in collagen-induced arthritis, but the question of which cell type acts as the initiator of the arthritis disease process remains controversial. Both cell types have been implicated through the observations that collagen-induced arthritis can be attenuated by treatment with mAbs to CD4 or the TCR, and that recipient mice develop arthritis after adoptive transfer of collagen-specific T cell lines or administration of anti-collagen Abs (17). Moreover, it has been shown that arthritis can be initiated in DBA/1j mice crossed with Rag-1 nullizous mice, which lack functional mature T and B lymphocytes, resulting in a milder form of disease (18). Clearly, the inconsistencies in the available data may be due to several factors including differences in the genetic background of the mice and the lack of methods that permit inhibition of one component of the immune system that is activated by CII without impairing the rest of the immune system.

Our recent results and those of others have shown that a FasL-transfected macrophage cell line is capable of inducing Ag-specific T cell depletion without affecting the host immune response to irrelevant Ags. This conclusion was further supported by two observations in this article. First, CII- and OVA-primed DBA/1j mice treated with CII-APC-AdFasLp35Tet leads to T cell unresponsiveness to CII but not OVA. Second, the therapeutic effects of prevention of CII-induced arthritis was significantly reduced in the mice treated with APC-AdFasLp35Tet without preincubation of the APCs with CII. Notably, APC-AdFasLp35Tet treatment in which the APCs were not pulsed with CII resulted in a marginal reduction of T cell infiltration in the joints and decreased T cells

FIGURE 6. Effect of AdCMVsTACI and CII-APC-AdFasL plus AdCMVsTACI on arthritis and production of anti-CII Abs. DBA/1j mice were immunized with CII and treated with AdCMVsTACI, CII-APC-AdFasLp35Tet, or combined treatment with CII-APC-AdFasLp35Tet plus AdCMVsTACI. Control DBA/1j mice were immunized with CII and treated with AdGFP or CII-APC-AdGFP plus AdGFP. A. The severity of arthritis development was scored from 0 to 4 as described in the text. Each bar represents the scores from five mice of each treatment. B. Sera were collected at the time of sacrifice (20 wk of age) and analyzed for anti-CII Ab by an ELISA using an IgG. The results represent the mean ± SEM of two individual determinations on five mice per group determined separately.

4170 SPECIFIC DEPLETION OF CII-REACTIVE T CELLS BY CELL-GENE THERAPY

Downloaded from http://www.jimmunol.org/ by guest on April 12, 2017
compared with CII-pulsed APCs. The nonspecific effects due to APC-AdFasLp35Tet treatment was not sufficient to suppress the OVA response. Therefore, CII-pulsed-APC AdFasLp35Tet specifically reduced CII-reactive T cells and CII arthritis. These results are consistent with our previous observation using an APC-FasL gene therapy protocol to eliminate the postinfectious arthritis that results after the administration of mycoplasma pulmonis (42). In these experiments, there were only a slight decrease in the total number of CD3+, CD4+, and CD8+ T cells, indicating that this postinfectious arthritis can be greatly reduced with only minimal reduction of the total T cell population.

In this report, by using this novel strategy, we show that T cells can play a dominant pathogenic role in CII-induced arthritis independent of the anti-CII autoantibody production. Our immunohistological analyses further indicate that both deletion of CII-activated T cells and blocking of B cell activation are necessary to completely prevent the development of CII-induced arthritis and further support the concept that the B cell compartment plays a significant, although secondary, role in the development of CII-induced arthritis. Our data show that a wide range of Dox doses can be used to prevent the development of CII-induced arthritis, implying that CII-specific T cells in both the early and late stages of activation are susceptible to apoptosis triggered by CII-APC-AdFasLp35Tet treatment. It will be of interest to further characterize the susceptibility of the CII-activated T cells at different stages in the development of arthritis. The information will provide a scientific basis for the initial starting point of CII-APC-AdFasLp35Tet treatment to achieve maximum deletion of Ag-activated T cells without impairment of host immune response to irrelevant Ags.

Our treatment initiated at wk 2 after CII immunization was based on our previous observation that spleen T cells reached maximum response to CII 2 wk after immunization (data not shown). Therefore, we predict that these T cells may be highly susceptible to Fas-mediated apoptosis. This is supported by our previously published data that the APC-AdFasL cell-gene therapy first migrates to the spleen where the APCs come into contact with activated T cells, resulting in apoptosis of these T cells. We are currently analyzing the initial migration and survival of CII-reactive T cells using T cells that express a receptor with high affinity for a nuclear imaging reagent (43, 44).

BlyS/TALL-1 is a potent B cell costimulatory factor which acts by direct binding and activation of its cell surface receptor on B cells (26, 28, 29). Transgenic mice that overexpress TALL-1 develop severe B cell hyperplasia and hypergammaglobulinemia (45). These mice also develop an autoimmune, lupus-like disease characterized by the presence of autoantibodies and immune complex deposits in the kidney. Wang et al. (30) have shown that administration of high-dose sTACI can inhibit CII arthritis. Our findings clearly demonstrate that TACI is one of the major molecules involved in the stimulation of anti-CII Ab production, as administration of recombinant adenosivirus expressing soluble TACI can completely block CII Ab production, but this is associated with only a minor reduction in the severity of the disease when used separately. The different results may be due to different methods for administration of sTACI which may result in different sera levels of sTACI. Treatment with AdCMV-sTACI likely resulted in significantly lower serum levels of sTACI (maximum level of 330 ng/ml) 1 wk after AdCMV-sTACI compared with mice receiving 100 µg i.v. administered three times a week for 6 wk (30).

Interestingly, CII-APC-AdFasLp35Tet treatment alone did not completely block anti-CII Ab production, and the similar results were obtained previously (24). This implies either that there was incomplete deletion of CII-reactive T cells or survival of the subpopulation of helper T cells that promote B cell production of anti-CII Abs. Alternatively, the T-B cell interaction could occur relatively early, perhaps at week 8 before CII-APC-AdFasLp35Tet treatment was initiated or at early time points after the treatment.

Acknowledgments
We thank Linda Flurry for excellent secretarial work, Dr. Fiona Hunter for editorial assistance, Dr. Uney generously provided AdrtTA construct, and Deidre Downs for critical review of this manuscript.

References


