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To investigate the antagonistic effect of IL-12 p40 on IL-12 activity in vivo, we generated transgenic (Tg) mice in which p40 gene was regulated by a liver-specific promoter. Three Tg mouse lines were generated, and they expressed the p40 transgene predominantly in liver. Serum p40 level was extremely high, and it consisted of mainly monomer and homodimer and also of higher m.w. complexes. These Tg mice did not show any apparent phenotypic difference from control littermates in lymphoid cells. Enhancement of NK cell lytic activity in spleen by administration of rIL-12 to these mice was greatly diminished. Ag-induced cytokine production was impaired: decreased production of IFN-γ and increased production of IL-4 and IL-10. Delayed-type hypersensitivity response was also significantly reduced. Moreover, these Tg mice showed increased susceptibility to the infection with an intracellular pathogen, blood-stage Plasmodium berghei XAT, which is an irradiation-induced attenuated strain of P. berghei NK65, presumably due to the decreased IFN-γ production. These results suggest that p40 functions as an IL-12 antagonist in vivo, and that Th1 responses in p40 Tg mice are significantly reduced. Thus, these Tg mice could be a useful model to evaluate the inhibitory effect of p40 on IL-12-mediated various immune responses in vivo. The Journal of Immunology, 1998, 160: 588–594.

Interleukin-12 is a potent immunoregulatory molecule that plays a pivotal role in the generation of Th1 response against a variety of intracellular pathogens and the pathogenesis of some Th1-associated autoimmune disorders as well (1–3). IL-12 is a heterodimeric cytokine (p70) composed of two disulfide-linked subunits, p35 and p40, that induces IFN-γ production by T cells and NK cells and promotes a generation of Th1 cells (1, 4–7). The p40 expression is highly regulated by various stimuli in monocytes/macrophages and dendritic cells, and the p40 is considered the regulatory component for IL-12 production and responsible for IL-12R binding (1, 4). For the generation of bioactive IL-12, the expression of both subunits within one cell is required, although their expression is differentially regulated (1, 4, 8).

It was demonstrated that p40 is produced as monomer and homodimer in liver and IL-12 p40 both in vitro (9, 10) and in vivo (11, 12). The p40 level in murine circulation was reported to remain high after LPS stimulation, whereas IL-12 production rapidly decreases (11, 12). These results indicate a critical role of p40 as a natural antagonist of IL-12. Indeed, mouse p40 has been demonstrated to bind to mouse IL-12R with an affinity similar to that of IL-12. However, it does not trigger biologic activation and inhibits IL-12-mediated responses by competitive binding to the IL-12R in vitro (13, 14). Human p40, which also exists in monomeric and dimeric forms, binds to the IL-12-R and acts as a competitive antagonist of IL-12 in vitro (15). It has been demonstrated very recently that locally produced mouse IL-12 p40 from a transplanted myoblast cell line suppresses Th1-mediated immune responses and prevents the allogeneic rejection of the myoblast (16). However, the ability of p40 as an IL-12 antagonist in various immune responses remains to be elucidated further under physiologic conditions in vivo.

In the present study, we generated p40 transgenic (Tg)3 mice predominantly expressing the transgene in liver and found that IL-12-mediated Th1 responses were reduced significantly due to the antagonistic activity of p40 in these p40 Tg mice. Thus, these mice would be useful to evaluate the function of p40 as an IL-12 antagonist in vivo.

Materials and Methods

Construction of IL-12 p40 transgene

The open reading frame (1 kb) of murine IL-12 p40 cDNA (kindly donated by Dr. M. Kobayashi, Genetics Institute, Cambridge, MA) was amplified using PCR and inserted into the EcoRI site of pLG1-SAP that contains the human serum amyloid P component (SAP) promoter and the rabbit β-globin gene (17), resulting in construction of the plasmid pLG1-SAP-IL-12 p40 (Fig. 1). Before injection into fertilized mouse eggs, the plasmid was digested with HindIII and XhoI, and the resultant 2.8-kb fragment of SAP-IL-12 p40 gene was isolated and used for microinjection.

Generation of IL-12 p40 Tg mice

Fertilized eggs were obtained from C57BL/6 mice, and several hundred molecules of the SAP-IL-12 p40 fragment (2.8 kb) were injected into the pronucleus of the fertilized eggs, as described (18). When the mice were at 4 wk of age, genomic DNA was extracted from a piece of the tail of each mouse and used to detect the transgene by PCR using the following two primers: one (β2 sense primer, 5’-TGGTGTCTCATCATTTTGGC-3’) corresponds to the 5’ untranslated region of rabbit β-globin gene, and the other (p40 antisense primer, 5’-TTGATGCCATCTACCTACGG-3’) corresponds to the IL-12 p40 gene (Fig. 1).

3 Abbreviations used in this paper: Tg, transgenic; DTH, delayed-type hypersensitivity; KLH, keyhole limpet hemocyanin; LN, lymph node; PE, phycoerythrin; PRBC, parasitized red blood cell; RT, reverse transcriptase; SAP, serum amyloid P component.
Reverse-transcriptase (RT) PCR analysis

Total RNA was extracted from various tissues by using the guanidine thiocyanate procedure (19). One microgram of total RNA was reverse transcribed into cDNA using SuperScript RT (Life Technologies, Gaithersburg, MD) by incubation for 1 h at 42°C in a reaction mixture of 50 mM Tris-HCl (pH 8.3) containing 75 mM KCl, 3 mM MgCl₂, 0.4 U/μl RNase inhibitor (WAKO Chemicals, Osaka, Japan), 0.2 mM dextoxynucleotide triphosphates, 1 mM DTT, and 0.8 μM RT from Moloney murine leukemia virus (Life Technologies) after annealing with oligo(dT) primer (Promega Corp., Madison, MD). To avoid detection of endogenous IL-12 p40 mRNA, the following two primers were used: one (β1 sense primer, 5'-GATCCCTGAGAACTTCCAGGCTC-3') corresponds to the 5' untranslated region of rabbit β-globin gene, and the other (p40 primer) corresponds to the IL-12 p40 gene (Fig. 1). The PCR was performed in 10 mM Tris-HCl (pH 9) containing 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dextoxynucleotide triphosphates, 0.1% Triton X-100, 0.5 μM each primer, and 0.025 U/μl Taq DNA polymerase (Toyobo, Osaka, Japan). Reactions for the p40 transgene or β-actin were subjected to 40 cycles consisting of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, or 35 cycles consisting of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, respectively. All PCR mixtures were subjected to denaturation at 94°C for 3 min before the first cycle, and to final extension at 72°C for 7 min after the last cycle. The amplified products were size fractionated by electrophoresis on a 1.5% agarose gel, followed by ethidium bromide staining for UV-assisted visualization and Southern blot hybridization with 32P-end-labeled p40 internal oligonucleotide probe (5'-TAAAGCCTTTTCAAGTGCGAGGCC-3'), as described (20).

ELISA for serum IL-12 p40 and p70

Blood was obtained from the eye artery, and serum was separated by centrifugation. The serum was assayed for murine IL-12 p40 by sandwich ELISA using IL-12 p40 Abs (C15.6 and C17.8, kindly provided by Dr. G. Trinchieri, The Wistar Institute, Philadelphia, PA), as described (16). Serial dilutions of murine IL-12 p40 (sp. act., 4.6 × 10⁶ U/mg, kindly donated by Dr. M. Kobayashi, Genetics Institute) were used as the standard. To detect p40, microtiter plate (Becton Dickinson, Mountain View, CA) was coated with anti-IL-12 p40 mAb (C15.6), followed by incubation with twofold serially diluted samples to be tested. After washing, the plate was incubated with biotinylated anti-IL-12 p40 mAb (C17.8) and subsequently with peroxidase-labeled streptavidin. The plate was then developed with o-phenylenediamine, and the level of p40 was determined photometrically by measuring OD₄₉₂ in reference to the standard tiration curve of murine rIL-12. Reagents for ELISA of murine IL-12 p70 (sp. act., 1.0 × 10⁸ U/mg) were kindly provided by Dr. D. H. Fuesky (Hoffmann-La Roche, Nutley, NJ). In this assay (21), rat anti-mouse IL-12 p70 mAb (SA5), peroxidase-labeled rat anti-mouse IL-12 p40 mAb (5C3), and rIL-12 were used as a coating Ab, developing Ab, and standard, respectively, and ELISA was performed as described above.

Western blot analysis

Serum IL-12 p40 was separated by SDS-PAGE (10%) under reducing or nonreducing conditions. The separated proteins were transferred to a polyvinylidene difluoride microporous membrane (Immobilon PVDF; Millipore Co., Bedford, MA). Transblots were incubated with biotinylated anti-mouse IL-12 p40 mAb (C17.8) and then with alkaline phosphatase-conjugated streptavidin. The Ab-reactive bands were visualized using 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT) phosphatase substrate system.

FACS analysis

One million cells were analyzed on a FACScan using a Lysis II software (Becton Dickinson) for data analysis. For two- or three-color analysis of thymus, LN, and spleen cells, PE anti-CD8 (53-6.7, rat IgG2a), PE anti-B220 (RA3-6B2, rat IgG2a), PE anti-NK1.1 (PK136, mouse IgG2a), FITC anti-Thy-1.2 (3O-H12, rat IgG2b), FITC anti-CD4 (14-2C11, hamster IgG), biotinylated anti-TCR-α-β (H57-597, hamster IgG) (all from Phar-Mingen, San Diego, CA), and biotinylated anti-CD4 (GIK.1, rat IgG2b), which was prepared using purified mAb from ascites on a protein G column, and streptavidin-Cy-chrome (PharMingen) were used. The mAbs specific for heat-stable Ag (HSA, M1/69, rat IgG2b), Pgp-1 (KM201, rat IgG1), IL-2Rα (PC61, rat IgG1), and c-kit (ACK-2, a gift from Dr. S. Nishikawa, Kyoto University, Kyoto, Japan) (22) were used in the form of culture supernatants of each hybridoma clone. FITC anti-rat IgG(F(ab')₂), (Life Technologies) was used as a second Ab. Dead cells positively stained with 7-aminofluorocynin D (Sigma Chemical Co., St. Louis, MO) were gated out.

NK cell lytic activity

YAC-1 lymphoma target cells were incubated for 1 h with Na₅²¹CrO₄ (Amersham, Arlington Heights, IL) and washed extensively. Spleen cells were used as effector cells and mixed with the target cells (1 × 10⁶ cells in 200 μl RPMI 1640 medium supplemented with 10% FCS, 5 × 10⁻³ M 2-ME, and 100 μg/ml kanamycin in 96-well round-bottom plates. Plates were incubated for 4 h at 37°C, and ⁵¹Cr release into the supernatants was determined in a gamma counter. The lytic activity of the cells was assayed at E/T ratios of 100, 50, 25, and 12.5. The specific ⁵¹Cr release (%) was determined as follows: [experimental release − spontaneous release]/(maximum release − spontaneous release) × 100. The maximum release was obtained by target cell lysis with 1% Triton X-100.

Cytokine production

Mice were injected i.c. in the hind footpads with keyhole limpet hemocyanin (KLH, 50 μg) emulsified with CFA. After 1 wk, popliteal lymph nodes (LNs) were removed and the cells were cultured in the medium containing 0.3 mg/ml of KLH at 6 × 10⁶ cells/ml. Culture supernatants were harvested after 48 h and assayed for the concentration of IFN-γ, IL-4, and IL-10 by ELISA kits obtained from PharMingen, according to the manufacturer’s instruction.

Delayed-type hypersensitivity (DTH) reaction

Mice were primed with 1 × 10⁶ SRBC suspended in 0.2 ml PBS by s.c. injection in the back (23). After 7 days, mice were injected s.c. in the left footpad with 1 × 10⁶ SRBC suspended in 20 μl PBS, and in the right footpad with 20 μl PBS as a control. Twenty four hours later, footpad swelling was measured with a caliper. The degree was calculated as the percentage of the swelling using the following formula: footpad swelling (%) = [(thickness of footpad injected with SRBC − thickness of footpad injected with PBS)/thickness of footpad injected with PBS] × 100.
RNA obtained from lymphoid organs such as spleen, LN, and transgene was detected when the RT-PCR was conducted using RNA obtained from various tissues and primers (of age, RT-PCR was performed using RNA obtained from various tissues (5–33.4 ng/ml) when mice were at 6 to 7 wk of age. Surprisingly, the mean concentration in sera from each mice was assayed for p40 in sandwich ELISA to examine whether p40 molecule was circulating in p40 Tg mice, p40 Tg B mouse (Fig. 2) was also observed slightly in kidney of p40 Tg A mouse, not of p40 Tg A and B lines, when they were at 5 to 6 wk of age, and RT-PCR was performed, followed by Southern blot analysis using the internal probe. The PCR product (357 bp) was detected predominantly in liver of these Tg mice (Fig. 2). The PCR product was also observed slightly in kidney of p40 Tg A mouse, not of p40 Tg B mouse (Fig. 2A), and in stomach of p40 Tg B mouse, not of p40 Tg A mouse (Fig. 2B). No apparent band due to the p40 transgene was detected when the RT-PCR was conducted using RNA obtained from lymphoid organs such as spleen, LN, and thymus, and also from lung and small intestine.

High serum p40 level in p40 Tg mice

To examine whether p40 molecule was circulating in p40 Tg mice, sera from each mice were assayed for p40 in sandwich ELISA when mice were at 6 to 7 wk of age. Surprisingly, the mean concentrations of p40 in p40 Tg A and B mice were extremely high: 46 ± 30.3 μg/ml (12–100.1 μg/ml, n = 12) and 14.9 ± 8.4 μg/ml (5–33.4 μg/ml, n = 22), respectively. In contrast, the mean concentration in control littersmates was 0.78 ± 0.28 ng/ml (0.38–1.10 ng/ml, n = 7). Furthermore, we tried to detect serum IL-12 p70 in p40 Tg mice and control littersmates in the expression of CD3, CD4, CD8, B220, Thy-1.2, and NK1.1 (data not shown). Thymocytes of Tg mice were normal in expression of HSA, IL-2Ra, CD44, and c-kit, typical markers for immature thymocytes (data not shown). In addition, the size of thymus and the absolute number of thymocytes were normal. Thus, no apparent abnormality of lymphocyte was noted in ontogeny of these p40 Tg mice.

Diminished enhancement of NK cell lytic activity by rIL-12 administration to p40 Tg mice

Since administration of rIL-12 was reported to enhance NK cell lytic activity in vivo (27), we first examined whether the lytic activity of spleen cells would be increased by the administration of rIL-12 in p40 Tg mice to evaluate the antagonistic activity of serum p40 in vivo. p40 Tg A mice and control littersmates were treated with rIL-12 (30 ng) or PBS by i.p. injection for 2 consecutive days, spleens were removed 24 h after the second injection, and these cells were assayed for lytic activity on YAC-1 cells. rIL-12 administration greatly enhanced the NK cell lytic activity in control littersmates (Fig. 4). However, no significant enhancement of the lytic activity was observed in p40 Tg mice (Fig. 4). Similar results were obtained when p40 Tg B mice were used (data not shown).
These results suggest that serum p40 in p40 Tg mice is functional as an IL-12 antagonist in vivo.

Reduced Th1 responses in p40 Tg mice

A number of evidences have been accumulated for a critical role of IL-12 in promoting Th1 responses both in vitro and in vivo (1). Therefore, we next investigated Ag-induced cytokine production of T cells from p40 Tg A mice in comparison with that from control littermates. Mice were immunized with KLH in CFA, draining LNs were removed 7 days later, and cytokine production of LN cells stimulated with KLH for 48 h was examined in ELISA. LN cells from KLH-primed p40 Tg mice were reduced markedly in the production of IFN-γ on KLH stimulation (Fig. 5). In contrast, Ag-induced IL-4 and IL-10 production of these cells was significantly increased (Fig. 5). Similar results were obtained when p40 Tg B mice were used (data not shown). These results indicate that in p40 Tg mice the response in Th1 cytokine production is decreased, and that in Th2 cytokine production is increased.

To further examine the effect of p40 on the Th1-mediated immune response, we investigated the cell-mediated response in terms of DTH reaction. Mice were primed s.c. with SRBC for 7 days and challenged by the SRBC injection into footpads for DTH reaction. The specific footpad swelling 24 h after the challenge was diminished significantly in p40 Tg A mice as compared with that in control littermates (Fig. 6). Similar results were obtained when p40 Tg B mice were used (data not shown). These results indicate that in p40 Tg mice the response in Th1 cytokine production is decreased, and that in Th2 cytokine production is increased.

Increased susceptibility of p40 Tg mice to malarial infection

IL-12 has been demonstrated to play an important role in the development of protective immunity involving Th1 responses, especially IFN-γ production, against intracellular pathogens such as Leishmania major, Listeria monocytogenes (1, 2), and blood-stage Plasmodium chabaudi AS (28) and P. berghei XAT (T. Yoshimoto et al., manuscript submitted). Therefore, we finally examined the susceptibility of p40 Tg mice to the infection with an intracellular pathogen, blood-stage P. berghei XAT. p40 Tg A mice and control littermates were inoculated i.v. with 1 × 10⁸ PRBC, and blood smears were examined for parasitemia. All control mice cleared PRBC in about 3 wk after two peaks of parasitemia (Fig. 7A). In p40 Tg mice, however, parasitized corpuscles were increased progressively in percentage (Fig. 7A). Three of five p40 Tg mice could not clear PRBC and eventually died of the infection, and the rest of these mice scarcely recovered from the infection. This increased susceptibility of p40 Tg mice would be attributable to the antagonistic activity of p40 against the function of bioactive...
IL-12 produced by the infection. To confirm this notion, p40 Tg A mice and control littermates were administrated i.p. with 100 ng of rIL-12 for 5 consecutive days from day 21 to day 4 after the inoculation, and parasitemia was examined. Appearance of parasitemia was greatly delayed in the control littermates treated with rIL-12, and PRBC were cleared in about 3 wk (Fig. 7B and T. Yoshimoto et al., manuscript submitted). In contrast, the rIL-12 administration did not delay the onset of the parasitemia in p40 Tg mice, and the parasitemia in these mice progressed similarly to that in untreated p40 Tg mice (Fig. 7B). Eventually, two of five p40 Tg mice could not clear PRBC and died, and the rest of these mice scarcely recovered. We have found recently that IFN-γ production in spleen, which is critically important for the protective immunity to blood-stage *P. berghei* infection, is highly dependent upon IL-12 produced by the infection (T. Yoshimoto et al., manuscript submitted). Therefore, spontaneous in vitro IFN-γ secretion of spleen cells in p40 Tg A mice infected with *P. berghei* XAT was examined at various time intervals after the inoculation to see the in vivo effect of p40 on the IFN-γ production during the infection. IFN-γ production of spleen cells in p40 Tg mice was reduced significantly compared with that of control littermates (Fig. 8), which presumably accounts for the reduced ability of p40 Tg mice to establish the protective immunity to blood-stage *P. berghei* XAT infection.

**Discussion**

In the present study, we generated Tg mice in which the p40 transgene was expressed predominantly in liver, but not in lymphoid organs, and demonstrated that p40 is a functional antagonist against the in vivo activity of IL-12 and that, therefore, Th1 responses are reduced significantly in the p40 Tg mice. The p35 mRNA was reported not to be expressed in liver and kidney, which was analyzed by Northern blot (29). We confirmed this by RT-PCR analysis (data not shown). Therefore, the p40 transgene expression is considered not to be accompanied by IL-12 p70 production because both p40 and p35 have to be produced in the same
cell for the production of IL-12 p70 (8), although we cannot formally rule out the possibility. As a matter of fact, no apparently increased serum IL-12 p70 level was observed in p40 Tg mice. In contrast, the serum p40 levels in our Tg mice were surprisingly high even as compared with the serum IFN-γ level (mean concentration: 3.533 ng/ml) in IFN-γ Tg mice, which were generated similarly using the SAP promoter (17). This may imply that p40 is stable in serum, which is advantageous for the clinical application of p40 as a therapeutic agent. The serum p40 consisted of mainly monomer (M₀ ≈ 45 kDa) and homodimer (M₀ ≈ 120 kDa), and also of higher m.w. complexes (M₀ ≈ 200 kDa). The electrophoretic mobility of serum p40 homodimer on nonreducing SDS-PAGE was much slower than that calculated according to the m.w., as previously described for p40 homodimer (14, 26). Monomer and homodimer of p40 and the minor molecules were also detected in the culture supernatant of murine myeloma cells (Ag8.653) transfected with p40 cDNA ligated to the expression vector, BCMGSNeo (30), whereas the higher m.w. complexes were not detected (data not shown). These results may indicate the potential complex formation of p40 with other serum molecules.

p40 Tg mice did not show any apparent phenotypic abnormality in lymphoid cells, which is consistent with the results in IL-12 p40- or p35-deficient mice that IL-12 seemed not to be required for normal T lymphocyte differentiation and maturation (31, 32). To evaluate the antagonistic activity of serum p40 in p40 Tg mice, we first examined the NK cell lytic activity of spleen cells after administration of rIL-12 and found that no significant enhancement of the lytic activity was observed in p40 Tg mice. These results suggest that serum p40 functions as an IL-12 antagonist in vivo toward NK cells, which are compatible with the in vitro results reported previously (14). Furthermore, we clarified significantly reduced Th1 responses in p40 Tg mice due to the antagonistic activity of serum p40 against IL-12 in terms of Ag-induced cytokine production and DTH reaction, to an extent comparable with that observed in IL-12 p40-deficient mice (31). In addition, the p40 Tg mice showed increased susceptibility to the infection with an intracellular pathogen, blood-stage P. berghei XAT, presumably due to the decreased IL-12-dependent IFN-γ production. We also examined the susceptibility of p40 Tg mice to the infection with another intracellular pathogen, L. monocytogenes. It was reported previously in murine L. monocytogenes infection that neutralization of IL-12 resulted in increased number of CFU recovered from spleens and livers on day 3 and 5 after the inoculation (33, 34). However, there were no significant differences in spleen and liver burdens of L. monocytogenes strain EG2 between p40 Tg mice and control littermates on days 2, 4, and 6 after the inoculation (data not shown). This is a contrast to the results of P. berghei XAT infection. Although further analysis is necessary, this different susceptibility of p40 Tg mice to these pathogens might result from different IL-12 dependency to develop the protective immunity to these pathogens. Thus, the p40 Tg mice would be useful to evaluate the applicability of the treatment with p40 to inhibit various immune responses mediated by IL-12 in vivo.

After this study was submitted, another report demonstrating the IL-12 antagonistic activity of rIL-12 p40 homodimer in vivo in the acute endotoxemia was published (26). The study showed that endotoxemic mice generate serum IL-12 p40 homodimer in quantities corresponding to approximately one-third of the p40 monomer, whose ratio seems to be roughly comparable with that in serum p40 molecules of p40 Tg mice (Fig. 3). Treatment of mice with 40 µg of purified rp40 homodimer at 18 and 2 h before endotoxin challenge was shown to result in increase (25 ng/ml) of serum p40, which was approximately 50-fold in excess of the peak heterodimer concentration attained during endotoxemia (0.5 ng/ml), and therefore in significant decrease of IFN-γ circulation (26). The authors concluded that p40 homodimer is a naturally occurring cytokine antagonist that is produced in vivo and that specifically modulates systemic inflammatory responses dependent on the paracrine effects of IL-12 heterodimer. Taken together with our data, these results further suggest that p40 homodimer functions as an IL-12 antagonist in vivo in IL-12-mediated various immune responses. We cannot precisely determine the concentration of p40 homodimer alone in p40 Tg mouse serum, whereas it should be much higher than that attained by the administration of rp40 homodimer. Therefore, our p40 Tg mice would be more useful to analyze the in vivo effect of p40 on IL-12-mediated various immune responses. Considering that p40 is potentially stable in serum and nonimmunogenic, p40 could be applied to treat diseases associated with enhanced IL-12 production, such as Th1-dependent autoimmune diseases and graft rejection in transplantation. For the evaluation of applicability of p40 treatment to these diseases, especially, in which administration of p40 over a prolonged period of time may be required, p40 Tg mice, which constitutively express high serum p40, would be an useful model. These applicabilities are currently under investigation.

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References


