Attenuation of bleomycin-induced pneumopathy in mice by monoclonal antibody to interleukin-12

TAKASHIGE MAEYAMA, KAZUYOSHI KUWANO, MASAYUKI KAWASAKI, RITSUKO KUNITAKE, NAOKI HAGIMOTO, AND NOBUYUKI HARA. Attenuation of bleomycin-induced pneumopathy in mice by monoclonal antibody to interleukin-12. Am J Physiol Lung Cell Mol Physiol 280: L1128–L1137, 2001.—We previously demonstrated essential roles of the Fas-Fas ligand (FasL) pathway in bleomycin-induced pneumopathy in mice. T lymphocytes and natural killer cells express FasL on activation and use it as a cytotoxic effector molecule. Because interleukin (IL)-12 is known to play a critical role in cell-mediated immunity, we investigated whether anti-IL-12 antibody treatment suppresses the development of this model. The anti-IL-12 antibody treatment decreased the number of apoptotic cells and the degree of inflammation and fibrosis in lung tissue. The results of RT-PCR showed that IL-12p40, IL-12 receptor (R)β2, interferon-γ, tumor necrosis factor-α, and FasL mRNAs were upregulated after bleomycin instillation. The upregulation of FasL, IL-12Rβ2, and tumor necrosis factor-α mRNA expression in lung tissue was suppressed by anti-IL-12 antibody treatment. The results of enzyme-linked immunosorbent assay showed that the levels of IL-12p40, but not of IL-12p70, were increased in lung tissue after bleomycin instillation. Although the increase in IL-12Rβ2 mRNA levels suggests that the T helper type 1 cell response may participate in lung injury, the increase in IL-12p40 supports T helper type 2 cell predominance in the fibrotic process of this model. The administration of anti-IL-12 antibody could be a novel therapy against lung injury and pulmonary fibrosis.

T helper type 1 cell; T helper type 2 cell; Fas-Fas ligand pathway; apoptosis

The administration of bleomycin has been extensively used as a model of pulmonary fibrosis. The acute pulmonary toxicity induced by bleomycin in vivo is DNA damage (10), which is known to induce apoptosis in vitro (22). The accumulation of lymphocytes in bleomycin-induced pneumopathy (3, 32) and the lack of inflammation and pulmonary fibrosis in athymic nude mice receiving bleomycin (26) suggest that T lymphocytes may participate in the pathophysiology of this model. Although the effect of eliminating T lymphocytes on the development of this model is controversial, the administration of anti-CD4+ and anti-CD8+ antibodies in vivo diminished pulmonary fibrosis in this model (23). These results suggest that a T cell-mediated immune reaction is important in the development of this model.

Interleukin (IL)-12 is an inducible, antigen-presenting cell-derived cytokine composed of 35- and 40-kDa subunits. IL-12 potentiates T cell- and natural killer (NK) cell-mediated cytotoxicity and stimulates interferon (IFN)-γ production and proliferation of NK cells and T cells (5, 27, 34). IL-12 also plays an important role in regulating T helper (Th) type 1 (Th1) cell-dependent immune responses by priming T cells for high IFN-γ production and inducing them to differentiate toward the Th1 subset (18, 20). However, only the p70 heterodimer of IL-12 has full biological activity, and coexpression of both the p40 and p35 subunits in the same cell is required for production of the biologically active p70 heterodimer. It has also been shown that the p40 subunit of IL-12 can antagonize the biological activity of the p70 heterodimer via competition for its receptor (4, 14). Huaux et al. (12) demonstrated that production of the p40 subunit and Th2 polarization may play important roles in the fibrotic responses in silica-induced pulmonary fibrosis in mice.

IL-12 and IL-4 are the key cytokines mediating the development of Th1 and Th2 cells, respectively. IL-4 participates in the development of fibrosis by inducing fibroblast proliferation and increased production of extracellular collagen. The IL-4-transgenic mouse is susceptible to bleomycin-induced pulmonary fibrosis (28). It has been shown that Th2 cytokines such as IL-4, IL-5, and IL-10 are predominantly present, and IFN-γ is decreased in idiopathic pulmonary fibrosis (IPF) (36). However, another study (35) has shown that not only IL-5 but also IL-2 and IFN-γ are significantly increased in IPF (35). Sharma et al. (29) demonstrated that CD3ζ+ lymphocytes and the production of Th1 lymphokines, IFN-γ, and IL-2, are increased in lung and lymph nodes after intratracheal instillation of bleomycin in mice. It also has been demonstrated that irradiation- or bleomycin-induced pulmonary fibrosis is

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associated with an overproduction of Th2 cytokines such as IL-4 (2) and IL-5 (7). The roles of Th1 and Th2 cytokines on the pathophysiology of pulmonary fibrosis in human disease and animal models remain to be determined.

Hagimoto et al. and Kuwano et al. previously demonstrated that Fas expression on bronchiolar and alveolar epithelial cells and the upregulation of Fas ligand (FasL) expression in infiltrating lymphocytes were present in bleomycin-induced pulmonary fibrosis in mice (9) and in IPF (16). Kuwano et al. (15) also demonstrated that the administration of a soluble form of Fas and anti-FasL antibody prevented apoptosis of epithelial cells and, subsequently, the development of fibrosis and found that Fas- or FasL-deficient mice were resistant to the induction of bleomycin-induced pneumopathy. These results suggest that apoptosis induced by the Fas-FasL pathway is essential in the pathophysiology of bleomycin-induced pneumopathy in mice.

The Fas-FasL pathway is a cytotoxic mechanism used by CD4+ T cells, CD8+ cytotoxic T lymphocytes, and NK cells. IL-12 potentiates T cell- and NK cell-mediated cytotoxicity by the efficient activation of these cells. IL-12 also induces the Th1 immune response, and Th1 cells mediate the cellular immune responses. Because the cytotoxicity mediated by FasL-expressing lymphocytes seems to be involved in bleomycin-induced pneumopathy in mice, we examined whether the anti-IL-12 antibody prevents cell-mediated immune responses and the cytotoxic effect of the Fas-FasL pathway. Furthermore, we also examined the effects of the anti-IL-12 antibody on the Th1 and Th2 cytokine expression and fibrotic response in lung tissue.

MATERIALS AND METHODS

Model of bleomycin-induced pulmonary fibrosis in mice. Five-week-old male C57BL/6 mice were purchased from the Laboratory for Animal Experiments (Kyushu University, Fukuoka, Japan). After measurement of their body weight, the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (Dinabot, Osaka, Japan). The anesthetized animals received 50 μl of a bleomycin hydrochloride (Nippon Kayaku, Tokyo, Japan) solution containing 1.0 U bleomycin/kg body wt dissolved in sterile saline intra-
One or two hundred micrograms per animal of sheep anti-mouse IL-12 monoclonal antibody, provided by Genetics Institute (Cambridge, MA), or 100 μg/animal of nonspecific sheep IgG (ICN Pharmaceuticals, Aurora, OH) were injected intravenously immediately after bleomycin instillation and 7 days after instillation. The anti-IL-12 antibody used in this research was generated by immunizing sheep with intact p70 murine IL-12 and binds to the p40 subunit of IL-12 as well as to the intact p70 heterodimer but not to the p35 subunit according to the manufacturer’s instructions. After treatment, the mice were returned to their cages and allowed food and water ad libitum. The mice were anesthetized either 7 or 14 days after bleomycin instillation and then killed. Samples of the right lung were excised to be processed for light microscopy. Samples of the left lung tissue were frozen in liquid nitrogen for the measurement of hydroxyproline content.

**Cell differential counts in bronchoalveolar lavage fluid.** A tracheotomy was performed in killed mice. After insertion of a tracheal tube, the trachea was lavaged five times with 1 ml of sterile saline at room temperature. The recovered fluid was filtered through a single layer of gauze to remove mucus. The cells in the bronchoalveolar lavage (BAL) fluid (BALF) were counted with a hemocytometer. Differential counts of BALF cells were performed on 200 cells stained with Diff-Quik (Baxter Diagnostics, Düdingen, Switzerland).

**Cytokine measurements in BALF and lung tissue.** IL-12p40 subunit levels in BALF and lung tissue were measured with a cytokine-specific ELISA obtained from Genzyme (Minneapolis, MN), and IL-12p70 heterodimer, IFN-γ, and IL-4 levels were also measured with ELISAs obtained from BioSource International (Camarillo, CA). For this assay, BAL was performed by infusing the lungs with 1 ml of sterile saline. The BALF was centrifuged, and the supernatant was stored at −280°C until used for ELISA. After BAL, a right lung sample was obtained and homogenized in PBS at 4°C. The homogenate was centrifuged at 3,000 rpm for 10 min, and the supernatant was stored at −80°C until used for ELISA. The minimum detectable dose of IL-12p40 and IL-12p70 was <4 pg/ml, and those of IFN-γ and IL-4 were <1 and <5 pg/ml, respectively.

**Histopathology of lung tissue.** After a thoracotomy, the pulmonary circulation was flushed with saline and the lungs were explored. The lung samples were fixed in 10% formalin overnight and embedded in paraffin. A 5-μm paraffin section was adhered to slides and stained with hematoxylin and
The pathological grade of inflammation and fibrosis in the whole area of the midsagittal section was evaluated under ×40 magnification. A pathological grade was determined according to the following criteria: 0, no lung abnormality; 1, presence of inflammation and fibrosis involving <25% of the lung parenchyma; 2, lesions involving 25–50% of the lung; and 3, lesions involving >50% of the lung.

Analysis of apoptosis. Apoptosis was detected by the terminal deoxynucleotidyltransferase dUTP nick end labeling (TUNEL) method with a commercially available kit (Takara Biomedicals, Kusatsu, Japan). After proteinase digestion and removal of endogenous peroxidase, the sections were incubated in a mixture containing terminal deoxynucleotidyltransferase and fluorescein isothiocyanate-labeled dUTP. The sections were then treated with peroxidase labeled with anti-fluorescein isothiocyanate antibody. The reaction products were developed with 3,3′-diaminobenzidine tetrahydrochloride and counterstained with methyl green. The number of cells positive for TUNEL in the whole area of the section was counted under a microscope at ×250 magnification.

Hydroxyproline assay. Samples of the lung tissue were frozen in liquid nitrogen, lyophilized with a freeze-dry system (LABCONCO, Kansas City, MO), weighed, and minced into a
immunohistochemistry for IL-12p40 and FasL. After deparaffinization, immunohistochemistry was performed with a modified streptavidin-biotinylated peroxidase technique with a Histofine SAB-PO kit from Nichirei. Nonspecific protein staining was blocked with rabbit serum or goat serum for 30 min at room temperature. The sections were then incubated with goat or mouse IgG replaced the specific antibody. The slides were washed, incubated with a streptavidin-biotinylated anti-rabbit, anti-goat, or anti-mouse IgG for 30 min, washed, and treated with 0.3% hydrogen peroxide in methanol for 30 min to inhibit the activity of any endogenous peroxide.

RNA preparation and analysis. Total RNA was prepared from lung tissue by an ISOGEN RNA extraction kit (Nippon Gene, Tokyo, Japan). The primers and probes used were trypolipidesterase, and 1.25 U of Taq polymerase (Takara Bio, Shiga, Japan). For the PCR analysis of RNA, cDNA was prepared by RT of 2 μl of each RNA sample in a 20-μl reaction volume containing 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 2 μM MgCl2, 0.1% Triton X-100, 1 mM dithiothreitol, 0.25 mM deoxynucleotide triphosphates, 5 μM random hexamer primers, 0.1 U/μl of ribonuclease inhibitor (Promega, Madison, WI), and 10 U/μl of Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Life Technologies). The reaction mixture was incubated at 42°C for 1 h and at 95°C for 5 min. The cDNAs were then diluted to 100 μl, and these cDNAs were used in all PCRs. The PCR amplifications were performed in a 50-μl reaction volume containing 5 μl of each cDNA, 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 1 mM dithiothreitol, 0.25 mM deoxynucleotide triphosphates, 5 μM random hexamer primers, and 0.1 U/μl of ribonuclease inhibitor (Promega, Madison, WI). The PCR amplifications were performed in a 50-μl reaction volume containing 5 μl of each cDNA, 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 1 mM dithiothreitol, 0.25 mM deoxynucleotide triphosphates, 5 μM random hexamer primers, and 0.1 U/μl of ribonuclease inhibitor (Promega, Madison, WI). The PCR amplifications were performed in a 50-μl reaction volume containing 5 μl of each cDNA, 10 mM Tris- HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 1 mM dithiothreitol, 0.25 mM deoxynucleotide triphosphates, 5 μM random hexamer primers, and 0.1 U/μl of ribonuclease inhibitor (Promega, Madison, WI).

The hybridization was performed according to the manufacturer’s directions. For control incubations, nonimmune goat or mouse IgG replaced the specific antibody.

RNA preparation and analysis. Total RNA was prepared from lung tissue by an ISOGEN RNA extraction kit (Nippon Gene, Tokyo, Japan). The primers and probes used were as follows: sense, 5′-TCTCGTGGCATTCATGAAACT-3′, antisense, 5′-CTTCTGGAACCCACGTCGTA-3′, and probe, 5′-GAGTCTTCTGGCCTC-3′, for β-actin; sense, 5′-TCTCGTGGCATTCATGAAACT-3′, antisense, 5′-TTTCTGGAACCCACGTCGTA-3′, and probe, 5′-GAGTCTTCTGGCCTC-3′, for β-actin; sense, 5′-GAGTCTTCTGGCCTC-3′, antisense, 5′-TTTCTGGAACCCACGTCGTA-3′, and probe, 5′-GAGTCTTCTGGCCTC-3′, for β-actin; sense, 5′-GAGTCTTCTGGCCTC-3′, antisense, 5′-TTTCTGGAACCCACGTCGTA-3′, and probe, 5′-GAGTCTTCTGGCCTC-3′, for β-actin. The hybridization was performed according to the manufacturer’s directions. The digoxigenin-labeled probe, which hybridized with the PCR products, was detected with the DIG nucleic acid detection kit (Roche Diagnostics, Tokyo, Japan). The hybridization was performed according to the manufacturer’s directions. The digoxigenin-labeled probe, which hybridized with the PCR products, was detected with the DIG nucleic acid detection kit (Roche Diagnostics, Tokyo, Japan). The hybridization was performed according to the manufacturer’s directions. The digoxigenin-labeled probe, which hybridized with the PCR products, was detected with the DIG nucleic acid detection kit (Roche Diagnostics, Tokyo, Japan). The hybridization was performed according to the manufacturer’s directions.
sity of RT-PCR products, ANOVA followed by Scheffe’s F-test was used. For comparison of pathological grade, Kruskal-Wallis test was used. P values of <0.05 were considered significant. Statistical analysis was performed with StatView J-4.5 (Abacus Concepts, Berkeley, CA).

RESULTS

Effect of anti-IL-12 antibody on BALF cell analysis. The number of total cells, macrophages, neutrophils, and lymphocytes in BALF at 7 and 14 days was significantly decreased in 100 or 200 μg/animal of anti-IL-12 antibody-treated mice compared with that in control IgG-treated mice after bleomycin instillation except for the number of neutrophils at 14 days in 100 or 200 μg/animal and lymphocytes at 14 days in 100 μg/animal of anti-IL-12 antibody-treated mice (Fig. 1). Because eosinophils were not found in BALF from saline-treated mice and were not increased after bleomycin instillation, the number of eosinophils in BALF is not presented in Fig. 1.

Effect of anti-IL-12 antibody on histological findings in lung tissue. The alveolar wall was beginning to thicken, with infiltration of neutrophils and lymphocytes 7 days after bleomycin instillation (Fig. 2b), compared with that in untreated mice (Fig. 2a). To identify the cell type of inflammatory cells, we observed hematoxylin and eosin-stained tissue under light microscopy with magnifications of ×400 to ×1,000. After 14 days, a large number of lymphocytes infiltrating into the lung interstitium, thickening of the alveolar septa, collapse of alveolar spaces, and proliferation of fibroblasts were observed (Fig. 2c). On the other hand, there were only minimal changes in

Effect of anti-IL-12 antibody on the results of TUNEL. Although the type of apoptotic cells was not clearly identified, some bronchiolar and alveolar epithelial cells or inflammatory cells in inflammatory lesions showed evidence of apoptosis as estimated by the TUNEL method 7 and 14 days after bleomycin instillation (Fig. 2f and g, respectively) but not in untreated mice (Fig. 2e). The signals positive for TUNEL at 14 days were abrogated in lung tissue of mice treated with 200 μg/animal of anti-IL-12 antibody (Fig. 2h). Figure 4 shows that the number of signals positive for TUNEL in lung tissue at 7 days was significantly decreased in lung tissue of mice treated with 200 μg/animal of anti-IL-12 antibody compared with that in mice treated with control IgG after bleomycin instillation. The number of signals positive for TUNEL at 14 days was significantly decreased in mice treated with 100 or 200 μg/animal of anti-IL-12 antibody compared with that of mice treated with control IgG after bleomycin instillation.

Effect of anti-IL-12 antibody on lung hydroxyproline content. Figure 5 shows the effect of anti-IL-12 antibody on hydroxyproline content in lung tissue, which represents the extent of fibrosis, 14 days after bleomycin instillation. There was a significant decrease in
lung hydroxyproline content of mice treated with 200 but not with 100 μg/animal of anti-IL-12 antibody compared with that in mice treated with control IgG after bleomycin instillation.

RT-PCR analysis. Because Th1 development may be mediated by IL-12, we determined whether the neutralization of IL-12 would result in a change in the Th1 and Th2 responses by examining the expression of cytokine mRNA. Figure 6 shows the representative results of RT-PCR, and Fig. 7 shows a summary of the results of RT-PCR. Although the expression of Fas mRNA was not changed, the expression of IL-12p40, IL-12Rβ2, IFN-γ, FasL, and TNF-α mRNAs was significantly increased on both days 7 and 14 in mice administered control IgG after bleomycin instillation compared with that in saline-instilled mice. The expression of IL-12Rβ2 and FasL mRNAs was significantly decreased on days 7 and 14 in the lung tissue of mice treated with 200 μg/animal of anti-IL-12 antibody compared with that in mice treated with control IgG after bleomycin instillation. The expression of TNF-α mRNA on day 14 after bleomycin instillation was significantly decreased in the lung tissue of mice treated with 200 μg/animal of anti-IL-12 antibody compared with that in mice treated with control IgG after bleomycin instillation. The expression of IFN-γ mRNA appeared to be decreased by the administration of anti-IL-12 antibody after bleomycin instillation, but it was not significant.

Cytokine measurement in BALF and lung tissue. Figure 8 shows the results of cytokine measurement in BALF and lung tissue. The levels of IL-12p40 in BALF and lung tissue were significantly increased in mice on days 7 and 14 after bleomycin instillation compared with those in saline-instilled mice. The administration of anti-IL-12 antibody did not significantly decrease these levels. The IL-12p70 level in lung tissue of anti-IL-12 antibody-treated mice was decreased compared with that in control IgG-treated mice on day 7 after bleomycin instillation, which was also less than that in saline-instilled mice. The levels of IL-12p70 on day 14 in BALF and lung tissue were not changed in mice administered either control IgG or anti-IL-12 antibody after bleomycin instillation compared with those in saline-instilled mice. The levels of IFN-γ and IL-4 in BALF were very low or not detectable. IFN-γ levels in lung tissue were significantly decreased on days 7 and 14 after bleomycin instillation compared with those in saline-instilled mice. The administration of anti-IL-12 antibody did not affect the levels of IFN-γ in lung tissue. IL-4 levels in lung tissue were not significantly changed after bleomycin instillation, except those on day 7 with anti-IL-12 antibody treatment after bleo-
mycin instillation compared with those in saline-instilled mice.

Immunochemistry for IL-12p40 and FasL protein. Positive signals for IL-12p40 were localized in the cytoplasm of macrophages in lung tissue 14 days after bleomycin instillation (Fig. 9a), whereas positive signals were weakly expressed in anti-IL-12 antibody-treated mice (Fig. 9b). Positive signals for FasL were detected mainly in infiltrating mononuclear cells after bleomycin instillation (Fig. 9c), whereas positive signals were weakly expressed in anti-IL-12 antibody-treated mice (Fig. 9d). No positive signal for IL-12p40 or FasL was detected in lung tissue from untreated mice (data not shown).

DISCUSSION

Bleomycin-induced pneumopathy is frequently used as a model of pulmonary fibrosis. The sequestration of T lymphocytes to the lung tissue after bleomycin administration is well demonstrated, and the role of T lymphocytes in this model is studied extensively. The importance of T cells in the pathophysiology of this model has been demonstrated (23, 26, 32). However, conflicting studies (26, 30) with athymic nude mice have also been reported. Zhu et al. (39) and Helene et al. (11) have demonstrated that pulmonary fibrosis can be induced in severe combined immunodeficient mice, suggesting that lymphocytes do not appear to be required for induction of this model. They also suggest that cells other than T cells, especially NK cells and alveolar macrophages, may play important roles in the development of this model. Sharma et al. (29) have demonstrated that eliminating NK1.1⁺ cells prevents bleomycin-induced pneumopathy, although it is not as effective as anti-CD3⁺ antibody. Therefore, NK cells may play some role, cooperating with T lymphocytes in bleomycin-induced pneumopathy. The results of these conflicting studies appear to show that the macrophages, T lymphocytes, and NK cells cooperate with each other in the development of this model, although the dependence on T cells is variable according to the mouse strains used in experiment (39). The administration of anti-IL-12 antibody may prevent the development of this model by inhibiting the activation and proliferation of both T cells and NK cells.

The administration of anti-IL-12 antibody reduced the number of macrophages, lymphocytes, and neutrophils in BALF and subsequently decreased the number of apoptotic cells, the pathological grade, and hydroxyproline content in lung tissue. It also suppressed upregulation of FasL mRNA in lung tissue of bleomycin-treated mice. Hagimoto et al. (9) previously demonstrated that FasL mRNA was expressed in infiltrating inflammatory cells, and FasL protein was expressed in these cells in this study. Because epithelial cell apoptosis induced by the Fas-FasL pathway seems to be essential in the model described by Kuwano et al. (15), these results may indicate that anti-IL-12 antibody downregulated the proliferation and activation of FasL-expressing cells and suppressed epithelial cell damage, apoptosis, and the development of this model. The requirement of IL-12 has also been reported in autoimmune diabetes in nonobese diabetic (NOD) mice (13). Inactivation of macrophages in NOD mice results in the prevention of diabetes along with a decrease in Th1 cells, an increase in Th2 cells, reduced expression of IL-12, and significant decrease of FasL (13). Furthermore, the administration of IL-12 reverses the prevention of diabetes in NOD mice (13). In addition, anti-IL-12 antibody prevents experimental autoimmune encephalomyelitis (17), experimental colitis (21), experimental autoimmune uveoretinitis (38), and collagen-induced arthritis (19) through inhibition of the development of pathogenic Th1 cells. Therefore, IL-12 may play an essential role in the development and activation of cytokotoxic Th1 cells that cause tissue-specific destruction in Th1 cell-mediated autoimmune diseases.

The relative Th1 and Th2 contribution to the development of bleomycin-induced pulmonary fibrosis has not clearly been defined. Although the production of IFN-γ and IL-2 is increased in lung and lymph nodes after intratracheal instillation of bleomycin in mice (29), it has also been demonstrated that irradiation- or bleomycin-induced pulmonary fibrosis is associated with an overproduction of Th2 cytokines (2, 7). We observed a significant increase in the IL-12p40 subunit and a decrease in the IL-12p70 heterodimer and IFN-γ on days 7 and 14 in lung tissue after bleomycin instillation. The IL-12p40 subunit antagonizes the biological
activity of IL-12p70 via competition for its receptor and suppresses Th1-mediated immune responses (5). Because pulmonary fibrosis progresses during this period, it is likely that Th2 polarization is dominant, at least in the fibrotic stage of this model. Therefore, one of the antifibrotic effects of anti-IL-12 antibody may be the inhibitory effect against the IL-12p40 subunit, although this antibody can bind to IL-12p70 and IL-12p40.

The IL-12Rβ2 subunit is preferentially expressed in Th1 cells, whereas the IL-12Rβ1 subunit is expressed in both Th1 and Th2 cells (25). We demonstrated that IL-12Rβ2 subunit mRNA was significantly increased after bleomycin instillation and that it was downregulated by administration of anti-IL-12 antibody. IFN-γ mRNA was significantly increased after bleomycin instillation and tended to be decreased by administration of the anti-IL-12 antibody. However, the expression of IFN-γ protein was not changed by the administration of anti-IL-12 antibody as assessed by ELISA. The transcriptional and translational processes in IFN-γ expression could be distinctly regulated.

Impaired IFN-γ release by pulmonary lymphocytes in patients with IPF has been reported (24). It has also been reported (40) that IFN-γ treatment was associated with improvements in patients with IPF. The therapeutic strategies of IFN-γ in these studies are based on antifibrotic effects of IFN-γ. IFN-γ inhibits the proliferation of lung fibroblasts and reduces the production of collagen in vitro (1), and exogenous IFN-γ ameliorates the bleomycin-induced pulmonary fibrosis in mice, probably through the downregulation of transforming growth factor-β (8). In this study, IFN-γ protein level was not affected by anti-IL-12 antibody. Anti-IL-12 antibody administration, which prevents the effects of IL-12p40 subunit, resulting in inhibiting Th2 polarization, may be a novel therapy against pulmonary fibrosis.

There are several studies (6, 33) showing the exacerbating effects of exogenous administration of IL-12 on Th1-mediated autoimmunity. However, Tarrant et al. (31) demonstrated that exogenous administration of IL-12 protects against a Th1-mediated autoimmune disease, experimental autoimmune uveitis. The early IL-12 administration elevates IFN-γ in the serum, and it strongly inhibits antigen-specific IFN-γ production, probably through inducing apoptosis of lymphocytes, indicating a suppressed Th1 response (31). Whether IL-12 administration protects or exacerbates the development of bleomycin-induced pneumonitis should be investigated in the future.

TNF-α has also been demonstrated to play a key role in bleomycin-induced pneumopathy in mice (23), in which depletion of lymphocytes by anti-CD4+ and anti-CD8+ antibody inhibits the induction of TNF-α and the development of fibrosis. TNF-α is produced by activated NK cells, T cells, and macrophages. In this study, TNF-α mRNA on day 14 in lung tissue was downregulated by the administration of anti-IL-12 antibody. One of the protective effects of anti-IL-12 antibody in this model may be through the downregulation of TNF-α mRNA expression.

In conclusion, although various factors such as macrophages and inflammatory cytokines are involved in bleomycin-induced pneumopathy in mice, IL-12 seems to be one primary contributor to the creation of cell-mediated immune responses, lung injury and apoptosis, and the development of pulmonary fibrosis. One of the beneficial effects of anti-IL-12 antibody in this model is likely to protect epithelial cells from activated T cell- and NK cell-mediated cytotoxicity because IL-12Rβ2 mRNA was increased after bleomycin instillation. Because the Th2 response mediated by IL-12p40 is predominant in fibrotic processes in this model, another one is likely to inhibit the fibrotic process mediated by Th2 polarization. Anti-IL-12 antibody could be a novel strategy against lung injury and pulmonary fibrosis. However, it will be important to investigate which cytokine, Th1 or Th2, is predominant before treatment because it appears variable from patient to patient or from stage to stage of the disease.

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REFERENCES


