Role of IP-10/CXCL10 in the progression of pancreatitis-like injury in mice after murine retroviral infection

Yusuke Kawauchi,1 Kenji Suzuki,1 Shiro Watanabe, Satoshi Yamagiwa,1 Hiroyuki Yoneyama,2 Gi Dong Han,3,6 Suresh S. Palaniyandi,4 Punniyakoti T. Veeraveedu,4 Kenichi Watanabe,4 Hiroshi Kawachi,3 Yoshiaki Okada,5 Fujio Shimizu,3 Hitoshi Asakura,1 Yutaka Aoyagi,1 and Shosaku Narumi2

Departments of 1Gastroenterology and Hepatology and 2Cell Biology, Institute of Nephrology, Niigata University Graduate School of Medical and Dental Sciences, Niigata; 3Department of Bacterial and Blood Products, National Institute of Infectious Diseases, Tokyo; 2Department of Molecular Preventive Medicine, University of Tokyo Graduate School of Medicine, Tokyo; 4Department of Clinical Pharmacology, Niigata University of Pharmacy and Applied Life Sciences, Niigata, Japan; and 6Department of Food Science and Technology, Yeungnam University, Gyeongsan, Republic of Korea

Submitted 4 January 2006; accepted in final form 1 April 2006

Kawauchi, Yusuke, Kenji Suzuki, Shiro Watanabe, Satoshi Yamagiwa, Hiroyuki Yoneyama, Gi Dong Han, Suresh S. Palaniyandi, Punniyakoti T. Veeraveedu, Kenichi Watanabe, Hiroshi Kawachi, Yoshiaki Okada, Fujio Shimizu, Hitoshi Asakura, Yutaka Aoyagi, and Shosaku Narumi. Role of IP-10/CXCL10 in the progression of pancreatitis-like injury in mice after murine retroviral infection. Am J Physiol Gastrointest Liver Physiol 291: G345–G354, 2006; doi:10.1152/ajpgi.00002.2006.—Exocrinopathy and pancreatitis-like injury were developed in C57BL/6 (B6) mice infected with LP-BM5 murine leukemia virus, which is known to induce murine acquired immunodeficiency syndrome (MAIDS). The role of chemokines, especially CXCL10/interferon (IFN)-γ-inducible protein 10 (IP-10), a chemokine to attract CXCR3+ T helper 1-type CD4+ T cells, has not been investigated thoroughly in the pathogenesis of pancreatitis. B6 mice were inoculated intraperitoneally with LP-BM5 and then injected every week with either an antibody against IP-10 or a control antibody. Eight weeks after infection, we analyzed the effect of IP-10 neutralization. Anti-IP-10 antibody treatment did not change the generalized lymphadenopathy and hepatosplenomegaly of mice with MAIDS. The treatment significantly reduced the number of IP-10- and CXCR3-positive cells in the mesenteric lymph nodes (mLN) but not the phenotypes and gross numbers of cells. In contrast, IP-10 neutralization reduced the number of mononuclear cells infiltrating into the pancreas. Anti-IP-10 antibody treatment did not change the numbers of IFN-γ+ and IL-10+ cells in the mLN but significantly reduced their numbers, especially IFN-γ− and IL-10− CD4+ T cells and IFN-γ−Mac-1+ cells, in the pancreas. IP-10 neutralization ameliorated the pancreatic lesions of mice with MAIDS probably by blocking the cellular infiltration of CD4+ T cells and IFN-γ−Mac-1+ cells into the pancreas at least 8 wk after infection, suggesting that IP-10 and these cells might play a key role in the development of chronic autoimmune pancreatitis.

autoimmune pancreatitis; Sjögren’s syndrome; murine acquired immunodeficiency syndrome; chemokines; interferon-γ-inducible protein 10

CHEMOKINES, which are chemotactic cytokines, control the essential process of the attraction of leukocytes to the tissues in inflammation (1, 18). The chemokine family comprises two major subfamilies, termed CXC and CC according to the arrangement of the first two conserved cysteines, which are separated by one amino acid and are adjacent, respectively (1, 18). Interferon (IFN)-γ-inducible protein of 10 kDa (IP-10/CXCL10) is a member of the CXC chemokine family and a potent chemoattractant for activated T lymphocytes, natural killer cells, and monocytes (6, 17). It is also considered as a regulator of T helper (Th)1 inflammatory responses (26). The expression of IP-10 was elevated in several diseases such as ulcerative colitis (34), hepatitis (21), multiple sclerosis (31), and Sjögren’s syndrome (SjS) (22), suggesting the involvement of IP-10 in the development of these diseases. It has been recently reported that IP-10 is expressed by β-cells of the islets of Langerhans, resulting in the preferential accumulation of CXCR3+ T cells into the pancreas in a virus-induced Type I diabetic mouse model (7). Information about the role of chemokines in pancreatic diseases, however, is limited and needs further investigation (2, 28). In the pathogenesis of chronic pancreatitis, especially with autoimmune etiology, the role of chemokines such as IP-10 has not been investigated thoroughly.

The LP-BM5 murine leukemia virus (MuLV) is a retrovirus that is known to induce profound immunodeficiency with splenomegaly and generalized lymphadenopathy in susceptible strains of mice, such as C57BL/6 (B6) mice, and occasionally brings about lymphoid malignancy (10, 14, 19). In the early phase of infection, hypergammaglobulinemia and polyclonal B and T cell activation are induced and autoantibodies such as anti-nuclear and anti-double-stranded DNA antibodies are detected in mice infected with the virus (10, 14, 19). In the late phase of infection, virus-infected B6 mice show symptoms similar to those of human acquired immunodeficiency syndrome (AIDS); therefore, they have been studied as a murine model of AIDs, termed as murine AIDS (MAIDS) (10, 14, 19). We have reported previously that systemic exocrinopathy resembling SjS was induced in systemic exocrine glands such as salivary glands and lachrymal glands and in the pancreas of virus-infected mice; thus we proposed that mice with MAIDS could be an animal model for SjS as well as AIDS (32, 33). In mice with MAIDS, the pancreatic lesions are the exocrine system-oriented inflammation characterized by cellular inflamma-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tration around the interlobular pancreatic ducts and acinar cell destruction (32, 33, 35), but with no damage of the endocrine system, that is, the islets of Langerhans (35). The pancreas-infiltrating cells comprise both Th1- and Th2-type CD4+ T cells, although with a predominance of Th2 cells over Th1 cells (35). Thus the pancreatic lesions of the mice have some similarities to autoimmune-related chronic pancreatitis, especially the lesions associated with SjS.

To clarify the role of IP-10 in the development of chronic pancreatitis with autoimmune etiology, especially associated with SjS, we investigated the effect of CXCL10 neutralization on pancreatic lesions of mice with MAIDS. Our results suggest that anti-IP-10 monoclonal antibody ameliorated the pancreatic lesions of mice with MAIDS.

MATERIALS AND METHODS

Animals. Four-week-old female B6 mice were purchased from Charles River Japan (Kanagawa, Japan) and maintained at the Animal Center of the Niigata University School of Medicine under specific-pathogen-free conditions. All animal experiments were performed according to the “Guide for Animal Experiments” of Niigata University School of Medicine.

Induction of MAIDS. LP-BM5 MuLV was prepared from the supernatant of cloned G6 cells infected with the retrovirus as reported previously (35). Four-week-old B6 female mice were injected intra-peritoneally with 0.3 ml of LP-BM5 MuLV virus stock solution. At 8 wk after virus inoculation, mice with MAIDS were killed by cervical dislocation under ether anesthesia, and their pancreases were removed for further analysis. For blocking experiments, PBS containing 100 μg/100 μl anti-CXCL10 monoclonal antibodies (36) or anti-human parathyroid-related peptide monoclonal antibodies, which was the IgG1 subclass-matched control monoclonal antibody, or PBS alone were administered intraperitoneally at the time of virus inoculation and once a week thereafter.

Monoclonal antibodies. For immunofluorescence (IF) and flow cytometric analyses, the following monoclonal antibodies were used: anti-CD4 (clone GK1.5, IgG2b), anti-CD8 (clone 53-6.7, IgG 2a), anti-B220 (clone RA3-6B2, IgG 2a), anti-Mac-1 (clone M-70.15, IgG2b), anti-mouse INF-γ (clone XMG1.2), and anti-mouse IL-10 (clone JES5-16E3). For immunostaining of IP-10 or CXCR3, goat polyclonal antibodies to IP-10 or CXCR3 (Santa Cruz Biotechnology; Santa Cruz, CA) were used.

Detection of LP-BM5 MuLV by PCR. The PCR method used for the detection of the virus was as reported previously (33).

Quantitative RT-PCR to detect cytokine mRNA. Total RNA was extracted from the mesenteric lymph node (mLN) and pancreas specimens with TRIzol (GIBCO-BRL) according to the standard protocol and reverse transcribed. Thereafter, cDNA was amplified using the ABI 7700 sequence detector system (Applied Biosystems; Foster City, CA) with a set of primers and probes corresponding to IFN-γ, IL-10, IP-10, CXCR3, and GAPDH as previously described (36).

Histopathological examination. Tissue samples were taken from the pancreas, fixed in 10% buffered formalin, and then embedded in paraffin wax blocks. Sections (4-μm thick) were made in the usual way and stained with hematoxylin and eosin. The stained sections were then examined by light microscopy.

The numbers of inflammatory cells in a high-power field (×400) were counted under a microscope, and the degree of pancreatitis was assessed from 0 to 4 as reported previously (11, 24).

IF staining procedure. Frozen sections of the pancreas were prepared in a cryostat and stained with several fluorescent dye-conjugated anti-mouse antibodies as described above. The sections were observed by fluorescence microscopy.

Double-IF staining procedure. For the simultaneous demonstration of cell surface antigens and cytokines, the IF staining method was as reported previously (35).

RESULTS

IP-10 neutralization did not prevent infection by the MAIDS virus. All mice infected with LP-BM5 MuLV developed characteristic MAIDS symptoms such as generalized lymphadenopathy and hepatosplenomegaly (n = 15), and neutralization of IP-10 did not change the course of MAIDS (n = 15). Eight weeks after the virus inoculation, there were no differences in the weights of the liver, spleen, and mLNs between mice with MAIDS injected with anti-IP-10 monoclonal antibody and those injected with control antibody (Fig. 1A). A defective LP-BM5 virus genome was detected in mice of both groups by

![Fig. 1. Effect of neutralization of interferon (IFN)-γ-inducible protein of 10 kDa (IP-10/CXCL10) on the course of murine acquired immunodeficiency syndrome (MAIDS). A: effect of neutralization of IP-10 on organs of mice with MAIDS. The weights of the liver, spleen, and mesenteric lymph nodes (LNs) of mice with MAIDS injected with control antibody (Ab) were increased at 8 wk after infection. However, they were not changed after the injection of anti-IP-10 monoclonal (m)Ab. Data are means ± SD. NS, not significant. B: neutralization of IP-10 did not prevent infection by the MAIDS virus. LP-BM5 murine leukemia virus (MuLV) was detected in the mesenteric LNs and pancreases of mice with MAIDS at 8 wk after infection. Template DNAs were extracted from frozen sections of the pancreas and then analyzed by PCR with P12 primer. The bands were obtained by running the PCR products in agarose gel. M, molecular size marker; lane 1, the mesenteric LN of an uninfected C57BL/6 (B6) mouse (negative control); lane 2, the pancreas of an infected B6 male (negative control); lane 3, the mesenteric LN of a mouse with MAIDS injected with control Ab; lane 4, the pancreas of a mouse with MAIDS injected with control Ab; lane 5, the mesenteric LN of a mouse with MAIDS injected with anti-IP-10 mAb; lane 6, the pancreas of a mouse with MAIDS injected with anti-IP-10 mAb.](http://ajpgi.physiology.org/doi/10.1152/ajpgi.05306.2004/supp)}
PCR in the same frozen sections of the mLNs and pancreas of mice with MAIDS as those used for immunohistochemical staining (Fig. 1B). P12 of the virus genome was not detected in untreated normal B6 mice (Fig. 1B).

Effect of IP-10 neutralization on the expression of IP-10 and CXCR3 by lymphoid cells in mice with MAIDS. In the mLN and pancreas of mice with MAIDS injected with control antibody, the expression levels of mRNA for IP-10 and its receptor CXCR3 were significantly increased compared with untreated B6 mice (Fig. 2A). Neutralization of IP-10 down-regulated the expression of mRNA for IP-10 and CXCR3 in the mLNs and pancreas of mice with MAIDS (Fig. 2, A and B). Next, we analyzed their expressions in the pancreas and mLN by IF. In normal mice, IP-10 and CXCR3 were detected neither in the mLN nor in the pancreas, with the exception of a few CXCR3+ cells scattered in the mLN (data not shown). The numbers of cells that expressed IP-10 and CXCR3 increased in the mLN and pancreas of mice with MAIDS injected with control antibody (Fig. 3I, A and B, and II, A and B). Double-color IF revealed that in the mLN, some IP-10+ cells were Mac-1+ cells and CXCR3+ cells were mainly CD4+ T cells (Fig. 3I, C and D). In the pancreas of mice with MAIDS, IP-10 and CXCR3 were mainly detected on some cells in an inflammatory cell focus around the pancreatic duct (Fig. 3II, A and B). Double-color IF showed that IP-10+ cells were not positive for CD4, CD8, B220, and Mac-1 (Fig. 3II, C). It also revealed that CXCR3+ cells were mainly CD4+ T cells (Fig. 3III, D).

Interestingly, IP-10 was also detected on some cells localized between the basal laminas that envelope each acinus of a minimal functional unit of the exocrine system of the pancreas (Fig. 3II, inset). Neutralization of IP-10 decreased the numbers of cells that expressed IP-10 or CXCR3 in the mLN (Fig. 3I, E and F) and pancreas (Fig. 3II, E and F) of mice with MAIDS.

Effect of IP-10 neutralization on mLN cells of mice with MAIDS. IF study of the mLN revealed that the numbers of CD4+, CD8+, B220+, and Mac-1+ cells were unchanged statistically by neutralization of IP-10 (Fig. 4A).

To reveal the systemic effect of IP-10 neutralization on cytokine production, we analyzed the number of IFN-γ- and IL-10-positive cells in the mLN of mice with MAIDS at 8 wk after infection by IF. We chose IFN-γ as a representative for a proinflammatory cytokine (or Th1 response) and IL-10 as a representative of an anti-inflammatory cytokine (or Th2). In mLNs of mice with MAIDS, there was no significant difference between the anti-IP-10 monoclonal antibody-treated group and the control antibody-treated group in IL-10- and IFN-γ-positive cells (Fig. 4B). IP-10 neutralization did not change the number of these cytokine-positive cells in the mLN of mice with MAIDS (Fig. 4B).

IP-10 neutralization ameliorated pancreatic lesions of mice with MAIDS. We have previously reported that periductal mononuclear cellular infiltration resembling autoimmune pancreatitis associated with SjS was detected in mice with MAIDS.
and the numbers of infiltrating cells and the grades of the lesions reached a peak at 8 wk after infection. To evaluate the effect of IP-10 neutralization, we, therefore, analyzed the pancreatic lesions of mice with MAIDS at 8 wk after infection. In mice with MAIDS injected with control antibody, inflammatory cells were detected around the pancreatic ducts, from where they progressively expanded, pressing the acinar architecture outward (Fig. 5A). At the interface lesions between the infiltrating cells and pancreatic parenchyma, destructed acinar cells were detected, but the degree of acinar cell destruction by infiltrating cells was rather mild (Fig. 5C). Peri-islet cellular infiltration was also observed (Fig. 5, A and C). Anti-IP-10 monoclonal antibody treatment clearly ameliorated the pathological lesions of the pancreas of mice with MAIDS (Fig. 5, B and D); the size of periductal cellular infiltration became smaller and the numbers of destructed acinar cells were also decreased (Fig. 5, B and D). Both the numbers of pancreas-infiltrating cells and the histological grading scores of the pancreatitis were significantly reduced by IP-10 neutralization (Fig. 5, E and F).

**IP-10 neutralization ameliorated pancreatic lesions through decreased migration of CD4$^+$T, Mac-1$^+$, and B220$^+$ cells in mice with MAIDS.** Eight weeks after infection, pancreas-infiltrating cells were composed of CD4$^+$ and CD8$^+$ T cells, Mac-1$^+$ macrophages, and B220$^+$ B cells but not natural killer cells or granulocytes. The major populations of infiltrating cells were composed CD4$^+$ T cells, Mac-1$^+$ macrophages, and B220$^+$ cells (Fig. 6, A, C, E, and G). The IF study showed that IP-10 neutralization significantly decreased the numbers of CD4$^+$ T, Mac-1$^+$, and B220$^+$ cells in pancreatic lesions of mice with MAIDS (Fig. 6, B, D, F, and G).

**IP-10 neutralization decreased the migration of IFN-$\gamma$- and IL-10-producing CD4$^+$T and Mac-1$^+$ cells in the pancreas of mice with MAIDS.** To reveal the effect of IP-10 neutralization on the immune response in the pancreas of mice with MAIDS, we analyzed the cytokine expression of IFN-$\gamma$ and IL-10. The
progression of MAIDS symptoms. Therefore, exocrine pancreatitis-like injury of mice with MAIDS might be a manifestation of several characteristic symptoms of MAIDS such as hepatomegaly, splenomegaly, systemic lymphadenopathy, and abnormal immunological reactions. In this study, we have shown that IP-10 neutralization ameliorated the pancreatic lesions of mice with MAIDS (Fig. 5) but prevented neither the infection nor the course of MAIDS (Fig. 1). These results suggest a different mechanism for the pathogenesis of systemic exocrinopathy including exocrine pancreatitis-like injury of MAIDS and the other MAIDS symptoms.

In the Toxoplasma gondii infection model, IP-10 neutralization inhibited the accumulation of effector T cells, resulting in a decreased ability to kill the parasite in target organs such as the liver, spleen, brain, and lung (12). IP-10 neutralization studies and a study on IP-10−/− mice also showed that the blockade of effector cell trafficking resulted in the breakdown of host defenses in neurotropic mouse hepatitis virus infection in the brain (5, 16). In the liver of patients with chronic active hepatitis C, we (21) have reported that IP-10 mRNA was expressed mainly in hepatocytes around intralobular focal and periportal piecemeal necrosis. These examples suggest that if the organ of the specific lesions induced by a particular infection was the same as the target organ of the infectious agent, IP-10 neutralization could ameliorate the organ lesions by inhibiting the trafficking of effector cells that eliminate the infectious agents from the target organ. In MAIDS, we reported previously that the virus was integrated in Ly-1+ B cells but not in T cells (9) or on parenchymal cells of the pancreas (unpublished observations), although several reports have shown that B cells (13) and macrophages (4) as well as T cells (15) can serve as targets for infection of the virus. Therefore, pancreatic tissues are not the direct target cells of LP-BM5 infection, and the IP-10 neutralization-induced amelioration of pancreatic lesions of MAIDS cannot be explained by the decreased accumulation of effector cells that eliminate LP-BM5 from the pancreas.

We and others have reported that systemic exocrinopathy including pancreatitis-like injury is the characteristic organ lesions of mice with MAIDS (10, 14, 19, 32, 33, 35); however, the target antigen in exocrine glands, which regulates the target organ specificity, has been unknown. Without knowing the target antigen, delineating the process of inflammatory cell infiltration provides a basis for understanding the development of the pancreatic lesions of MAIDS. The blocking activity of the anti-IP-10 monoclonal antibody used in this study was confirmed in the chemotactic assay and in some other murine models depicting acute colitis (27) and encephalomyelitis (20). We confirmed that the anti-IP-10 monoclonal antibody had no cross-reactivity with other chemokines such as the monokine induced by IFN-γ, macrophage inflammatory protein-1α, or mature dendritic cells (36). In this study, therefore, we examined the expression of IP-10 and its receptor CXCR3 in the mLN and pancreas of mice with MAIDS. In the mLN and pancreas of MAIDS, IP-10 was mainly expressed on Mac-1+ cells and CXCR3 was mainly detected on CD4+ T cells. Anti-IP-10 treatment clearly reduced the number of these cells in both the mLN and pancreas. Our results suggest that cell trafficking into the pancreas of mice with MAIDS is carried out by the interaction between IP-10 and CXCR3 of CD4+ cells. In the virus-induced Type 1 diabetes mouse model, it has been

Fig. 4. Effect of neutralization of IP-10/CXCL10 on lymphocyte subpopulations in the LNs of mice with MAIDS. A: quantitative analysis of immune cells in the LNs by immunofluorescence (IF) for CD4, CD8, Mac-1, and B220. B: quantitative analysis of IFN-γ and IL-10 expression in the mesenteric LNs of mice with MAIDS at 8 wk after infection. Each focus of cellular infiltration was examined for the presence of CD4, CD8, B220, Mac-1, IFN-γ, and IL-10 cells. Final results are presented as the numbers of cells per high-powered microscope field. Representative findings (means ± SD; n = 10) from 3 independent experiments are shown.

levels of expression of the mRNAs of both cytokines in the pancreas were significantly increased after infection, and IP-10 neutralization decreased them (Fig. 7A). IF showed that IP-10 neutralization significantly decreased the numbers of IFN-γ- and IL-10-positive cells in the pancreas of mice with MAIDS (Fig. 7B).

Next, by the double-color IF method, we characterized the phenotypes of cells producing these cytokines. IFN-γ and IL-10 were mainly present on CD4+ T cells (Fig. 8, A and C) and Mac-1+ cells (Fig. 8, E and G) but not on B220+ cells or CD8+ T cells (data not shown). We did not detect the expression of IFN-γ or IL-10 in the pancreas of normal B6 mice (data not shown).

The numbers of IFN-γ- and IL-10-positive CD4+ T cells were significantly reduced in mice with MAIDS by IP-10 neutralization (Figs. 7C and 8, B and D). Additionally, IP-10 neutralization significantly reduced the numbers of IFN-γ-positive Mac-1+ cells in the pancreas of mice with MAIDS (Figs. 7D and 8F). The numbers of IL-10-positive Mac-1+ cells became smaller by IP-10 neutralization, but there was no statistical significance between the mice with or without treatment (Figs. 7D and 8, G and H).

DISCUSSION

We have shown that systemic exocrinopathy including exocrine pancreatitis-like injury developed concordantly with the...
shown that β-cells of the islets of Langerhans produce chemokines including IP-10 with preferential attraction to T cells via CXCR3 (7). In our colitis model and those of others, IP-10 expression was confirmed in the colon epithelial cells, and IP-10 neutralization was shown to ameliorate the colitis with decreased CXCR3<sup>+</sup> cells in the colon (27, 29). In addition, Sugai et al. (22) reported that IP-10 is expressed on duct epithelial cells in salivary glands of patients with SjS. Contrary to these reports, we could not detect IP-10 expression on acinar cells or duct epithelial cells but found that IP-10 and CXCR3 were mainly expressed on cells in inflammatory foci around pancreatic ducts. Thus the lack of expression of IP-10 on target acinar cells and duct epithelial cells might be attributed to the mild pancreatitis-like injury of mice with MAIDS. Interestingly, IP-10 was also detected on cells localized between the basal laminae that envelope each acinus of a minimal functional unit of the exocrine system of the pancreas (Fig. 3II, A, inset). These cells disappeared together with the decreased numbers of pancreas-infiltrating cells after the neutralization of IP-10 (Fig. 3II, A and C). Therefore, we need to identify the nature of the IP-10<sup>+</sup> cells localized between basal laminae around each acinus, which are supposed to play a pivotal role in the recruitment of CXCR3<sup>+</sup> inflammatory cells into the pancreas of mice with MAIDS.

Recently, a Th1 and Th2 imbalance has been considered as one of the important mechanisms in the development of some autoimmune diseases (25). In our previous studies on the experimental models of encephalomyelitis (20), hepatitis (36), and Thy1.1 glomerulonephritis (8), we reported that anti-IP-10 monoclonal antibody treatment did not affect the cytokine environment of Th1/Th2 polarization. Considering these previous reports together with the observations of this study, it is conceivable that the IP-10 neutralization-induced amelioration of the pancreatic lesions of MAIDS did not result from the rectification of the cytokine environment of Th1/Th2 imbalance but rather from blockade of trafficking of inflammatory cells into the pancreas.
In MAIDS, pancreas-infiltrating cells are mainly composed of CD4+ T cells, B220+B cells, and Mac-1+ macrophages (Fig. 6). Neutralization of IP-10 clearly and selectively decreased the numbers of these cells in the pancreas but not in the mLN of mice with MAIDS (Figs. 3–8). Flow cytometric analyses revealed the reciprocal quantitative change of cell phenotype between the mLN and pancreas. That is, IP-10 neutralization increased the numbers of CD3+, CD4+, and αβT cells as well as Mac-1+ cells in the mLN but decreased the numbers of those cells in the pancreas of mice with MAIDS (unpublished observations). These results suggest that IP-10 plays a pivotal role in the migration of inflammatory cells between the pancreas and mLN. IP-10-CXCR3 interactions, with Th1-dependent immunity, have been observed in several
inflammatory diseases, including multiple sclerosis (23, 30) and inflammatory bowel diseases (34). In animal models of these diseases, IP-10 neutralization or gene disruption of IP-10 clearly showed that amelioration of the diseases was achieved mainly by blocking CXCR3+ cell trafficking into the IP-10-expressing target organs (5, 27, 29, 36). The ligands CXCL9, CXCL10, and CXCL11 bind to the CXCR3 receptor and share the ability to activate biochemical and functional events in target cells. All these ligands recruit CXCR3+ cells; hence, neutralization of any of these ligands may not be sufficient to significantly abrogate the underlying biology of CXCR3+ cells, but the use of CXCR3-deficient mice and IFN-γ neutralization will elucidate the role of these cells and cytokines in our model in future study. In this study, neutralization of IP-10 decreased not only the numbers of IFN-γ+ CD4+ T cells but also IL-10-CD4+ T and IFN-γ+Mac-1+ cells (Figs. 7 and 8). In an autoimmune diabetic mouse model injected with islet-specific Th1 CD4+ T cells, insulin was induced by the first accumulation of CD4+ T cells followed by infiltration of Mac-1+ cells (3). The inflammatory cells that infiltrated initially to the pancreas, therefore, might be the organ-specific autoreactive CXCR3+ IFN-γ+CD4+ T cells, and they then produce other cytokines and chemokines to attract the other IL-10+CD4+ T cells and IFN-γ+Mac-1+ cells into the pancreas of mice with MAIDS. In this study, we analyzed the effect of IP-10 neutralization only at one time point; however, the effects of the treatment on virus-induced inflammatory infiltrate and cytokine expression are most likely time dependent. Thus we need to analyze the kinetics of the responses in future study. IL-10+Mac-1+ cells in the pancreatic lesions of mice with MAIDS were not significantly reduced by neutralization of IP-10 (Fig. 8), and the function of these cells in the formation of the lesions should be elucidated in future study. In conclusion, IP-10 neutralization could be a unique organ-specific therapeutic strategy for chronic pancreatitis, especially autoimmune pancreatitis associated with SjS.

ACKNOWLEDGMENTS

We thank Dr. Xiu-Hua Yang and Norio Honda for technical assistance and Dr. Minoru Nomoto and Dr. Terasu Honma for helpful discussions.

GRANTS

This work was supported by grants from the Ministry of Education and Science and Technology and the Ministry of Health, Welfare, and Labor of the Government of Japan.

REFERENCES

4. Cheung SC, Chattopadhyay SK, Hartley JW, Morse 3rd HC, and Pitha PM. aberrant expression of cytokine genes in peritoneal macro-

Fig. 7. Effects of blockade of IP-10/CXCL10 on cytokine production by inflammatory cells infiltrating the pancreas in mice with MAIDS. A: real-time quantitative PCR of IFN-γ and IL-10 mRNA expression in the pancreas of mice with MAIDS. Each amount was normalized to the level of GAPDH, and the final relative values are expressed relative to calibrator on day 0. B: numbers of pancreas-infiltrating cells that expressed IFN-γ and IL-10 at 8 wk after infection. Stained cryostat sections of 3 focuses of cellular infiltration of the individual pancreas were examined by counting the numbers of cells expressing IFN-γ and cells expressing IL-10. Three mice were analyzed for each time point. In control normal B6 mice, a negligible number of cells expressed IFN-γ or IL-10, so data are not shown. C and D: quantitative analysis of IFN-γ and IL-10 expression patterns of CD4+ and Mac-1+ cells that infiltrated the pancreas at 8 wk after infection. Stained cryostat sections of 3 focuses of cellular infiltration of the individual pancreas were examined by counting the numbers of CD4+ cells expressing IFN-γ or IL-10 (C) and Mac-1+ cells expressing IFN-γ or IL-10 (D). Three mice were analyzed for each time point. In control normal B6 mice, a negligible number of cells expressed IFN-γ or IL-10, so data are not shown. *Data are significantly different between mice injected with anti-IP-10 mAb and those injected with control Ab as determined by Student’s t-test (P < 0.01).


IP-10 neutralization ameliorates pancreatitis in mice


