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Autoimmune Pancreatitis Mouse Model

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Abstract

Autoimmune pancreatitis (AIP) is a chronic fibro-inflammatory disorder of the pancreas. However, extensive clinico-pathological analyses have revealed that AIP is, in reality, a pancreatic manifestation of a newly described systemic disease known as IgG4-related disease (IgG4-RD). IgG4-RD is characterized by enhanced local and systemic IgG4 antibody (Ab) responses as well as inflammation involving multiple organs, including the pancreas, bile ducts, and salivary glands. Although mice lack the IgG4 Ab subtype, autoimmune-prone MRL/Mp mice treated with repeated injection with polyinosinic-polycytidylic acid (poly (I:C)) provide an experimental model of AIP. These mice exhibit massive destruction of pancreatic architecture associated with pancreatic immune cell infiltration and fibrosis. Moreover, this experimental AIP may be accompanied by involvement of multiple organs as well as elevation of serum levels of autoAbs; they thus resemble humans with IgG4-RD. Thus, elucidation of the molecular mechanisms accounting for the development of experimental AIP can potentially provide new insights into the immuno-pathogenesis of human IgG4-related AIP.

Keywords

Autoimmune pancreatitis; IgG4-related disease; Plasmacytoid dendritic cells

Introduction

IgG4-related disease (IgG4-RD) is a newly established disease entity first proposed by Japanese physicians (Kamisawa and Okamoto, 2006). It is a chronic fibro-inflammatory condition characterized by enhanced serum levels of IgG4 antibody (Ab) and by massive infiltration of IgG4-expressing plasma cells into the affected organs (Kamisawa et al., 2015; Stone et al., 2012). Thus, enhanced IgG4 Ab responses are one of the most prominent features of IgG4-RD. Another important feature of this disorder is the presence of inflammation in multiple organs and tissues such as the pancreas, bile duct, salivary glands, periorbital tissues, kidneys, lungs, lymph nodes, and retroperitoneum (Okazaki and

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Umehara, 2017). IgG4-RD has a distinct histopathological signature that is very important for its diagnosis; this includes the presence of three key pathological findings: lymphoplasmacytic infiltration including IgG4-expressing plasma cells, storiform fibrosis, and obliterative phlebitis (Kamisawa et al., 2015; Stone et al., 2012). As physicians' awareness and recognition of IgG4-RD expand rapidly, the numbers of patients diagnosed with IgG4-RD is increasing.

Autoimmune pancreatitis (AIP) is a chronic fibro-inflammatory disease of the pancreas. It is classified into two types, type 1 (lymphoplasmacytic sclerosing pancreatitis, LPSP) and type 2 (idiopathic ductcentric pancreatitis, IDCP), based on pathological findings (Kamisawa et al., 2013). Type 1 AIP (also called LPSP) is characterized by dense lymphoplasmacytic infiltration, storiform fibrosis, and obliterative phlebitis. This is accompanied by massive accumulation of IgG4-expressing plasma cells and, in some patients with elevated levels of serum IgG4 and involvement of other organs, including bile ducts, salivary glands, and kidney. In contrast, infiltration of IgG4-expressing plasma cells is absent in the pancreas of type 2 (also called IDCP) AIP. Thus, it is now generally accepted that only type 1 AIP is a pancreatic manifestation of systemic IgG4-RD.

Although clinico-pathological analyses of patients with IgG4-RD have helped us to establish diagnostic criteria that establish the presence of this disease, our understanding of its immuno-pathogenesis is very limited. Initial studies of IgG4-RD focused on adaptive immune responses rather than innate immune responses since IgG4-RD is characterized by enhanced adaptive IgG4 Ab responses. These studies established that abnormal T helper type 2 (Th2), regulatory T cell (Treg), and T follicular helper (Tfh) responses as well as the generation of plasmablasts are implicated in the immuno-pathogenesis of this disorder (Akitake et al., 2010; Akiyama et al., 2015; Della-Torre et al., 2015; Zen et al., 2007). More recently, the pathological role played by abnormal innate immune responses in IgG4-RD have been examined using an experimental model of AIP, consisting of MRL/Mp mice subjected to repeated injections of polyinosinic-polycytidylic, poly (I:C) (Arai et al., 2015; Watanabe et al., 2017). Extensive analyses of this experimental AIP revealed that chronic fibro-inflammatory responses of the pancreas depend upon the activation of plasmacytoid dendritic cells (pDCs) with the ability to produce a large amount of IFN- α and IL-33. It should be noted that pDCs expressing both IFN- α and IL-33 are also present in the pancreas of patients with IgG4-RD and that pDCs isolated from such patients promote IgG4 Ab production by B cells (Arai et al., 2015; Watanabe et al., 2017). Thus, this experimental model of AIP is very useful for the elucidation of immuno-pathogenesis of IgG4-related AIP despite the fact that mice lack IgG4 Ab subtype. This unit reviews methods to study IgG4-related AIP in mice, using the MRL/Mp model.

Basic Protocol

Induction of autoimmune pancreatitis in MRL/Mp mice

Spontaneous development of AIP is seen in female MRL/Mp mice at 34-38 weeks old (Kanno et al., 1992). The incidence of AIP was reported to be around 70 %. On the other hand, male MRL/Mp mice spontaneously develop AIP at later time points such as 45-50 weeks and the incidence is less than 40 % (Kanno et al., 1992). Poly (I:C) is a prototypical

Toll-like receptor 3 (TLR3) ligand with the ability to induce systemic type I IFN responses. Repeated injection of poly (I:C) accelerates the development of AIP. The pancreas of MRL/Mp mice treated with repeated injection with poly (I:C) exhibits destruction of pancreatic acinar architecture, massive immune cell infiltration and fibrosis. These histological findings are similar to those of human AIP. Moreover, extra-pancreatic involvement such as the bile duct and salivary glands is seen in this experimental model (Qu et al., 2002; Yamashina et al., 2012). Finally, autoAbs against the pancreatic secretory trypsin inhibitor, carbonic anhydrase II, and lactoferrin, all of which are elevated in patients with human AIP, can also be detected in the serum of MRL/Mp mice treated with repeated injection with poly (I:C) (Asada et al., 2010; Okazaki et al., 2000). It should be noted that the development of AIP in MRL/Mp mice is independent of Fas-Fas ligand interaction since MRL/Mp *lpr/lpr* mice bearing Fas deletion mutant gene and wild type MRL/Mp mice exhibit comparable sensitivity to AIP (Qu et al., 2002). Thus, this experimental model of AIP shares important immunological features with human IgG4-related AIP.

Materials

Six to eight week old female MRL/Mp Mice (Japan SLC or Charles River Laboratory)

Poly (I:C) high molecular weight (HMW) (InvivoGen)

Endotoxin-free physiological water (InvivoGen)

PBS

10% Formalin (Wako Laboratory Chemicals)

27-29-G needles

1-ml syringes

Surgical instruments for removal of lymphoid organs

Preparation of poly (I:C)—Each mouse receives intra-peritoneal (IP) injection of poly (I:C) (100 µg) twice a week for a total of 14-16 times. Each mouse receives a total volume of 100 µl per IP injection.

1. Add 50 ml of the endotoxin-free physiological water to the 50 mg of poly (I:C) vial.
2. Mix the solution by pipetting.
3. Heat the mixture for 10 minutes at 65-70 C and then let the solution cool for one hour at room temperature to achieve proper annealing.
4. Prepare the sterile stock solution of poly (I:C) (1 mg/ml). Store this sterile stock solution at -20 C in small aliquots.

Injection of poly (I:C)

- 5 Thaw the frozen poly (I:C) stock solution at room temperature. Carefully aspirate the poly (I:C) stock solution into a 1-ml syringe attached with 27-29 G needle. Perform IP injection of 100 μ l of poly (I:C) stock solution.
- 6 Inject 100 μ l of poly (I:C) stock solution intraperitoneally twice a week (Monday and Thursday or Tuesday and Friday) for a total of 14-16 times.

Collection of pancreas and blood

- 7 Euthanize mice three hours after the final IP injection and collect blood by cardiac puncture or retro-orbital bleeding.
- 8 Surgically remove the pancreas.

The pancreas is attached to the spleen; therefore, gently lift the spleen to identify the underlying pancreas. The whole pancreas extends horizontally toward the duodenum.
- 9 Fix half of the excised pancreas in formalin for pathologic examination and place the other half of the excised pancreas into PBS for the isolation of pancreatic mono-nuclear cells.
- 10 Subject the pancreatic tissue fixed in formalin to appropriate slide preparation and staining with hematoxylin & eosin. Stain slides with Sirius Red or α -smooth muscle actin (α -SMA) for evaluation of pancreatic fibrosis.

Stained slides obtain should reveal AIP, i.e., destruction of acinar architecture, infiltration of immune cells, and fibrosis (Figure 1).

Support Protocol

Isolation of pancreatic mono-nuclear cells

Immunological analyses of pancreatic mono-nuclear cells (PMNCs) obtained from MRL/Mp mice with experimental AIP allows direct evaluation of the pancreatic immune response and inflammation occurring in the pancreas. Isolation of PMNCs from the inflamed pancreas is described below.

Materials

PBS without CaCl_2 or MgCl_2
HBSS without CaCl_2 or MgCl_2
RPMI1640
IM HEPES
Collagenase (Wako Laboratory Chemicals)
DNase I (Roche)
Percoll (GE-Healthcare)

70 µm cell strainer (BD Falcon)

FITC-conjugated B220 Ab (eBioscience)

PE-conjugated PDCA-1 Ab (eBioscience)

Preparation of collagenase digestion medium

1. Prepare stock solution of collagenase.
Dissolve 400 mg of collagenase in 20 ml of PBS (20 mg/ml) and store this stock solution at -20C.
2. Prepare stock solution of DNase I.
Dissolve 20 mg of DNase I in 20 ml of PBS (1 mg/ml) and store this stock solution at -20C.
3. Prepare digestion medium:
437.5 ml RPMI1640
50 ml Heat-inactivated FBS
12.5 ml IM HEPES (final concentration 25 mM)
Digestion medium can be stored at 4C in one month.
4. Prepare collagenase digestion medium:
100 ml digestion medium + 5 ml collagenase stock solution + 1 ml DNase I stock solution.

Isolation of PMNCs

- 5 Remove the pancreas and put it in a petri dish containing PBS.
- 6 Cut the pancreas into 3 mm pieces and put them into a 50 ml Falcon tube containing 20 ml of PBS.
- 7 Centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.
- 8 Add 10 ml of HBSS and centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.
- 9 Add 10 ml of HBSS and centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.
- 10 Add 10 ml of collagenase digestion medium containing the collagenase and DNase I and then shake for 30 minutes at 150 rpm at 37C.
- 11 Centrifuge at 30G for 5 minutes at 4C. Collect the supernatant to remove the debris.
- 12 Centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.
- 13 Add 10 ml of HBSS to the pellet and centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.

- 14** Add 10 ml of HBSS to the pellet and let the solution drain through 70 μ m filter into 50 ml Falcon tube. Transfer the solution into 15ml of Falcon tube.
- 15** Centrifuge at 1500 rpm for 5 minutes at 4C. Discard the supernatant.
- 16** Add 5 ml of 30 % of Percoll solution to the pellet and centrifuge for 30 minutes at 1800 rpm at room temperature. Discard the supernatant.
- 17** Add 10 ml of HBSS to the pellet and wash twice by centrifugation for 5 minutes at 1500 rpm at 4C.
- 18** Cell-pellet can be used for flow-cytometric analysis or for the culture experiments. Approximately 2×10^6 cells are obtained from the whole pancreas of poly (I:C)-treated MRL/Mp mice.

Accumulation of pDCs into the pancreas can be visualized by flow-cytometric analysis by staining with FITC-conjugated B220 Ab and PE-conjugated PDCA-1 Ab (Figure 2).

PMNCs can be isolated by enzymatic digestion of the pancreatic tissue as described above and then subjecting the digested cell mixture to appropriate density gradient centrifugation.

Reagents and Solutions

Digestion medium

437.5 ml RPMI1640

50 ml Heat-inactivated FBS

12.5 ml IM HEPES (final concentration 25 mM)

Digestion medium can be stored at 4C in one month.

Commentary

Background Information

Repeated IP injection of poly (I:C) into MRL/Mp mice results in the development of AIP, a pancreatic inflammation characterized by three key pathological findings, massive destruction of acinar architecture, infiltration of immune cells, and the development of fibrosis (Arai et al., 2015; Watanabe et al., 2017). The development of sialoadenitis and cholangitis, which often simultaneously occur in patients with IgG4-related AIP, are also observed in this experimental model of AIP. Thus, repeated IP injection of poly (I:C) into MRL/Mp mice causes experimental AIP accompanied by extra-pancreatic lesions (Qu et al., 2002; Yamashina et al., 2012). Other manifestations of human AIP such as elevated serum levels of autoAbs against the pancreatic secretory trypsin inhibitor, carbonic anhydrase II, and lactoferrin, can also be detected in the serum of MRL/Mp mice treated with repeated injection with poly (I:C) (Asada et al., 2010; Okazaki et al., 2000). Thus, collectively, this experimental murine AIP share many immunological features with human IgG4-related AIP except for the fact that the former lacks IgG4 Ab responses. Extensive analysis of this

interesting animal model of murine AIP could provide new insights into the immunopathogenesis of human IgG4-related AIP and lead to the identification of novel therapeutic targets of this disorder.

Flow-cytometric analysis of PMNCs obtained from the digested pancreas of mice with AIP identifies the type of immune cell that migrate into the pancreas in this model. These are composed of a wide variety of immune cells such as CD3⁺ T cells, B220⁺ B cells, CD11b⁺ or Gr-1⁺ myeloid cells (Arai et al., 2015; Watanabe et al., 2017). Of note, massive infiltration of the inflamed pancreas by pDCs defined as PDCA-1⁺ B220^{low} is also one of the most prominent features of this model (Figure 2). pDCs are unique DCs in that they have been shown to have the ability to produce type I IFNs and marked increases in the levels of type I IFN has been reported in both the pancreas and the serum of mice with experimental AIP in addition to prototypical pro-inflammatory cytokines such as TNF- α and IFN- γ (Arai et al., 2015; Watanabe et al., 2017). Therefore, this experimental AIP model could be used to elucidate the mechanisms by which activated pDCs mediate autoimmunity.

Critical Parameters and Troubleshooting

In general, the methods described in this section are very straightforward and the chance of experimental failure is very low.

Experimental AIP does not develop or too mild—It is important to use female MRL/Mp mice since male MRL/Mp mice are resistant to the induction of experimental AIP. Ensure female MRL/Mp mice six-eight week old. MRL/Mp mice are available from Japan SLC (Arai et al., 2015; Watanabe et al., 2017) or Charles River Laboratory (Schwaiger et al., 2014). Do not use MRL/Mp *lpr/lpr* mice bearing Fas deletion mutant gene. Another critical point is the preparation of poly (I:C). Ensure that you have prepared poly (I:C) HMW properly according to the protocol.

The yields of PMNCs are too low—It is important to prepare collagenase digestion medium as described in the protocol and digest the pancreas tissue enough at the indicated condition. Another important point is low-speed centrifugation to remove debris. After low-speed centrifugation collect supernatant, not the pellet.

Anticipated Results

Repeated IP injection of poly (I:C) into MRL/Mp mice results in the development of AIP characterized by massive destruction of pancreas acinar architecture, infiltration of immune cells, and fibrosis in hematoxylin and eosin (H&E) staining (Figure 1 top panel). Pancreatic fibrosis can be evaluated by Sirius Red or α -smooth muscle actin (α -SMA) staining. Positive areas for Sirius Red or α -SMA is markedly increased in the pancreas of MRL/Mp mice treated with poly (I:C) (Figure 1 middle and bottom panels).

A wide variety of immune cells such as CD3⁺ T cells, B220⁺ B cells, CD11b⁺ or Gr-1⁺ myeloid cells accumulate into the pancreas of this experimental AIP (Arai et al., 2015; Watanabe et al., 2017). Of note, massive infiltration of pDCs defined as PDCA-1⁺ B220^{low} is one of the most prominent features of this model (Figure 2).

Time Considerations

The development of AIP requires IP injection of poly (I:C) twice a week for a total of 14-16 times; this indicates that it usually takes 7-8 weeks to induce experimental AIP. Isolation of PMNCs will take around four-five hours after the removal of pancreas.

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Figure 1. Pathological findings of experimental autoimmune pancreatitis

MRL/Mp mice received intraperitoneal poly (I:C) injection twice a week for a total of 14-16 times. Pancreas tissues were removed and then subjected to H&E, Sirius Red, and α -smooth muscle actin (α -SMA) staining. Massive destruction of acinar architecture, infiltration of immune cells, and fibrosis are seen in the pancreas of MRL/Mp mice treated with poly (I:C). The development of pancreas fibrosis is confirmed by Sirius Red and α -SMA staining.

Figure 2. Accumulation of plasmacytoid dendritic cells into the pancreas

MRL/Mp mice received intraperitoneal poly (I:C) injection twice a week for a total of 14-16 times. Pancreas mononuclear cells were isolated and then subjected to flow-cytometric analysis. Massive accumulation of plasmacytoid dendritic cells (pDCs) defined as pDC Ag-1 (PDCA-1)⁺ B220^{low} is seen in the pancreas of MRL/Mp mice treated with poly (I:C).