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AAV-IL-22 modifies liver chemokine activity and ameliorates portal inflammation in murine autoimmune cholangitis

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ABSTRACT

There remain significant obstacles in developing biologics to treat primary biliary cholangitis (PBC). Although a number of agents have been studied both in murine models and human patients, the results have been relatively disappointing. IL-22 is a member of the IL-10 family and has multiple theoretical reasons for predicting successful usage in PBC. We have taken advantage of an IL-22 expressing adeno-associated virus (AAV-IL-22) to address the potential role of IL-22 in not only protecting mice from autoimmune cholangitis, but also in treating animals with established portal inflammation. Using our established mouse model of 2-OA-OVA immunization, including α-galactosylceramide (α-GalCer) stimulation, we treated mice both before and after the onset of clinical disease with AAV-IL-22. Firstly, AAV-IL-22 treatment given prior to 2-OA-OVA and α-GalCer exposure, i.e. before the onset of disease, significantly reduces the portal inflammatory response, production of Th1 cytokines and appearance of liver fibrosis. It also reduced the liver lymphotropic chemokines CCL5, CCL19, CXCL9, and CXCL10. Secondly, and more importantly, therapeutic use of AAV-IL-22, administered after the onset of disease, achieved a greater hurdle and significantly improved portal pathology. Further the improvements in inflammation were negatively correlated with levels of CCL5 and CXCL10 and positively correlated with levels of IL-22. In conclusion, we submit that the clinical use of IL-22 has a potential role in modulating the inflammatory portal process in patients with PBC.

1. Introduction

IL-22 is a member of the IL-10 cytokine family, produced by a variety of immune cells, including T helper (Th) 17, Th22, γδ T, NKT, and innate lymphoid cells (ILCs) [1,2]. The IL-22 receptor (IL-22R) is a heterodimer that consists of IL-22R1 and the IL-10 receptor β subunit and is expressed by non-hematopoietic cells such as hepatocytes, keratinocytes, and epithelial cells of lung and intestine [3]. IL-22 has a protective role in several experimental models of hepatic injury, including T cell–mediated hepatitis, liver ischemia-reperfusion injury, bacterial and parasitic infection, acute and chronic alcohol-induced liver damage and liver fibrosis [4–10]. IL-22 has also been shown to modulate inflammation in other autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), psoriasis, experimental autoimmune uveitis and experimental autoimmune myocarditis [11–18]. However, IL-22, while protective before the onset of collagen induced arthritis, can exacerbate pathology if administered during the course of disease [19,20]. This dual role of IL-22 is also reflected in some murine models of psoriasis-like skin inflammation [15,16].

Our laboratory has extensively studied a murine model of primary biliary cholangitis (PBC) induced following immunization with a mimotope of the inner lipoyl domain of the major mitochondrial autoantigen of PBC. This mimotope of PDC-E2 is coined 2-octynoic acid (2-OA). Mice immunized with 2-OA coupled to either BSA or OVA, and stimulated with α-galactosylceramide (α-GalCer) develop high titer anti-mitochondrial antibodies (AMAs) and autoimmune cholangitis with lymphocytic infiltrates, portal inflammation, granuloma formation, bile duct damage, and fibrosis [21,22]. In the study herein, we investigated whether IL-22 could

Abbreviations: 2-OA-OVA, 2-octynoic acid conjugated ovalbumin; AAV, adeno-associated virus; AMAs, anti-mitochondrial antibodies; MNCs, mononuclear cells; PBC, primary biliary cholangitis; TU, transduction unit.

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modulate the natural history of autoimmune cholangitis by intravenously injecting mice either before or after the establishment of hepatic pathology with an IL-22 expressing recombinant adeno-associated virus (AAV-IL-22). Recombinant AAV is an appropriate vector for gene transfer *in vivo* based upon its replication defective nature and capability of infecting a broad range of cell types including non-dividing cells. It is retained *in vivo* as concatemers for long-term expression and elicits only a mild immune response compared with the older use of adenovirus [23]. We report herein that the use of IL-22 not only prevents clinical autoimmune cholangitis but, more importantly, significantly reduces the portal inflammatory response even in mice with established clinical pathology. We submit that the mechanisms defined herein, namely the downregulation of specific chemokine pathways, suggest that IL-22 may be an attractive therapeutic venue for investigative study in humans with PBC.

2. Materials and methods

2.1. Experimental mice

Female C57BL/6 mice aged 7–9 weeks were obtained from the National Laboratory Animal Center, Taiwan and mice maintained in the Animal Center of the College of Medicine, National Taiwan University. All experiments were performed following approval of The Institutional Animal Care and Use Committee (IACUC) of National Taiwan University College of Medicine and College of Public Health.

2.2. Preparation of AAV-IL-22

IL-22 cDNA was expressed and reversed from activated mouse T cells and cloned to adeno-associated virus vector (AAV-DJ). AAV-DJ is a recombinant AAV produced by a complex library of hybrid capsids from 8 different wild-type viruses (Cell Biolabs, San Diego, CA, USA). Earlier work has demonstrated that AAV-DJ vectors are not only superior to HBD-negative wild-type viruses (up to 100,000-fold superior to AAV-8 or AAV-9), but are also substantially better than AAV-2 (Cell Biolabs) [24]. Briefly, the 540-bp cDNA containing murine IL-22 was inserted into a recombinant adeno-associated viral vector (pAAV-IRES-GFP). IL-22 inserted pAAV-IRES-GFP plasmid was co-transfected with pAAV-DJ and pHelper at a ratio of 1:1:1 into the adenovirus packaging AD293 cell line. Viruses were purified from infected cells 42–48 h after infection by three freeze–thaw cycles followed by Hi-Trap Heparin column. Viral titers (transduction unit, TU) were measured by GFP expression in infected 293T cells using flow cytometry. Throughout these studies a mock AAV was used as a control; it did not contain a transgene in the expression cassette.

2.3. Experimental protocol

Female mice, at 7–9 weeks of age, were intraperitoneally immunized with 2-OA-OVA in the presence of complete Freund's adjuvant (CFA, Sigma–Aldrich, St. Louis, MO, USA) and subsequently boosted at weeks 2, 4, 6 and 8 with 2-OA-OVA in incomplete Freund's adjuvant (IFA, Sigma–Aldrich). Two μg of α-galactosylceramide (Funakoshi, Tokyo, Japan) were injected with the first and second 2-OA-OVA immunizations. AAV-IL-22 was administered to mice at 3 days before (the preventive study) or at 3 weeks after (the therapeutic study) the first 2-OA-OVA immunization. Three weeks was chosen as the time period for the therapeutic phase of this study because at 3 weeks following initial immunization with 2-OA-OVA, mice exhibit florid portal inflammation (see below). Sera were obtained on all mice at 10 weeks post-immunization and levels of IL-22 were measured by ELISA. In addition in nested subgroups, mice were sacrificed at 5 weeks post immunization for hepatic chemokine assays; additional mice were sacrificed 10 weeks post-immunization for liver histopathology, definition of mononuclear cell phenotypes, cytokine profiles and titers of AMAs. All experiments were performed a minimum of 2–3 times with group sizes of 11–15 mice. Prior to this experimental protocol, two pilot studies were undertaken. Firstly, we assessed the basal level of expression of IL-22 as well as hepatic IL-22 by qRT-PCR in mice either immunized with 2-OA-OVA using our standard protocol or, for the purpose of controls, immunized with saline only. In addition, to verify the activity of AAV-IL-22, naive mice were injected with either AAV-IL-22, mock virus or normal saline and the levels of sera IL-22 and hepatic IL-22 mRNA quantitated at 10 weeks of age.

2.4. Determination of serum AMAs

Serum titers of IgM and IgG anti-PDC-E2 autoantibodies were measured by ELISA using our well standardized recombinant PDC-E2 [25]. Briefly, purified mouse recombinant PDC-E2 at 10 μg/ml in carbonate buffer (pH 9.6) was coated onto ELISA plates at 4 °C overnight. After blocking with 1% casein (Sigma–Aldrich), diluted sera was added for 2 h at room temperature. In parallel one positive pool serum was diluted serially and added to each plate to constitute an internal standard. HRP-labeled anti-mouse IgG or IgM (Invitrogen, Camarillo, CA, USA) diluted 1/10,000 in blocking buffer were added for detection of mouse antibodies. Optical density was read at 450 nm.

2.5. Liver mononuclear cell quantitation

Livers were perfused with PBS containing 0.2% BSA (PBS/0.2% BSA), passed through a 100 μm nylon mesh, and re-suspended in PBS/0.2% BSA. The parenchymal cells were removed as pellets after centrifugation at 50 g for 5 min and the non-parenchymal cells isolated using Histopaque-1077 (Sigma–Aldrich) [25] or 40% and 70% Percoll (GE HealthCare Biosciences, Quebec, Canada). After centrifugation, collected cells were washed with PBS/0.2% BSA and viability of cells was confirmed by trypan blue dye exclusion. Subsets of liver mononuclear cells were measured by flow cytometry. Before staining cells, with a previously defined optimal dilution of monoclonal antibodies (Abs), the cells were pre-incubated with anti-CD16/32 (clone 93) to block non-specific FcRγ binding. The following Abs were used in this study: anti-CD3, anti-CD4, anti-CD8a, anti-CD19, anti-NK1.1 (Biolegend, San Diego, CA, USA). For Foxp3 staining, after the cell surface staining, Foxp3 intracellular staining was performed using a commercially available kit from Biolegend. Stained cells were assessed on a FACSCalibur using FlowJo software (Tree Star, Inc., Ashland, OR, USA). Optimal concentrations of the mAbs were used throughout and all assays included positive and negative controls.

2.6. IL-22 levels and IL-22R1 expression

IL-22 expression in liver and IL-22R1 expression in liver mononuclear cells were detected by reverse transcription PCR. TRI^{zol} reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) was used to isolate RNA. cDNA was prepared using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) to reverse transcribe the isolated RNA. PCR were amplified for 35 cycles (1 min at 95 °C, 1 min at 66 °C, and 1 min at 72 °C) and the products detected by 2% agarose ethidium bromide gel. H_2O was used as a negative control, and LPS treated liver as a positive control. Serum levels of IL-22 were assayed by ELISA (R&D Systems, Minneapolis, MN, USA) and using positive and negative controls.

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