EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

IL-4 Receptor Alpha Signaling through Macrophages Differentially Regulates Liver Fibrosis Progression and Reversal

Shih-Yen Weng [a,b], Xiaoyu Wang [a,b], Santosh Vijayan [a,b], Yilang Tang [b,c], Yong Ook Kim [a,b], Kornelius Padberg [a,b], Tommy Regen [b,c], Olena Molokanova [a,b], Tao Chen [a,b], Tobias Bopp [b,d], Hansjörg Schild [b,d], Frank Brombacher [e], Jeff R. Crosby [f], Michael L. McCaleb [f], Ari Waisman [b,c], Ernesto Bockamp [a,b], Detlef Schuppan [a,b,g,*]

[a] Institute of Translational Immunology, University Medical Center, Johannes Gutenberg University, Mainz, Germany
[b] Research Center for Immunotherapy (FZI), University Medical Center, Johannes Gutenberg University, Mainz, Germany
[c] Institute for Molecular Medicine, University Medical Center, Johannes Gutenberg University, Mainz, Germany
[d] Institute for Immunology, University Medical Center, Johannes Gutenberg University, Mainz, Germany
[e] International Center for Genetic Engineering and Biotechnology, Institute of Infectious Disease and Molecular Medicine, South African Medical Research Council, Cape Town, South Africa
[f] Ionis Pharmaceuticals, Carlsbad, CA, United States
[g] Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States

ARTICLE INFO

Article history:
Received 29 August 2017
Received in revised form 22 January 2018
Accepted 23 January 2018
Available online xxxx

Keywords:
Fibrosis
IL-4 receptor alpha
Liver
Macrophage
MMP12
Progression
Reversal

ABSTRACT

Chronic hepatitis leads to liver fibrosis and cirrhosis. Cirrhosis is a major cause of worldwide morbidity and mortality. Macrophages play a key role in fibrosis progression and reversal. However, the signals that determine fibrogenic vs fibrolytic macrophage function remain ill defined. We studied the role of interleukin-4 receptor α (IL-4Rα), a potential central switch of macrophage polarization, in liver fibrosis progression and reversal. We demonstrate that inflammatory monocyte infiltration and liver fibrogenesis were suppressed in general IL-4Rα$^{-/-}$ as well as in macrophage-specific IL-4Rα$^{-/-}$ (IL-4Rα$^{\Delta LysM}$) mice. However, with deletion of IL-4Rα$^{\Delta LysM}$ spontaneous fibrosis reversal was retarded. Results were replicated by pharmacological intervention using IL-4Rα-specific antisense oligonucleotides. Retarded resolution was linked to the loss of M2-type resident macrophages, which secreted MMP-12 through IL-4 and IL-13-mediated phospho-STAT6 activation. We conclude that IL-4Rα signaling regulates macrophage functional polarization in a context-dependent manner. Pharmacological targeting of macrophage polarization therefore requires disease stage specific treatment strategies.

Research in Context: Alternative (M2-type) macrophage activation through IL-4Rα promotes liver inflammation and fibrosis progression but speeds up fibrosis reversal. This demonstrates context dependent, opposing roles of M2-type macrophages. During reversal IL-4Rα induces fibrolytic MMPs, especially MMP-12, through STAT6. Liver specific antisense oligonucleotides efficiently block IL-4Rα expression and attenuate fibrosis progression.

1. Introduction

Chronic liver diseases can progress to cirrhosis, which is characterized by excessive accumulation of scar tissue (extracellular matrix, ECM) that is mainly composed of fibrillar collagens, glycoproteins and proteoglycans and that leads to severe distortion of the liver vascular architecture (Schuppan and Afdhal, 2008). While activated myofibroblasts and hepatic stellate cells (HSC) are the major producers of the fibrotic scar, their fibrogenic activation and proliferation depends on a complex interplay with other resident or recruited cells and their secreted factors. Here, immune cells, which promote or attenuate fibrogenesis, have become targets of antifibrotic treatments (Friedman et al., 2013; Schuppan and Kim, 2013; Trautwein et al., 2015).

Abbreviations: Arg1, Arginase 1; α-SMA, alpha–smooth muscle actin; ASO, antisense oligonucleotide; CCL2, chemokine (C-C motif) ligand 2; CCl_4, carbon tetrachloride; Col1a1, procollagen α1(I) transcript; DAPI, 4,6-diamidino-2-phenylindole; DKO, double knockout; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSC, hepatic stellate cell; HYP, hydroxyproline; IFNγ, interferon-gamma; IL-4Rα, interleukin-4 receptor alpha; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; MRC1, mannose receptor C type 1 (CD206); PDGF, platelet-derived growth factor beta; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; STAT3/STAT6, signal transducer and activator of transcription 3 or 6; TGFβ1, transforming growth factor beta 1 transcript; TIMP, tissue inhibitor of metalloproteinase 1; TNF-α, tumor necrosis factor alpha; WT, wildtype.

* Corresponding author at: Institute of Translational Immunology, Research Center for Immune Therapy, University Medical Center, Johannes Gutenberg University, Langenbeckstraße 1, 55131 Mainz, Germany.
E-mail address: detlef.schuppan@unimedizin-mainz.de (D. Schuppan).

https://doi.org/10.1016/j.ebiom.2018.01.028

Importantly, recent studies of highly effective antiviral therapies in hepatitis B or C patients proved cirrhosis to be a reversible condition, but the detailed mechanism underlying reversal remain unclear (Chang et al., 2010; D'Ambrosio et al., 2012; Marcellin et al., 2013).

As precursors of macrophages and dendritic cells, blood monocytes are recruited to sites of injury by cytokines such as CCL2, CCL3 and CCL6, which are abundant in patients with chronic liver diseases and cirrhosis (Karlmark et al., 2009; Marra et al., 1998; Shimizu et al., 2001). In advanced CCl_4-induced liver fibrosis, there is a massive recruitment of CCL2/CCR2-guided monocytes that develop into $CD11b^+$ $F4/80^+$ $Ly\text{-}6C^{hi}$ monocytic macrophages (Karlmark et al., 2009). These infiltrating cells produce cytokines and chemokines with potent pro-inflammatory or direct fibrogenic effects on HSC and (myo)fibroblasts, such as IL-1β, CCL2, CCL5, CCL7, CCL8, CXCL4, TNFα, TGFβ-1, and PDGF-BB (Barron and Wynn, 2011; Mehal and Schuppan, 2015; Ramachandran et al., 2012). With cessation of the damaging stimulus and by yet ill-defined mechanisms, these cells can undergo a shift towards an anti-inflammatory and fibrolytic resident macrophage phenotype.

Resident macrophages may originate from $Ly\text{-}6C^-$ $CCR2^-$ monocytes and have the potential to transform into classically (M1) or alternatively (M2) polarized macrophages in vivo (Egawa et al., 2013). M1- and M2-type polarizations can be induced in vitro by cytokines that also favor T helper (Th)1 vs Th2 cell polarization, i.e., by IFNγ and IL-12 vs IL-4 and IL-13, respectively (Jaguin et al., 2013). M1-type macrophages are considered pro-inflammatory and potentially antifibrotic, while the role of M2-type macrophages, which can be subdivided into at least 5 subtypes, in inflammation and fibrosis is less well defined (Gundra et al., 2017). The various M2-subtypes may exhibit either pro- or anti-fibrotic activity (Borthwick et al., 2016; Gordon and Martinez, 2010; Murray et al., 2014; Sica et al., 2014). This and other observations suggest that the overall functional relevance of the classical M1 and M2 paradigm is limited (Murray et al., 2014). Therefore, also the utility of therapeutic approaches using classical Th1/M1 vs Th2/M2 polarizing cytokines (Barron and Wynn, 2011; Duffield et al., 2013) remains unclear.

IL-4Rα that responds to both IL-4 and IL-13 should represent a central switch for M1- towards M2-type macrophage polarization. Since its cell specific function in inflammation and fibrosis has not been clearly investigated, we extensively characterized its role during liver fibrosis progression and regression, including its myeloid cell specific deletion and therapeutic inhibition. We show that macrophage IL-4Rα regulates fibrosis progression and reversal discordantly through modulation of macrophage subsets, indicating that antifibrotic therapies targeting IL-4Rα require disease stage specific pharmacological intervention.

2. Materials and Methods

2.1. Mice

Il4ra$^{-/-}$, *Il4*/*Il13* double knockout (DKO), *Il4ra*$^{fl/fl}$ and $LysM^{Cre}$ mice were on a Balb/c background (Herbert et al., 2004; McKenzie et al., 1999; Mohrs et al., 1999). Balb/c wild type mice were obtained from Janvier (Saint Berthevin Cedex, France). For the generation of *Il4ra*$^{\Delta CD4}$ mice ($CD4^{Cre}$*Il4ra*$^{flox/flox}$) with T cell-specific deletion of IL-4Rα, $CD4^{Cre}$ mice were mated with *Il4ra*$^{flox/flox}$ mice. To generate *Il4ra*$^{\Delta LysM}$ mice ($LysM^{Cre}$*Il4ra*$^{flox/-}$) with myeloid-specific deletion of IL-4Rα, $LysM^{Cre}$*Il4ra*$^{-/-}$, mice were bred with *Il4ra*$^{flox/flox}$ mice. Littermates of $LysM^{Cre}$*Il4ra*$^{flox/-}$ and *Il4ra*$^{flox/-}$ were used as controls in all related studies. All mice were housed under specific pathogen-free conditions. All experiments were performed with female 8- to 16-week-old mice and approved by the ethical committee of the Government of Rhineland Palatinate under the reference number 2317707/G12–1-007.

2.2. Fibrosis Models

Escalating CCl_4 doses (0.875 mL/kg; week 1–3, 1.75 mL/kg; week 4–6, 2.5 mL/kg) were given via oral gavage three times per week during 6 weeks as described (Popov et al., 2011), to induce a primarily parenchymal liver fibrosis. Fibrosis reversal was examined at 1, 2 or 4 weeks after the last CCl_4 treatment. For therapeutic intervention a 3 week CCl_4-regimen was chosen. Diet-induced liver fibrosis, resembling human nonalcoholic steatohepatitis, was induced in 8-week old male mice that were fed a methionine and choline deficient (MCD) diet for 8 weeks. Mice on a methionine and choline sufficient diet (MCS) served as controls (Wang et al., 2018).

2.3. In Vivo and In Vitro Application of Antisense Oligonucleotides (ASOs)

All 2nd-generation chimeric antisense nucleotides (ASOs) were produced by Ionis pharmaceuticals (Calsbad, CA) with base modifications as described (Seth et al., 2008). Thus to enhance binding affinity and avoid degradation by nucleases, the phosphorothioate backbone nucleotides were synthesized with 2-O-methoxyethyl (MOE) or locked 2-O, 4-C-((*S*)-ethylidene) (cEt) modifications in the ribose backbone (3–5 bases at both ends), while the internal 10 nucleotides remained unchanged. ASOs were administered 3 times per week (40 mg/kg in a volume of 10 μL PBS per g BW) by intraperitoneal injection. In short-term progression experiments (CCl_4 for 3 weeks) mice were treated with ASOs during the 3 weeks. Alternatively, ASO treatment was started off CCl_4 for one week to assess efficacy during fibrosis regression. ASO sequences were as follows: Control ASO (Ionis 141923): 5′-CCTTCCCTGAAGGTTCCTCC-3′; IL-4Rα ASO1 (Ionis 629123): 5′-AGTAG GTAGGACAACA-3′; IL-4Rα ASO2 (Ionis 231894): 5′-CCGCTGTTC TCAGGTGACAT-3′; STAT6 ASO: (Ionis 195428), 5′-CCACAGAGACATG ATCTGGG-3′.

2.4. Hydroxyproline (Hyp) Determination

300 mg liver tissue (150 mg from the right and 150 mg from the left liver lobe) was lysed in 5 mL HCl (6 N) at 110 °C for 16 h as previously described(Popov et al., 2005). Briefly, the 5 μL of hydrogenated sediment and Hyp standards (5 points of serial dilutions) were incubated with 50 μL citrate acetate buffer (5% citric acid, 7.24% sodium acetate, 3.4% NaOH and 1.2% glacial acetic acid, pH 6.0) and 100 μL of chloramine-T solution (14.1 mg/mL chloramine T hydrate, 10% n-propanol, 80% citrate acetate buffer) for 30 min at room temperature. Then 100 μL of Ehrlich's reagent (0.17 g/mL 4-dimethylaminobenzaldehyde dissolved in 20.7% perchloric acid and 70.5% n-propanol) were added to the mixture and incubated at 65 °C for 30 min. The reactants were measured for the absorbance of 550 nm. Hyp concentrations in liver tissue were determined by their absorbance relative to the standards after subtraction of the reaction after and before Ehrlich's reagent incubation.

2.5. Quantitative PCR

Liver samples (100 mg; 50 mg from the right and 50 mg from the left liver lobe) were lysed in Trizol (Invitrogen, Carlsbad, CA) and 500 ng total RNA was used for reverse transcription (Quanta Bio, Gaithersburg, MD). cDNAs were subjected to qPCR using either the Taqman or SYBR green technology (Applied Biosystems, Foster City, CA) and run on a StepOnePlus real-time PCR system (Applied Biosystems, Foster City, CA). Gene expression was normalized to expression of *Gapdh*. Primers and probes are specified in the Supplemental Information.

2.6. Histology and Immunohistochemistry

Liver tissue was fixed in 4% Roth-Histofix (Carl Roth, Karlsruhe, Germany), dehydrated, embedded in paraffin, sectioned and rehydrated as described (Popov et al., 2005). For Sirius Red staining, rehydrated sections were exposed to 0.1% Direct Red (Sigma-Aldrich, St Louis, MO) in saturated picric acid for 30 min. Slides were examined under the microscope using a 100× magnification and in each case 10 random fields

Please cite this article as: Weng, S.-Y., et al., IL-4 Receptor Alpha Signaling through Macrophages Differentially Regulates Liver Fibrosis Progression and Reversal, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.01.028