Research Article

JOURNAL OF HEPATOLOGY

IL-23 prevents IL-13-dependent tissue repair associated with Ly6C^{lo} monocytes in *Entamoeba histolytica*-induced liver damage

Jill Noll[1,†], Elena Helk[1,†], Helena Fehling[1], Hannah Bernin[1], Claudia Marggraff[1], Thomas Jacobs[1], Samuel Huber[2], Penelope Pelczar[2], Thomas Ernst[3], Harald Ittrich[3], Benjamin Otto[4], Hans-Willi Mittrücker[5], Christoph Hölscher[6], Frank Tacke[7], Iris Bruchhaus[1], Egbert Tannich[1], Hannelore Lotter[1,*]

[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany;* [2]*Department of Molecular Immunology and Gastroenterology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [3]*Department and Clinic for Diagnostic and Interventional Radiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [4]*Department of Bioinformatics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [5]*Institute of Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* [6]*Division of Infection Immunology, Research Center Borstel, Borstel, Germany;* [7]*Department of Medicine III, University Hospital Aachen, Aachen, Germany*

Background & Aims: The IL-23/IL-17 axis plays an important role in the pathogenesis of autoimmune diseases and the pathological consequences of infection. We previously showed that immunopathologic mechanisms mediated by inflammatory monocytes underlie the severe focal liver damage induced by the protozoan parasite, *Entamoeba histolytica*. Here, we analyze the contribution of the IL-23/IL-17 axis to the induction and subsequent recovery from parasite-induced liver damage.

Methods: *IL-23p19*$^{-/-}$, *IL-17A/F*$^{-/-}$, *CCR2*$^{-/-}$, and wild-type (WT) mice were intra-hepatically infected with *E. histolytica* trophozoites and disease onset and recovery were analyzed by magnetic resonance imaging. Liver-specific gene and protein expression during infection was examined by qPCR, microarray, FACS analysis and immunohistochemistry. Immuno-depletion and substitution experiments were performed in *IL-23p19*$^{-/-}$ and WT mice to investigate the role of IL-13 in disease outcome.

Results: Liver damage in infected *IL-23p19*$^{-/-}$, *IL-17A/F*$^{-/-}$, and *CCR2*$^{-/-}$ mice was strongly attenuated compared with that in WT mice. *IL-23p19*$^{-/-}$ mice showed reduced accumulation of *IL-17* and *CCL2* mRNA and proteins. Increased numbers of IL-13-producing CD11b^{+}Ly6C^{lo} monocytes were associated with disease attenuation in *IL-23p19*$^{-/-}$ mice. Immuno-depletion of IL-13 in *IL-23p19*$^{-/-}$ mice reversed this attenuation and treatment of infected WT mice with an IL-13/anti-IL-13-mAb complex supported liver recovery.

Conclusions: The IL-23/IL-17 axis plays a critical role in the immunopathology of hepatic amebiasis. IL-13 secreted by CD11b^{+}Ly6C^{lo} monocytes may be associated with recovery from liver damage. An IL-13/anti-IL13-mAb complex mimics this function, suggesting a novel therapeutic option to support tissue healing after liver damage.

Keywords: IL-23/IL-17 immunopathology; Parasite-induced liver damage; Regenerative Ly6C^{lo} monocytes; IL-13-dependent liver repair.

Received 9 July 2015; received in revised form 13 January 2016; accepted 16 January 2016; available online 22 January 2016

* Corresponding author. Address: Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Str. 74, D-20359 Hamburg, Germany. Tel.: +49 40 42818 475; fax: +49 40 42818 512.

E-mail address: lotter@bnitm.de (H. Lotter).

† These authors contributed equally as joint first authors.

Abbreviations: IL, interleukin; CCR2, C-C chemokine receptor 2; mAb, monoclonal antibody; *E. histolytica*, *Entamoeba histolytica*; ALA, amebic liver abscess; TNF, tumor necrosis factor; Th, T helper; CCL2, C-C chemokine ligand 2; Ly6C, lymphocyte antigen 6C; NOS2, nitric oxide synthase 2; Arg1, arginase 1; IFN, interferon; CD, cluster of differentiation; IL-23R, IL-23 receptor; TLR, toll like receptor; MRI, magnet resonance imaging; Nr4a1, Nuclear Receptor Subfamily 4, Group A, Member 1; MFI, mean fluorescence intensity; Ki-67, marker of proliferation Ki (Kiel) -67 or MKI67 (gene); IL-13^{tg}, IL-13, transgenic mice.

Introduction

In recent years, significant advances have been made in understanding the pivotal role of the interleukin (IL)-23/T helper (Th)-17 axis in host protection and autoimmunity [1,2]. While the IL-23/Th17 axis is involved in protection against several pathogens [3–5], it is also responsible for immune-mediated diseases, including autoimmune encephalitis among others [2]. Despite its role in protection against various pathogens, infection-induced pathological changes can occur as an adverse side effect after activation of this pathway [6,7]. Amebic liver abscess (ALA), a severe form of focal liver destruction caused by the protozoan *Entamoeba histolytica* (*E. histolytica*) might serve as both an example and a model for studying the relevance of the IL-23/IL-17 axis to the immunopathology underlying parasite-induced liver damage. The disease occurs in tropical and subtropical areas throughout

the world and mainly affects adult males [8,9]. A murine model for ALA shows besides parasite-specific effector molecules, tumor necrosis factor (TNF) released from Kupffer cells and inflammatory monocytes contribute to the disease development [10]. The IL-23/Th17 pathway might be of critical importance in this disease because it acts upstream of the cytokines and effector cells relevant to liver damage.

The heterodimeric cytokine IL-23 comprises two subunits, a unique p19 subunit and the p40 subunit [11], and is essential for Th17 cell differentiation, expansion, and survival [12]. The IL-23 receptor (IL-23R) complex comprises two subunits, IL-23R and IL-12Rβ1, and is present on a variety of immune cells [13]. IL-23 mediates expansion of IL-17 (IL-17A/F)-expressing T cell populations [14]. IL-17 and TNF exhibit strong synergy in promoting inflammation [15], and recent studies show that IL-17 is not only involved in the recruitment of neutrophils, but also in the migration and infiltration of monocytes through induction of C-C chemokine ligand 2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1) [16–18]. CCL2 is produced by a variety of cells in the liver according to the type of injury and stimulus, including hepatocytes and macrophages [19], hepatic stellate cells [20], Kupffer cells and endothelial cells [21].

In mice, blood-circulating monocytes express cluster of differentiation molecule 11b (CD11b) and the lymphocyte antigen 6C (Ly6C), on their surface [22]. Ly6C^{hi} monocytes are referred to as "classical" or "proinflammatory" monocytes, and enter inflamed or injured tissue. In a proinflammatory "type I" milieu, which is characterized by increased local concentrations of interferon (IFN)γ and TNF, these cells differentiate into proinflammatory activated macrophages that express nitric oxide synthase (NOS)2, and TNF and can initiate immunopathology [22]. The other major monocyte subset, namely "non-classical Ly6C^{lo}" or "regenerative" monocytes, develop in a "type 2" milieu (IL-4 and IL-13) and can further polarize into arginase 1 (Arg1)- and F4/80-expressing macrophages [23,24]. However, the enormous plasticity and diversity of monocyte-macrophage-dendritic cell populations will lead to a new nomenclature in the future [25]. In cases of acute organ impairment, such as that associated with partial hepatectomy or toxin-induced liver injury, IL-4/IL-13 make a substantial contribution to liver regeneration [26]; however, in cases of chronic inflammation, IL-13 acts as a pro-fibrotic mediator [27,28].

Here, we examined the role of the IL-23/Th17 axis in a murine model of parasite-induced liver damage. We observed a significant reduction in ALA pathology in *IL-23p19*$^{-/-}$, *IL-17A/F*$^{-/-}$, and *CCR2*$^{-/-}$ mice, which was accompanied by reduced expression of corresponding chemokines and cytokines and an increase in the number of Ly6C^{lo} cells in the livers of *IL-23p19*$^{-/-}$ and *CCR2*$^{-/-}$ mice immediately post-infection (p.i.); these mice showed significantly better recovery from liver damage. Liver regeneration in *IL-23p19*$^{-/-}$ mice was associated with increased production of IL-13 by CD11b^{+}Ly6C^{lo} monocytes among others. Depleting IL-13 in *IL-23p19*$^{-/-}$ mice reversed this attenuation. However, treating wild-type (WT) mice with an IL-13/anti-IL-13-mAb complex after infection-induced liver injury strongly supported the healing process.

Materials and methods

Mice

C57BL/6 (WT), *IL-17A/F*$^{-/-}$, and *CCR2*$^{-/-}$ mice were bred and kept under specific pathogens-free conditions in the animal facility at the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. *IL-23p19*$^{-/-}$ and IL-13 transgenic (IL-13^{tg}) and respective C57BL/6 control mice were bred in the animal facilities at the University of Kiel, Germany, and the Research Center Borstel, Germany, respectively. All mouse strains were backcrossed against the genetic C57BL/6 background for more than 10 generations. Mice were male and aged 8–12 weeks. Animal experiments were approved by the review board of the State of Hamburg, Germany, and conducted in accordance with institutional and Animal research: Reporting of *in vivo* experiments (ARRIVE) guidelines.

Induction of ALA and monitoring of disease course by magnet resonance imaging (MRI)

Mice were infected by intrahepatic injection of 1.25×10^5 *in vitro*-cultured trophozoites of the highly pathogenic cell line B, generated from *E. histolytica* trophozoites (HM-1:IMSS) [29] as described previously [10,30]. For MRI measurements, mice were pre-anesthetized with a mixture of 1.5% isoflurane/oxygen in a closed box, placed on a small animal slide (warmed at 37 °C), and measured under constant inhalation of the isoflurane/O_2 mixture using a nose mask at a flow rate of 500 ml/min. Abscess course was monitored by MRI using a small animal 7tesla MRI scanner (Bruker) with a T2-weighted turbo spin echo sequence (T2TSE). OsiriX Imaging Software DICOM Viewer (Opensource version 32-bit 4.1.1) was used to measure total abscess volume, calculated by measuring the region of interest (ROI) in each slice showing the abscess on transversal sections of the abdomen.

qPCR

Total liver RNA was isolated using the RNeasy Mini Kit (Qiagen). cDNA synthesis was accomplished using Maxima® First Strand cDNA Synthesis Kit for RT-qPCR (Thermo Scientific). qPCR was performed using Maxima® SYBR Green qPCR Master Mix (Thermo Scientific). Accumulation levels were calculated using the $2^{-\Delta\Delta Ct}$ method [31] and normalized to the housekeeping genes ribosomal protein S9 (*RPS9*) and glycerinaldehyd-3-phosphate dehydrogenase (*GAPDH*). Primer sequences are shown in the Supplementary materials.

Measurement of CCL2 serum levels

The CCL2 concentration in the serum of experimental mice was measured using a Cytometric-Bead-Array and a Mouse MCP-1 Flex Set (BD).

Flow cytometry analysis of lymphocytes

The following fluorochrome-labeled antibodies and the corresponding isotype control antibodies were purchased: B220, CD3, CD4, CD8, CD11b, CD11c, CD90.2, CD127, F4/80, γδ TCR, IL-23R, Ly6C, Ly6G, NK1.1, NKp46, and Siglec-F (BioLegend); Arg1 (R&D Systems); and IL-13 and IL-17 (eBioscience). Isolation and analysis of liver and blood lymphocytes is described in the Supplementary materials.

Immunohistology

Paraffin-embedded sections of liver tissue obtained at day 3 p.i. were stained with hematoxylin and eosin (H&E) and *E. histolytica* trophozoites were visualized using pooled polyclonal rabbit sera raised against recombinant *E. histolytica* antigens. Neutrophils were stained with the 7/4-mAb (Cedarlane), macrophages with an F4/80-mAb (Serotec), monocytes with a CD11b-mAb (Abcam), proliferation with a Ki67-mAb (BD), Arg1 with mouse anti-Arginase 1 (BD), and nitric oxide synthase (NOS) with anti-NOS2 (Abcam) (see Supplementary materials for methodological details).

Microarray analysis

Total liver RNA was isolated from WT and *IL-23p19*$^{-/-}$ mice at 6 h p.i. as described above, and RNA integrity checked on an Bioanalyzer 2100 (Agilent). Microarray analysis was performed using Affymetrix Mouse Gene ST 2.0 arrays and the WT PLUS Reagent Kit (Affymetrix). Sample amplification, hybridization, washing, and staining was performed according to the manufacturer's instructions. Arrays were scanned on a GeneChip Scanner 3000 7G (see Supplementary materials for details).

Immuno-depletion and substitution of IL-17 and IL-13

Depletion of IL-17A (in WT mice) and IL-13 (in *IL-23p19*$^{-/-}$ mice) was performed by i.p. injection of 100 μg anti-mouse IL-17A and anti-IL-13-mAb (R&D) in 100 μl PBS 1 day prior to infection. Control WT and *IL-23p19*$^{-/-}$ mice received 100 μg