Institut Pasteur

Microbes and Infection 17 (2015) 564–574

www.elsevier.com/locate/micinf

Original article

Bacillary replication and macrophage necrosis are determinants of neutrophil recruitment in tuberculosis

Teresa Repasy [a], Nuria Martinez [a], Jinhee Lee [a], Kim West [a], Wenjun Li [b], Hardy Kornfeld [a,*]

[a] Department of Medicine, University of Massachusetts Medical School, Worcester, MA, USA

[b] Division of Preventive and Behavioral Medicine, University of Massachusetts Medical School, Worcester, MA, USA

Received 30 December 2014; accepted 27 March 2015

Available online 7 April 2015

Abstract

We previously determined that burst size necrosis is the chief mode of mononuclear cell death in the lungs of mice with tuberculosis. The present study explored the link between infection-induced necrosis of mononuclear phagocytes and neutrophil accumulation in the lungs of mice challenged with one of four *Mycobacterium tuberculosis* strains of increasing virulence (Rv*ΔphoPR* mutant, H37Ra, H37Rv and Erdman). At all time points studied, Erdman produced the highest bacterial load and the highest proportion and number of *M. tuberculosis*-infected neutrophils. These parameters, and the proportion of TUNEL-positive cells, tracked with virulence across all strains tested. Differences in neutrophil infection were not reflected by levels of chemoattractant cytokines in bronchoalveolar lavage fluid, while interferon-γ (reported to suppress neutrophil trafficking to the lung in tuberculosis) was highest in Erdman-infected mice. Treating Erdman-infected mice with ethambutol decreased the proportion of mononuclear phagocytes with high bacterial burden and the ratio of infected neutrophils to infected mononuclear cells in a dose-dependent manner. We propose that faster replicating *M. tuberculosis* strains cause more necrosis which in turn promotes neutrophil recruitment. Neutrophils infected with *M. tuberculosis* constitute a biomarker for poorly controlled bacterial replication, infection-induced mononuclear cell death, and increased severity of immune pathology in tuberculosis.
© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: Tuberculosis; Neutrophils; Macrophages; Cell death

1. Introduction

We previously reported that necrotic cell death of mononuclear phagocytes (MPs) infected with *Mycobacterium tuberculosis* Erdman is associated with high intracellular bacterial burden and is the predominant form of cell death in tuberculosis (TB) disease in vivo [1]. Data from in vivo and in vitro experiments support the concept of burst size cytolysis in MPs harboring 20–40 bacilli [1–3]. This necrotic cell death mode relies on mycobacterial genes regulated by the PhoPR 2-component system but it is independent of Esx-1 [2]. Neutrophils comprised roughly half of all *M. tuberculosis*-infected lung leukocytes 3 weeks after aerosol challenge with Erdman. Bacterial burden increases logarithmically at this early stage of TB disease, prior to the full expression of adaptive immunity in the lung [1]. At later time points, when total lung bacillary load is held to a plateau level by host immunity, the proportion of MPs permissive for *M. tuberculosis* replication (and therefore susceptible to burst size necrosis) falls by ~90%. In the same interval post-infection (p.i.) the proportion of neutrophils amongst all *M. tuberculosis*-infected phagocytes falls to ~10%. Mice lacking interferon gamma (IFN-γ) fail to restrict Erdman replication and have a rising proportion of infected neutrophils until death. These results suggest a model where neutrophils are recruited to TB lesions in response to signals associated with burst size necrosis of MPs.

* Corresponding author. Department of Medicine, LRB-303, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA. Tel.: +1 508 856 2646; fax: +1 508 856 7883.

E-mail address: Hardy.Kornfeld@umassmed.edu (H. Kornfeld).

http://dx.doi.org/10.1016/j.micinf.2015.03.013

1286-4579/© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

In the present study, we investigated the relation between *M. tuberculosis* replication and neutrophil recruitment to the lung. Wild-type C57BL/6 mice were challenged with one of four *M. tuberculosis* strains differing in virulence: Erdman, H37Rv, H37Ra, or an H37Rv mutant lacking the *phoPR* coding region (RvΔ*phoPR*). This approach was necessary because there are no known inhibitors of burst size necrosis or any mouse strains resistant to this mode of infection-induced cell death. Results confirmed that bacterial replication during the innate phase of TB defense correlated with the total number of lung leukocytes harboring acid-fast bacilli (AFB), the proportion of *M. tuberculosis*-infected (AFB^{+}) leukocytes that were neutrophils and the distributions of AFB burden per cell in MPs and neutrophils. The data from this study support a model where the rate of *M. tuberculosis* replication and consequent burst size necrosis of MPs promotes neutrophil recruitment to TB lesions despite potentially counter-regulatory cytokine signals.

2. Materials and methods

2.1. Mice

C57BL/6 mice (Jackson Laboratory) were housed in a pathogen-free environment at Animal Medicine facility at The University of Massachusetts Medical School (UMMS). Experiments were conducted in accordance with National Institutes of Health guidelines regarding laboratory animals and followed protocols approved by the Institutional Animal Care and Use Committee and the Institutional Biosafety Committee at UMMS.

2.2. Mycobacteria and infection

M. tuberculosis Erdman, H37Rv, H37Ra (provided by H. Remold, Brigham and Women's Hospital) and RvΔ*phoPR* mutant (provided by K. Papavinasasundaram, UMMS) [2] were used in this study. Aerosol infections delivered ~100 colony forming units (CFU) of Erdman, H37Rv or H37Ra and ~400 CFU of RvΔ*phoPR* (Glas-Col Inhalation Exposure System).

2.3. CFU assay

Lung homogenates were serially diluted with PBS-Tween (0.05%) and plated in duplicate on Middlebrook 7H11 agar plates supplemented with Middlebrook OADC Enrichment. Plates were incubated at 37 °C and CFU were counted 2, 3 and 4 weeks after plating.

2.4. Cell preparation

Whole lung leukocytes were isolated from infected mice as previously described [4]. Single lung cell suspensions were used for flow cytometry and cytospin preparation for AFB staining.

2.5. Enumeration of intracellular bacteria

Intracellular AFB were enumerated from isolated lung leukocytes as described Repasy et al. [1].

2.6. Flow cytometry

Cell suspensions were incubated in CD16/CD32 mAb (BD Biosciences) to block Fc binding. The following reagent and conjugated antibodies (eBioscience) were used: CD11b-PerCP-Cyanine5.5 (M1/70), CD45-APC (30-F11), Ly-6G-Alexa Fluor 700 (RB6-8C5); Live/Dead Fixable Dead Cell Stain Kit (Invitrogen). Data acquisition by BD LSRII flow cytometer and analysis with FlowJo software (TreeStar). Excluding dead cells and lymphocytes by forward and side scatter, neutrophils were gated on CD45^{+} cells and defined as Ly-6G^{hi} CD11b^{hi} cells.

2.7. Histology, TUNEL assay and immunostaining

Lungs of infected mice were inflated with 10% buffered formalin and fixed for 24 h. Paraffin embedded sections were stained with hematoxylin and eosin (H&E) or terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay (In Situ Cell Death Detection Kit, Roche). For immunostaining, primary Ab against myeloperoxidase (MPO; LS Bio) and secondary Ab Alexa 555 (Invitrogen) were used, along with In Situ Cell Death Detection Kit with fluorescein-dUTP (Roche) to detect TUNEL^{+} cells. Lung sections were analyzed using Nikon Eclipse E400 Microscope equipped with a Nikon DS-Ri1 camera using NIS-Elements Microscope Imaging Software or Spot Advance Software.

2.8. Cytokine measurement

Lungs of infected mice were flushed three times with lavage fluid (0.2% BSA 0.2 mM EGTA in PBS) then filter-sterilized. Preparation of cell-free lung lysates were performed as described [4]. Bronchoalveolar lavage (BAL) fluid and supernatant were stored at −80 °C until used. BAL fluid and lung lysates were individually assayed for IFN-γ, IL-1α, IL-1β, IL-17, JE (CCL2), KC, MIP-2, (R&D Systems) and S100A8/A9 (CusaBio) by ELISA following the manufacturers' protocols.

2.9. Ethambutol treatment

Four days p.i. Erdman-infected mice were treated with ethambutol (EMB; Sigma–Aldrich) ad libitum in drinking water at concentrations of 67, 200 and 600 µg/ml. Water bottles containing EMB were replaced weekly and treatment continued for 11 days until 2 week p.i. time point.

2.10. Statistical analysis

For Fig. 1D, the data followed an approximate Poisson distribution; therefore we compared the median values by strain at each time point and bin using a Poisson regression model, and expressed the differences as ratios to a reference group and tested their statistical significance. For all other data, statistical analysis was performed using SigmaPlot v11.0 (Systat Software, Inc.). One-way ANOVA with Tukey or

Download English Version:

https://daneshyari.com/en/article/3414626

Download Persian Version:

https://daneshyari.com/article/3414626

Daneshyari.com