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Microbes and Infection xx (2017) 1–9

www.elsevier.com/locate/micinf

Original article

Contribution of intercellular adhesion molecule 1 (ICAM-1) to control *Mycobacterium avium* infection

Rafaella R. de Paula [a,1], Fábio V. Marinho [a,1], Julia S. Fahel [a], Sergio C. Oliveira [a,b,*]

[a] *Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil*
[b] *Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais (INCT-DT), Conselho Nacional de Desenvolvimento Científico e Tecnológico, Ministério de Ciência Tecnologia e Inovação, Salvador, Bahia, Brazil*

Received 30 March 2017; accepted 14 September 2017
Available online ■ ■ ■

Abstract

Mycobacterium avium is a facultative intracellular opportunistic pathogen especially relevant in cases of people living with AIDS. The aim of this study was to evaluate the role of intercellular adhesion molecule 1 (ICAM-1) in the inflammatory response against *M. avium* infection. Mice deficient for ICAM-1 (ICAM KO) and infected with *M. avium* presented increased bacterial load in the spleen, liver and lungs compared to C57BL/6. Moreover, ICAM deficient mice presented reduced granuloma area in liver at 30 days post-infection with reduced numbers of lymphocytes and granulocytes. The assessment of *in vitro* cytokine production by ICAM KO spleen cells showed lower levels of IFN-γ compared to C57BL/6, whereas TNF-α remained unaltered. Additionally, the production of IFN-γ in liver and spleen tissues was also diminished in ICAM-1 KO mice. Interestingly, a persistent reduction in IFN-γ production was observed in $CD3^+NK1.1^+$ cells of ICAM-1 deficient mice compared to wild-type animals. Together, these results demonstrate the importance of ICAM-1 in the efficient control of *M. avium* infection and granuloma formation and highlights its role on $CD3^+NK1.1^+$ cell population as important for IFN-γ production during infection.

Keywords: ICAM-1; *Mycobacterium avium*; NKT cells; Lymphocyte activation

1. Introduction

The genus *Mycobacterium* comprises diverse bacteria ranging from highly pathogenic species such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* to opportunistic pathogens such as nontuberculous mycobacteria (NTM). NTM are ubiquitously found in the environment being present in domestic and wild animals as well as in milk, food and water distribution systems enabling close contact with human population [1,2]. In the mid-1990s *Mycobacterium avium* and other NTM caused a series of infections in patients with HIV, particularly those with low $CD4^+$ T cell counts, emerging as opportunistic pathogens [3,4]. Recent evidences reveal a rise in the incidence of infections caused by *M. avium* and related nontuberculous species, with bacteria from the *M. avium* complex being the most predominantly worldwide [5,6].

The immune response against *M. avium* relies on both innate and acquired immunity. Once in the host, *M. avium* is detected by pattern recognition receptors (PRRs), among which Toll-like receptors (TLRs) 2, 6 and 9 are specially relevant [7–9]. Macrophages are mycobacteria preferential host cell and innate receptors and adapter molecules such as MyD88 and IRAK4 act as key elements in triggering the inflammatory process [10,11]. However, in order to promote

* Corresponding author. Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Pampulha, Belo Horizonte, Minas Gerais, Brazil. Fax: +55 31 3409 2666.
E-mail address: scozeus@icb.ufmg.br (S.C. Oliveira).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.micinf.2017.09.005

bactericidal activity, these cells require activation by proinflammatory cytokines such as IFN-γ from $CD4^+$ and $CD8^+$ T lymphocytes [12].

The intercellular adhesion molecule-1 (ICAM-1) is a typical transmembrane glycoprotein constitutively expressed in the endothelium and immune cells. It is also present in the membrane of activated cells promoting cell adhesion between lymphocytes and antigen presenting cells (APCs) or lymphocytes and target cells [13–15]. Therefore, this molecule plays two distinct roles during the immune response: it participates in the process of transendothelial migration and in the establishment of the immunological synapse. The expression of ICAM-1 and other adhesion molecules is stimulated by cytokines such as IFN-γ, TNF-α and IL-1 as well as by exogenous agents such as LPS from Gram-negative bacteria [16].

In this study, we demonstrate that ICAM-1 participates in lymphocyte activation and inflammation during *M. avium* infection. Although, ICAMs have been widely studied in APCs and some clinically relevant pathologies such as multiple sclerosis, rheumatoid arthritis, insulin-dependent diabetes mellitus and cancer, little is known about its importance in the context of activation and function of T lymphocytes during infectious diseases. Herein, we defined the role of ICAM-1 during *M. avium* infection using genetically deficient mice (ICAM-1 KO). Our results demonstrate that ICAM-1 is important for the control of bacterial growth, granuloma formation and IFN-γ production by host cells. Furthermore, this study highlights the role of $CD3^+NK1.1^+$ cell population as an important source of IFN-γ during infection. Taken together, these results provide a better understanding of ICAM-1 participation in the immune response against mycobacterial infection, and place it in perspective for specific immune interventions against inflammatory diseases.

2. Materials and methods

2.1. Experimental animals

C57BL/6 wild-type mice were obtained from the Federal University of Minas Gerais (UFMG) animal facility. ICAM KO mice were gifted by Dr. João S. Silva from the University of São Paulo, School of Medicine, Ribeirão Preto, Brazil. All mice were housed in a specific pathogen-free laboratory facility and were divided in groups of five animals each. Experiments were performed according to protocols that were approved by the Animal Studies Committee (protocol 145/2012).

2.2. Bacterial strains and growth conditions

The low-virulence *M. avium* strain 2447, which exhibits a smooth-transparent morphotype [17], were grown in Middlebrook 7H9 broth that contained 0.05% Tween 80 and 0.2% glycerol and was supplemented with 10% albumin-dextrose-catalase (ADC). Cultures were harvested by centrifugation at the mid-exponential phase, re-suspended in saline with 0.05% Tween 80, briefly sonicated, and stored at −70 °C until use.

2.3. In vivo *infection and measurement of bacterial burden*

Eight-week-old mice were infected intravenously with 1×10^6 colony forming units (CFU) of *M. avium* strain 2447. The bacterial loads in the spleen, liver and lungs were determined after 30 and 100 days of infection. Briefly, organs were aseptically collected and homogenized in 0.05% Tween 80 distilled water. Samples were serially diluted and plated in Middlebrook 7H10 agar medium supplemented with 10% oleic acid-ADC. CFU counting was determined after 10 days of incubation of the agar plates at 37 °C, as previously demonstrated [9].

2.4. Splenocyte cultures

Cells obtained from the spleens of infected mice were washed with saline and the erythrocytes were lysed with a hemolytic solution (155 mM NH_4Cl, 10 mM $KHCO_3$, pH 7.2). Splenocytes were seeded at 10^6/well into 96-well plates with RPMI 1640 (Gibco, Carlsbad, CA, USA) supplemented with 2 mM L-glutamine, 25 mM HEPES, 10% heat-inactivated FBS (Gibco, Carlsbad, CA), penicillin G sodium (100 U/ml), and streptomycin sulfate (100 μg/ml). Cells were stimulated with *M. avium* (MOI 5:1) and either Con A (5 μg/ml) or *Escherichia coli* LPS (1 μg/ml; Sigma–Aldrich, St. Louis, MO). Unstimulated cells were used as negative controls. After 48 h or 72 h of culture, TNF-α and IFN-γ levels were measured, respectively, in the cell supernatants by ELISA, using the Duoset kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.5. Measurement of IFN-γ in spleen and liver tissues

Spleen and liver from infected mice were collected 30 days post-infection. A sample of a 100 mg of each organ was homogenized in 1 ml of cytokine extraction solution (0.1 mM PMSF, 0.1 mM benzethonium chloride, 10 mM EDTA, 20 KI aprotinin A and 0.05% Tween 20 diluted in PBS) using a tissue homogeneizer (TE-102, Tecnal, Brazil). After homogenization, samples were centrifuged for 3000 g, 20 min at 4 °C and the supernatants were collected for IFN-γ detection by ELISA using the Duoset kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

2.6. Flow cytometry analysis

For intracellular detection of IFN-γ ex vivo, splenocytes from WT and ICAM-1 KO mice were harvested after 30 days of infection with *M. avium* 2447 and adjusted to 1×10^6 cells/well. Cells were previously treated with brefeldin A (1 μg/well, Sigma–Aldrich) for 4 h and then stained for the surface markers CD4, CD8, CD3 or NK1.1 simultaneously. Following surface staining, all samples were stained for intracellular IFN-γ. Briefly, cells were incubated for 20 min with anti-mouse CD16/32 to block Fc receptors (eBioscience, San Diego, CA) in FACS buffer (PBS, 0.25% BSA, 1 mM NaN3)

Please cite this article in press as: de Paula RR, et al., Contribution of intercellular adhesion molecule 1 (ICAM-1) to control *Mycobacterium avium* infection, Microbes and Infection (2017), https://doi.org/10.1016/j.micinf.2017.09.005