Immunobiology 216 (2011) 1054–1063

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

Trichinella spiralis infection rapidly induces lung inflammatory response The lung as the site of helminthocytotoxic activity

María V. Gentilini[a], Guillermo G. Nuñez[a], María E. Roux[b], Stella M. Venturiello[a,*]

[a] *Microbiology, Immunology and Biotechnology Department, School of Pharmacy and Biochemistry, University of Buenos Aires, IDEHU-CONICET, Buenos Aires, Argentina*
[b] *Cardiological Investigation Institute, Dr. Taquini, ININCA-UBA-CONICET, Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form 10 January 2011
Accepted 15 February 2011

Keywords:
Helminthocytotoxic activity
Inflammation response
Lung
Mucosal immunology
Trichinella spiralis

ABSTRACT

In the present work, we studied the kinetics of the appearance of different immunological parameters in the lungs during the intestinal phase of infection with *Trichinella spiralis*. We also evaluated the lung's role in the retention and death of this helminth in its migratory stage. To study these parameters, we used lung extracts, lung cell suspensions and rat lung tissue sections.

During the intestinal phase of infection (days 0–13 post-infection, p.i.), an inflammatory response is elicited in the lungs, which reflects humoral, cellular and functional changes. These changes included an increased number of mast cells and eosinophils and the local production of IL-4, IL-5, IL-10, TNFα, IFNγ, IL-13, CCL11 and CCL28. We found hyperplasia of the bronchus-associated lymphoid tissue (BALT). Total and specific IgA, IgE, IgG1 and IgG2a were detected locally. The retention of the migratory larvae in the lung, together with the *ex vivo* cytotoxic capacity of the lung cells and antibodies present in the lung extracts, suggested that the lung was one of the immune defense organs against the pathogen's migration stage.

Introduction

Different helminth species possess an obligatory route through the lungs and induce an alteration of the immunological status (Silveira et al. 2002; Reece et al. 2006). The relationship between the appearance of such processes and their influence on the course of infection has not been described. Lung manifestations during these phases are not common, suggesting tolerance by the host.

During the systemic migration toward the striated muscles, a number of migrant newborn larvae (NBL) of *Trichinella spiralis* migrate through different organs (Despommier et al. 2005), i.e., the lungs. Harley and Gallicchio (1971) studied the migration of *T. spiralis* in orally infected rats and isolated the NBL from the lungs. However, they did not determine whether the parasites were only passing through or were retained in such organs. According to the literature, the lungs would act as a parasite entrapment and destruction site (Binaghi et al. 1981; Wang and Bell 1986; Bruschi et al. 1992). These findings suggest that the lungs provide a suitable environment for retention and defense processes.

The effector immune mechanism against NBL is related to the presence of antibodies (Abs) that have specificity against the NBL surface antigen (Ag) and leukocytes that function as effector cells through antibody-dependent cell-mediated cytotoxicity (ADCC) reactions (Kazura 1981; Gansmüller et al. 1987; Venturiello et al. 1993, 1995). Nevertheless, the precise location in the host's body where such mechanisms occur remains unknown. Because the size of the NBL is on the same order of magnitude as lung blood capillaries, NBL retention may occur in this organ, where the parasite might be attacked by effector cells and specific Abs. Only the larvae capable of evading this mechanism would invade the striated muscle.

In our previous work, we demonstrated that on its migratory route through the lung, *T. spiralis* NBL evoke an inflammatory allergic response together with bronchus-associated lymphoid tissue (BALT) and goblet cells hyperplasia (Venturiello et al. 2007). The parameters of the development of this inflammatory process remain to be elucidated.

The mucosal immune system is an integrated system, and the immunization at one site may produce a protective immunity at another site (Lamm and Phillips-Quagliata 2002; Roux et al. 2003;

Abbreviations: BALT, bronchus-associated lymphoid tissue; NBL, newborn larvae; ADCC, antibody-dependent cell-mediated cytotoxicity; Abs, antibodies; Ag, antigen; Igs, immunoglobulins; ML, muscle larvae; p.i., post-infection; LTEs, lung tissue extracts; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; ML-ESP, muscle larvae excretory–secretory products; Ig LSCs, immunoglobulins lung secreting cells; PR, positivity ratios.

* Corresponding author at: Cátedra de Inmunología, Departamento de Microbiología, Inmunología y Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Buenos Aires, Argentina. Tel.: +54 11 4964 8259; fax: +54 11 4964 0024.
E-mail address: sventuri@ffyb.uba.ar (S.M. Venturiello).

0171-2985/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2011.02.002

Holmgren and Czerkinsky 2005). Our results suggested that the inflammatory process observed during *T. spiralis* infection in the lung results from a signal that originated in the gut due to the penetration of the muscle larvae (ML) into the gut epithelium, which develops an inflammatory process that ends with the rejection of the adult worms from such mucosa (Ierna et al. 2008).

Taking into account this evidence and previous findings, we aimed to characterize the kinetics of the appearance of the immunological parameters involved in the development of the inflammatory process in the lung during the intestinal phase of *T. spiralis* infection in rats and to evaluate the role of the lung in the retention and death of the parasite.

Materials and methods

Animals and infection

Two-month-old female Wistar rats were orally infected through a gastric canula with 2000 ML per rat. The ML were obtained from the muscle tissue of Swiss mice by the artificial digestion method (Nuñez et al. 2005). As controls, uninfected animals were administered PBS orally by gastric canula. A minimum of five animals per were used in each experiment. The animals were provided with water and food *ad libitum*, and they were exposed to 12 h light–dark cycles; the room temperature was kept at 21 ± 1 °C. All the experiments were approved by the Review Board of Ethics of the Instituto de Estudios de la Inmunidad Humoral (IDEHU) and conducted in accordance with the guidelines establish by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

Histological and immunohistochemical analysis of lung tissue

The lower respiratory tract was removed from the animals (n = 5–6/day p.i.) that were sacrificed on days 1, 2, 3, 6 and 13 p.i. and from the controls, and the removed tissue was subjected to the Sainte-Marie technique (Sainte-Marie 1961). Paraffin from the tissue sections was removed as previously described (Venturiello et al. 2007).

The Giemsa (Biopur, Rosario, Argentina) staining was employed to visualize the BALT, and Alcian Blue 8GX (CI 74240; Mallinckrodt, St. Louis, MO, USA)-PAS/Gill's Hematoxylin (Biopur) was used to assess the hyperplasia and the number of goblet cells. The goblet cell count was performed in the epithelium located close to the BALT and in the primary and secondary bronchial epithelium at 400× magnification. The results were expressed as the number of goblet cells/100 epithelial cells. The eosinophil and mast cell counts in the lung parenchyma were completed in sections stained with the modified Luna's methodology (Tomasi et al. 2008) and Toluidine blue (CI 50240; Merck, Darmstadt, Germany) (Tomasi et al. 2003), respectively. The eosinophils and mast cells were counted in 100–150 randomly selected fields for each sample at 400× magnification employing a grid with a known area (62,500 μm^2 at 400× magnification) attached to a microscope ocular (Olympus, Tokyo, Japan). The results were expressed as the number of cells/mm^2.

To determine the phenotype of the cells present in the BALT, the lung tissue sections were incubated with the following antisera: (1) IgE$^+$ cells: a goat anti-rat IgE serum (Bethyl Laboratories, Inc., Montgomery, TX, USA), followed by a FITC-conjugated anti-goat IgG serum (Sigma, St. Louis, MO, USA); (2) CD4$^+$ cells: a mouse anti-rat CD4 mAb (Pharmingen, San Diego, USA), followed by a FITC-conjugated F(ab′)$_2$ anti-mouse IgG serum (ICN, Cappel, Aurora, OH, USA); and (3) IgA$^+$ cells: a FITC-conjugated anti-rat IgA serum (Bethyl Laboratories). The number of cells in the BALT was recorded for 15 fields at 1000× magnification using a fluorescence microscope (Olympus, Tokyo, Japan) by two independent observers.

Obtention of lung tissue extracts (LTEs)

The LTEs were obtained on days 1, 2, 3, 6 and 13 p.i. using the Perfext method (Villavedra et al. 1997) with slight modifications to detect total and specific immunoglobulins (Igs), cytokines and chemokines. Briefly, the rats (n = 5/day p.i.) were bled and infused with PBS plus heparin (5000 UI/ml) into the heart. The perfused organs were cut into small pieces, placed in an extraction solution containing 90 mM CHAPS (Research Organics, Cleveland, USA) in PBS and protease inhibitors (EDTA-free Complete, Roche Diagnostics, Mannheim, Germany) at 2 μl/mg of tissue and frozen at −70 °C. After thawing, the extraction was performed overnight at 4 °C using a homogenizer. After centrifugation, the supernatants were collected, filtered through a 0.22 μm filter (Millipore Co, Bedford, MA, USA), aliquoted and kept frozen at −70 °C until use.

Lung cell suspensions

The perfused lungs were obtained as described above and removed. The cell suspensions were prepared by cutting the tissue into small pieces and later digested in RPMI (Gibco, Grand Island, NY, USA) plus collagenase A (0.5 mg/ml, Roche Diagnostics), DNase (0.1 mg/ml, Roche Diagnostics), L-glutamine (1.46 g/100 ml, Gibco), penicillin (100 IU/ml) and streptomycin (100 μg/ml, Gibco) for 40 min at 37 °C with occasional shaking. The tissues were homogenized, and the cell suspensions were passed through a 40-μm nylon sieve. The remaining erythrocytes were lysed with isotonic ammonium chloride buffer. The cell suspensions were resuspended and washed twice with PBS plus 5 mM EDTA (Gibco) and 3% fetal calf serum (FCS, Gibco), and the cells were counted using a hemocytometer and Trypan blue dye (bioMérieux, Marcy L'Etoile, France). The cell viability was invariably higher than 95%.

Finally, the cell suspensions were suitably resuspended in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 100 μg/ml streptomycin, 100 IU/ml penicillin (Gibco) and 5% FCS (Gibco).

Detection of NBL in the lungs

To detect the NBL in tissue sections, we used histochemical techniques and immunofluorescence using a reference human anti-NBL surface serum followed by a FITC-conjugated anti-human serum (Sigma). The *T. spiralis* in the stage of NBL was also recovered from the lung cell suspensions (n = 5/studied day) that were obtained as described above and subsequently counted in grooved Petri dishes and observed using a microscope by two independent observers. To assure that the enzymatic treatment did not kill the larvae, the normal lungs spiked with viable NBL were also processed as positive controls. Because the NBL are shed into the bloodstream and lymphatic circulation from the gut by the adult worms on and after day 5 p.i. (Denham and Martínez 1970; Despommier et al. 2005), these methodologies were conducted on days 6 and 13 p.i.

ELISPOT assay

To detect the presence of the total and specific anti-muscle larvae excretory–secretory products (ML-ESP) IgA, IgE, IgG1 and IgG2a lung secreting cells (Ig LSCs), ELISPOT assays were performed on days 3, 6 and 13 p.i. as previously described (Czerkinsky et al. 1983) with slight modifications. Briefly, 96-well nitrocellulose-bottom microtiter MultiScreen HTS plates (Millipore) were coated overnight at 4 °C with ML-ESP (50 μg/ml) obtained as described by Nuñez et al. (2000) or the following capture antisera: goat anti-rat IgA serum (Bethyl Laboratories); sheep anti-rat IgE serum (Bethyl Laboratories); and goat anti-γ1 or goat anti-γ2a chain serum (Bethyl Laboratories). After washing in sterile PBS, the plates