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PATHOLOGIC CHANGES IN LUNGS CAUSED BY MEXICAN ISOLATES OF *TRYPANOSOMA CRUZI* IN THE ACUTE PHASE OF INFECTION IN MICE

VALERY G. MELNIKOV, FRANCISCO FIERRO VELASCO, FRANCISCO ESPINOZA GÓMEZ, FERNANDO GUZMÁN RODRÍGUEZ, AND OXANA R. DOBROVINSKAYA*

Faculty of Medicine, and Center for Biomedical Research, University of Colima, Colima, Colima, Mexico; Faculty of Medicine, Autonomous University of Guadalajara, Guadalajara, Jalisco, Mexico

Abstract. Chagas' disease, which is caused by the protozoan parasite *Trypanosoma cruzi*, is a significant public health problem in the Americas. Its clinical presentation varies significantly in different geographic regions. Using experimental infection in mice, we studied the pathologic changes in lungs in the acute phase of the disease caused by three Mexican isolates of *T. cruzi*. Clusters of parasites and inflammatory reactions were found in the walls of conducting airways and pulmonary vessels. Inflammation was more intense in the small vessels. Although the parasites were not found in the alveolar walls, severe pathologic changes in these structure were observed and included alveolar wall thickening and inflammatory infiltration. Furthermore, serous liquid, fibrin fibers, hyaline membranes, and erythrocytes were found in the alveolar spaces. The pathomorphologic changes observed in the infected mice are consistent with pneumonitis.

INTRODUCTION

American trypanosomiasis, or Chagas' disease in humans, is caused by the protozoan parasite *Trypanosoma cruzi* and represents a significant public health problem in many regions of the Americas. An estimated 16–18 million persons are infected, and approximately 50,000 new cases are reported each year.[1] In recent years, impressive progress has been attained in the control of triatomine vectors transmitting the parasites.[2] Nevertheless, there are still serious problems with this disease. Infected persons gradually develop acute, indeterminate, and chronic phases of disease. The course of the acute phase, characterized by an abundant parasitemia, is frequently mild with unspecific symptoms that may be confused with other infections. However, serious neurologic, cardiac, and pulmonary complications occasionally occur.[3–7] Specifically, children and patients with immune deficiencies represent the group at greatest risk in the acute phase.[7,8] Clinical manifestations of the chronic phase may range from an absence of symptoms to severe disease with cardiovascular and gastrointestinal involvement.[9] In general, development of the chronic phase, its clinical presentation and severity may depend on the course and complications of the acute period. Since traditionally used antichagasic drugs such as nifurtimox and benznidazole are effective in the acute phase and ineffective in the chronic phase, the correct diagnostic and treatment in the acute phase is of primary importance.[10,11]

Since the discovery of *T. cruzi* by Carlos Chagas in 1909,[12] most clinical studies of this disease were concentrated in Brazil and Argentina. Therefore, a clinical description of the evolution of Chagas' disease was done basing on the data obtained in patients infected with the strains distributed in these regions. However, significant variations in the course of the infection in different geographic regions were observed.[13] These differences are thought to be caused by genetic diversity of the vector, genetic differences in host organisms, and heterogeneity of *T. cruzi*.[14–20] Therefore, detailed characterization of the strains isolated in distinct geographic areas is very important. Mexican strains are of the special interest because they have been studied less intensively than in other disease-endemic countries, and also because of extensive migration of people from Mexico to United States, where disease could be transmitted in a silent fashion by asymptomatic infected blood donors.[9,21]

Mice susceptible and resistant to *T. cruzi* are widely used as an adequate experimental models for studying American trypanosomiasis.[15,22–24] Biologic behavior and tissue tropisms of different *T. cruzi* strains have been characterized in these murine models.[18,22,25] Animal models permit detailed temporal analysis of pathologic changes in tissues in different phases of infection that is not possible in humans. In general, all *T. cruzi* strains have demonstrated tropisms for muscle and nerve tissue. However, the presence of the parasite has also been demonstrated in ear, skin, spleen, kidney, pancreas, and liver.[23] In lungs of mice infected with South American strains of *T. cruzi*, amastigotes were detected only in the muscular stratum of pulmonary blood vessels.[7,23] Pulmonary pathologic changes caused by this parasite was limited to focal inflammation.

Conversely, cases of acute edema of hypertensive lung, pneumonitis and pneumopathy have been reported.[3,6,7] Our work documents the pathologic changes in lungs of mice during the acute phase of infection caused by three isolates of *T. cruzi* from different regions of Mexico. These data are useful in understanding the pathophysiology of Chagas' disease and for clinical prognosis.

MATERIALS AND METHODS

Mice and parasites. Male BALB/c mice were obtained from our breeding facility, housed in light- and temperature-controlled conditions, and given food and water *ad libitum*. All experimental procedures followed the ethical standards for investigation of experimental pain in animals.[26] All protocols and experimental procedures were reviewed and approved by the Committee for Bioethics and Biosafety of the University of Colima.

The CH4 isolate of *T. cruzi* was isolated from a patient with chagasic cardiomegaly and provided by Dr. Mario Barrera (Instituto Hideo Noguchi, Mérida, Yucatan, Mexico). The Albarrada and Zarco isolates were isolated by our group from *Triatominae* intra-domestic vectors collected in urban areas of Colima State from houses in which inhabitants were seropos-

* Address correspondence to Dr. Oxana R. Dobrovinskaya, Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio s/n Col. Villa San Sebastián, Colima, Col. Mexico, CP 28045. E-mail: oxana@cgic.ucol.mx

itive for *T. cruzi*. All isolates were maintained *in vitro* in liver infusion tryptose (LIT) medium at 29–31°C.[27]

To obtain bloodstream trypomastigotes, two-week-old mice were infected with a intraperitoneal inoculation of 10^5 metacyclic trypomastigotes from late stationery phase (degraded) LIT cultures. Parasitemia was monitored every three days. Blood samples were collected from the tail vein to determinate the number of parasites per milliliter of blood. When maximum parasitemia was reached, the mice were humanely killed, blood was recollected from the hearts, and the concentration of the parasites was estimated. All experimental groups of 8–10-week-old mice were injected intraperitoneally with 10^5 bloodstream trypomastigotes of *T. cruzi* per mouse.

In preliminary experiments, parasitemia and mortality were studied. For each *T. cruzi* isolate, 20 animals were infected. The level of parasitemia was monitored every three days. After the peak of parasitemia was reached, the concentration of parasites in the bloodstream gradually decreased and finally stabilized at a low level. The period up to parasitemia stabilization was considered the acute phase.

Histopathologic studies. For histopathologic analysis, 40 animals were inoculated with CH4 and two groups of 20 animals each were inoculated with the Albarrada and Zarco isolates. The number of animals per group was determined taking into account the mortality caused by each isolate in preliminary experiments: 30% for both Zarco and Albarrada and 50% for CH4. The parasitemias in these experimental groups were also monitored. They were coincident with the parasitemia curves described for each strain in preliminary experiments. Two days before the maximum parasitemia was expected, surviving animals (20, 14, and 13 animals inoculated with the CH4, Zarco, and Albarrada isolates, respectively) were humanely killed with an overdose of sodium pentobarbital. Organs and tissue samples were collected, fixed in 10% buffered-formalin solution for three days, embedded in paraffin, and processed for routine histologic analysis. Tissue sections (3 μm) were stained with hematoxylin and eosin, and examined by optical microscopy using digital imaging with an Axioscop microscope, an MR5 digital camara, and Axiovision software version 4.1 (Carl Zeiss, Göttingen, Germany). Organs from healthy, untreated, saline-injected mice of the same age as experimental animals were also examined. We did not detect any pathologic changes in lungs of animals in these control groups.

Statistic analysis. Fisher's exact test and EPIDAT 2.0 (Pan American Health Organization, Washington, DC) were used for frequency comparisons of different types of pulmonary lesions in three groups of experimental animals.

RESULTS

General observations of biologic behavior and tissue tropism. The peak parasitemia was reached in all groups of infected animals on days 20–24 after parasite inoculation. Isolates differed in their maximum (mean ± SD) parasitemias: $2.2 \pm 0.8 \times 10^6$ parasites/mL for Albarrada, $4.2 \pm 1.5 \times 10^6$ parasites/mL for Zarco, and $8.5 \pm 2.6 \times 10^6$ parasites/mL for CH4. Seventy percent of mice infected with the Albarrada or Zarco strains and 50% of mice infected with the CH4 strain survived. All three isolates showed cardiomuscular tropism. Besides, parasites were detected in the lungs, muscular layers of pulmonary airways, and vessels of all experimental groups of mice (Figure 1). Tropism for lungs was most pronounced in Albarrada strain–infected group: parasites were detected in these organs in 90% of the infected animals. Parasites were present in the lungs of 50% and 32% of the animals infected with the Zarco and CH4 isolates, respectively.

Histopathologic findings in lungs. *Alveoli.* Although parasites were not in alveoli, the changes in these structures at a histologic level were noteworthy in all groups of infected animals (Figure 2). Hypercellularity and edema of alveolar walls resulting in their thickening were frequently observed. In extreme cases, the walls were approximately 10 times thicker than normal walls. The hypercellularity may be caused by proliferation of type II pneumocytes and macrophages. Mononuclear infiltrates with few lymphocytes were found. In

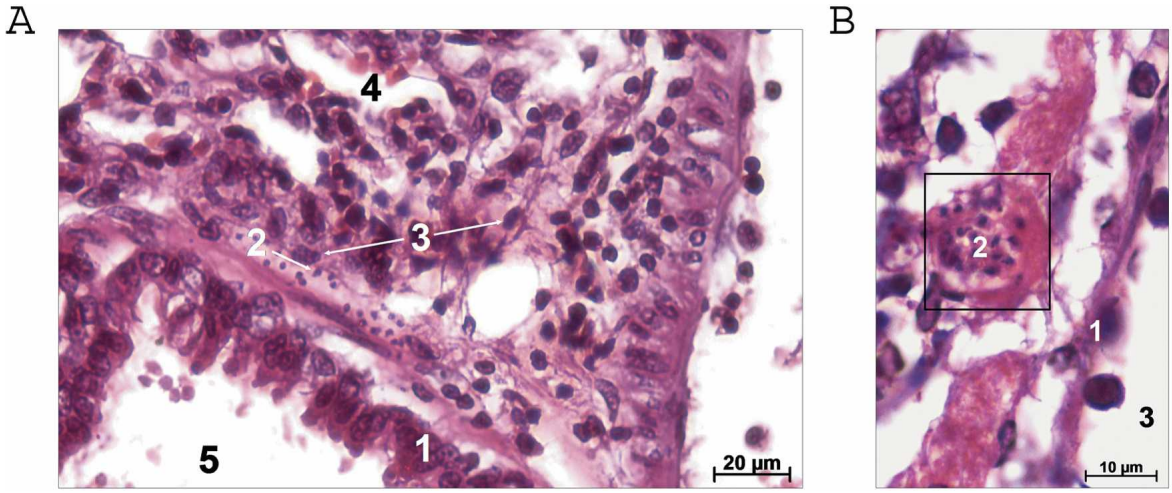

FIGURE 1. *Trypanosoma cruzi* parasitism lung structures. **A**, Parasites in the bronchial wall: 1 = bronchial epithelium; 2 = amastigotic nest in the muscular layer; 3 = immunocompetent cells; 4 = alveolar space; 5 = bronchial lumen. **B**, Parasites in the muscular cell of a pulmonary vessel: 1 = endothelial cells; 2 = amastigotic nest in the muscular tunica of the vessel; 3 = vessel lumen. This figure appears in color at www.ajtmh.org.

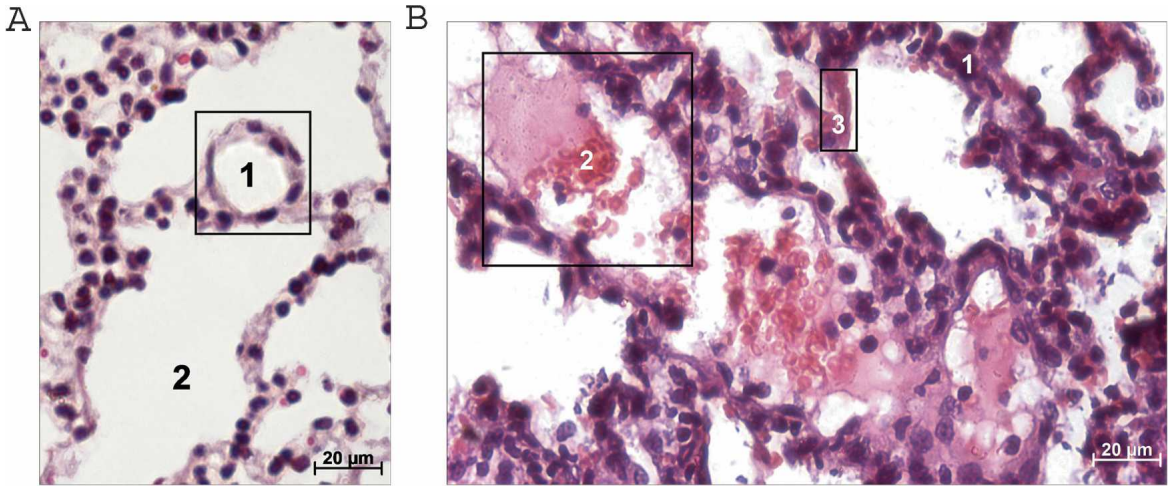

FIGURE 2. Alveolar pathologic changes caused provoked by *Trypanosoma cruzi* (Albarrada isolate). **A**, Normal mouse lung: 1 = small vessel; 2 = alveolar space. **B**, Abnormalities on day 27 of infection: 1 = hypercellularity of alveolar walls; 2 = alveolar spaces containing red blood cells, detritus, fibrous exudation, and mononuclear cells; 3 = alveolar sinusoid capillary distended by erythrocytes within it. This figure appears in color at www.ajtmh.org.

some animals infiltrates were relatively dense. Hyperemic capillaries were observed and the alveolar lining was prominent. Serous liquid, fibrin fibers, hyaline membranes, and erythrocytes were found within alveolar spaces. All of these abnormalities caused reduction of respiratory areas in extensive regions of the lungs.

Bronchi. Histopathologic analysis showed abnormalities in bronchi, with some distinctive changes depending on bronchi size. In large bronchi, most infected mice showed parasite infiltration without a significant inflammatory reaction. In medium and small bronchi, where the parasites were also present, the inflammatory reaction was pronounced (Figure 1A). Bronchial walls showed thickening and edema, resulting in lumen reduction. In some cases, the inflammatory process affected only muscular and submucous straits, thickening the walls due to the severe infiltration by lymphocytes, polymorphonuclear leukocytes, and macrophages. In some cases, mostly around the bronchioles, where the parasites were not found, we also observed peribronchiloitis presented as a surrounding heavy infiltration, mainly by lymphocytes, with edema of bronchiolar walls. In these animals, inflammatory cells were also found in well-preserved mucous and muscular straits, without any alterations in the bronchiole lumen.

Vessels. Most infected animals showed infiltration of the walls of large vessels with extensive clusters of parasites. The inflammatory response was extensive and associated with moderate interstitial edema that extended to the subendothelial space (Figure 3B). Walls of medium vessels were infiltrated irregularly with lymphocytes, and few polymorphonuclear leukocytes, which resulted in formation of irregular prominences in the walls of the lumen of vessels. In addition, interstitial edema, which was prominent in some areas, such as the subendothelial space, was observed. Vasculitis of medium veins was observed, but only in the group of animals infected with the Albarrada isolate. It was characterized by parasites in the myocytes of the muscular stratum accompanied by an inflammatory reaction, interstitial edema, and rupture of muscle fibers. The endothelial lining of small vessels

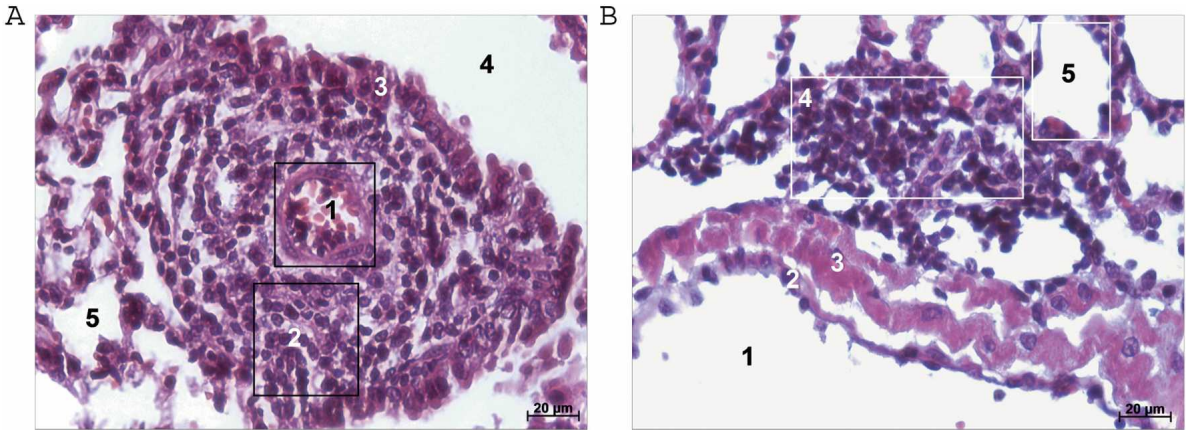

FIGURE 3. Vascular pathologic changes caused by *Trypanosoma cruzi* in the lungs. **A**, Small vessel perivascular infiltrate: 1 = vessel; 2 = mononuclear cells; 3 = bronchial epithelium; 4 = bronchial lumen; 5 = alveolar space. **B**, Localized large vessel perivascular infiltration: 1 = vascular lumen; 2 = endothelial cells; 3 = muscular stratum; 4 = mononuclear cells; 5 = alveolar space. This figure appears in color at www.ajtmh.org.

was prominent, with lymphocytes and a few polymorphonuclear leukocytes within its walls (Figure 3A).

Comparison of histopathologic changes in lungs caused by different *T. cruzi* isolates. Although histopathologic findings in lungs in all three groups of mice infected with different Mexican isolates of *T. cruzi* were similar, some variations in intensity of the changes were observed. Noteworthy was that the CH4 isolate, which had the highest level of parasitemia and caused severe damage of large vessels in our experimental model, produced only a mild inflammatory reaction in alveoli. Likewise, cellularity in alveolar walls was less pronounced when compared with changes caused by the Albarrada or Zarco isolates. In the group infected with the Zarco isolate, pulmonary tissue showed mild, generalized lung congestion, hypercellularity in the alveolar walls, proliferation of alveolar macrophages, and focal mononuclear infiltration in muscle of large bronchi. No brochiolitis or small vessel perivasculitis were seen in tissues obtained from animal groups infected with the CH4 or Zarco isolates.

Statistical analysis showed that the difference between experimental groups in parasite distribution in lung structures and the degree of the inflammatory reaction were not statistically significant (Table 1). Hemorrhaging in alveoli was more extensive in the groups infected with the Albarrada and Zarco isolates. The Zarco isolate caused significantly more pulmonary hemorrhaging than the Albarrada isolate. Although the Albarrada isolate showed the lowest level of parasitemia, it caused extensive changes in the lungs, in addition to these findings, such as bronchiolitis and perivasculitis of small vessels. We also observed moderate proliferation of alveolar macrophages and clusters of proliferating lymphocytes.

DISCUSSION

Tissue tropisms and pathologic changes caused by South American strains of *T. cruzi* in the mammalian host have been previously studied.[23,28] In the study with use of Brazilian strain of *T. cruzi* routinely maintained in laboratory mice, scattered amastigotes and patchy moderate inflammation with predominance of mononuclear cells were detected in myocytes of large pulmonary vessels between 20 and 30 days after parasite inoculation.[23] However, changes in alveoli or perivascular infiltrates have not been described. Strains of *T. cruzi* isolated from *Didelphis marsulialis* in Venezuela showed only mild parasitism and inflammation in the lungs of infected mice in the acute phase of infection.[28] Some Mexican strains of *T. cruzi* have been characterized genetically,[29] but data regarding their biologic behavior, tissue tropisms, and pathomorphologic changes caused in host organs and tissues are limited.[24] Pulmonary pathomorphology, which is caused by Mexican strains of *T. cruzi*, has not been studied.

In the present study, we have used the isolates from two geographic areas of Mexico: Colima (on the Pacific coast) and the Yucatan peninsula. In the state of Colima, the presence of *T. cruzi* in triatomine vectors has been known since 1939.[30] Recent studies have confirmed transmission of American trypanosomiasis in Colima, with a point seroprevalence of 2.4%.[31] The significant coincidence of intra-domestic triatominae vectors with cases of American trypanosomiasis, both asymptomatic and clinical, has been reported.[31] Although genetic characterization of some strains from this area was has been conducted,[29] the present work is the first study on tissue tropism and pathomorphologic changes caused by this parasite.

All three isolates used in this study showed cardiomyotropism, which is similar to most Mexican strains.[25] In contrast to findings of previous studies with South American strains,[23,28] we have detected numerous amastigotic nests in lungs and in the muscular stratum of all conducting branches of large bronchi and bronchioles. These clusters of parasites were accompanied by mononuclear inflammatory infiltrates. As a result, the walls of conducting airways showed thickening, edema, and significant lumen reduction. Although parasites were not observed in the respiratory zones of lungs (small terminal bronchioles and alveoli), serious pathologic changes in alveolar walls, including hypercellularity and mononuclear and lymphocyte infiltrates, were observed. Extensive alveolar hemorrhaging was detected. These findings represent a classic complex of changes characteristic of pneumonitis.

Although pathologic changes in vessels caused by *T. cruzi* in the acute phase of infection have been previously demonstrated,[32] pathmorphologic studies in pulmonary circulation system have not been conducted. We observed clusters of parasites and inflammatory infiltrates in the walls of pulmonary vessels, which resulted in a decrease in the lumen of vessels that was more extensive in small vessels and capillaries. In the group infected with the Albarrada isolate, perivasculitis of medium veins and small vessels was observed. As a result, venous pulmonary hypertension can be predicted in these mice.

Our findings suggest that *T. cruzi* isolates from different areas of Mexico cause more severe pulmonary lesions in mice lungs than strains from South America. Pulmonary pathologic

TABLE 1
Morphologic changes observed in lung structures in mice 20–24 days after intraperitoneal inoculation of *Trypanosoma cruzi*

T. cruzi isolate	No. of mice	Presence of amastigotes		Presence of inflammatory cells			Alveolar hemorrhage
		Vessels	Bronchi	Perivascul	Bronchial	Alveolar	
CH4 (Yucatán)	20	7	4	7	4	12	6
Albarrada (Colima)	13	8	5	3	3	9	6
Zarco (Colima)	14	3	6*	3	2	12†	11†
Saline (control)	21	0	0	0	0	0	0§
Total	68	18	15	13	10	34	23

* Regarding presence of parasites in vessels and bronchi, the difference between groups infected with the CH4, Albarrada, and Zarco isolates was not statistically significant.
† No statistically significant differences were observed in relation to inflammatory infiltration between all three experimental groups ($\chi^2 = 0.93$, $P = 0.33$).
‡ Alveolar hemorrhage was more pronounced in the two Colima isolates than in the Yucatan isolate ($\chi^2 = 5.1$, p = 0.02) and was more significant in the Zarco strain in contrast with the Albarrada and Yucatan strains ($\chi^2 = 7.32$, $P = 0.006$, odds ratio = 6.41, 95% confidence interval = 1.48–27.64).
§ Statistical comparison of experimental groups with control was omitted because there were no pathologic changes in the control group.

changes coincided with maximum parasitemia and febrile reactions. Some differences in tissue distribution and degree of pathologic manifestations were observed between isolates. Pulmonary hemorrhaging produced by the Colima isolates was more pronounced. These differences in pathomorphology of pulmonary tissue may be due to regional specificities of isolates studied. In addition, the Colima isolates were obtained from triatomine vector, whereas the CH4 isolate was obtained from a human host. However, triatominae were collected in houses in which asymptomatic or clinical cases of Chagas' disease were diagnosed. Therefore, the *T. cruzi* stocks isolated from these insects and used in our study could be adapted to humans.

Other protozoan parasites may also cause pulmonary disorders in murine models. Infection with *T. evansi*, the causative agent of surra disease, which affects predominantly hoofed animals, causes gradual development of intrabronchus inflammation, aggregation of inflammatory cells around the alveoli, congestion of bronchioles, septal edema, atrophy of alveolar walls, migration of macrophages, and emphysema in the lungs of the infected rats.[33] *Neospora caninum* has been reported to cause interstitial pneumonitis.[34] The final stage of maturation of the erythrocytic schizonts of *Plasmodium* in rodents may occur in lungs, affecting circulation rate, but not causing inflammation.[35] When immunosuppressive mice were infected with *Toxoplasma gondii*, parasites were found in the lungs, but pathomorphologic studies of these organs were not conducted.[36]

Knowledge of the distribution of the parasite in lung structures and pathomorphologic changes is important in the clinical prognosis and treatment of patients with Chagas' disease. Our study presents a detailed description of pulmonary histopathology in the acute phase of *T. cruzi* infection, when peak parasitemia is reached. Analysis of the pulmonary lesions produced by different protozoan parasites in the murine model shows the characteristic changes for each disease. In American trypanosomiasis caused by Mexican strains, these changes include the presence of abundant amastigotic nests in the muscular stratums of conducting airways and vessels, accompanied by extensive inflammatory infiltrates and severe widespread alveolar hemorrhages. Additional studies are needed to show the relationship between pulmonary lesions observed in infected mice and human clinical cases. However; based on our observations in mice, respiratory complications, pulmonary edema, acute bronchitis, bronchiolitis, peribronchiolitis, and pneumonitis could be expected in human patients. However, the clinical presentation must be discriminated from atypical pneumonia or bronchopneumonia. Furthermore, pathomorphologic changes reported in the present work could cause pulmonary hypertension that could result in a dilation of the right ventricle without affecting the left ventricle. In the clinic practice, right ventricular involvement is a typical characteristic of Chagas' disease, and it has been reported especially in the early stages. In clinical investigations of chagasic patients, pulmonary hypertension was associated with right ventricular dilation in many cases and has been attributed to left ventricular failure.[37] However, our data on pathologic changes in lungs during the acute phase of disease suggests that the possible mechanism could involve primary damage to the pulmonary vascular system.

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Authors' addresses: Valery G. Melnikov, Francisco Espinoza Gómez, and Fernando Guzmán Rodríguez, Facultad de Medicina, Universidad de Colima, Av. Universidad 333, Col. Las Víboras, Colima, Col. Mexico, CP 28040. Telephone/fax: 52-312-316-1099, E-mails: melnik@cgic.ucol.mx, fespin@cgic.ucol.mx, and gozman21@hotmail.com. Francisco Fierro Velasco, Facultad de Medicina, Universidad Autónoma de Gudalajara, Av. Ángel Liaño 500, Zapopan, Guadalajara, Jalisco, Mexico, CP 45110, Telephone/fax: 52-333-813-1571, E-mail: fierro@ucol.mx. Oxana R. Dobrovinskaya, Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio s/n Col. Villa San Sebastián, Colima, Col. Mexico, CP 28045, Telephone/fax: 52-312-3161 129, E-mail: oxana@cgic.ucol.mx.

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