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Infection with *Toxoplasma gondii* Increases Atherosclerotic Lesion in ApoE-Deficient Mice

Luciane R. Portugal,[1] Luciana R. Fernandes,[1] Giovana C. Cesar,[1] Helton C. Santiago,[1] Dirce R. Oliveira,[1] Neide M. Silva,[2] Andrea A. Silva,[3] Joseli Lannes-Vieira,[3] Rosa M. E. Arantes,[4] Ricardo T. Gazzinelli,[1,5] and Jacqueline I. Alvarez-Leite[1]*

Department of Biochemistry and Immunology[1] and Department of Pathology,[4] Institute of Biological Sciences, Federal University of Minas Gerais, and Laboratory of Immunopathology, René Rachou Research Center, Oswaldo Cruz Foundation,[5] Belo Horizonte, and Immunoparasitology Laboratory, Biomedical Sciences Institute, Federal University of Uberlândia, Uberlândia,[2] Minas Gerais, and Department of Immunology, Institute Oswaldo Cruz, Oswaldo Cruz Foundation, Rio de Janeiro, Rio de Janeiro,[3] Brazil

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***Toxoplasma gondii* is an intracellular protozoan that elicits a potent inflammatory response during the acute phase of infection. Herein, we evaluate whether *T. gondii* infection alters the natural course of aortic lesions. ApoE knockout mice were infected with *T. gondii*, and at 5 weeks of infection, serum, feces, and liver cholesterol; aortic lesion size, cellularity, and inflammatory cytokines; and levels of serum nitrite and gamma interferon (IFN-γ) were analyzed. Our results showed that serum cholesterol and atherogenic lipoproteins were reduced after *T. gondii* infection. The reduction of serum levels of total cholesterol and atherogenic lipoproteins was associated with increases in the aortic lesion area, numbers of inflammatory cells, and expression of monocyte chemoattractant protein 1 and inducible nitric oxide synthase mRNA in the site of lesions as well as elevated concentrations of IFN-γ and nitrite in sera of *T. gondii*-infected animals. These results suggest that infection with *T. gondii* accelerates atherosclerotic development by stimulating the proinflammatory response and oxidative stress, thereby increasing the area of aortic lesion.**

Atherosclerotic lesions are characterized by progressive accumulation of lipids, macrophages, natural killer (NK) cells, T and B cells, smooth muscle cells, and fibroproliferative elements in the intima of arteries (30). However, hypercholesterolemia and, mainly, high serum levels of low-density lipoprotein (LDL) are considered an absolute prerequisite for lesion establishment. Several genetic and environmental risk factors have been found to contribute to the development of atherosclerosis (2, 22, 27, 33). Among these factors, the immune system is one of the most important. Innate and adaptive immune responses modulate both the rate of lesion progression and composition of atherosclerotic lesions. ApoE knockout (KO) mice crossed into a recombination activating gene (Rag)-deficient background, which lack B and T lymphocytes, had an 80% decrease in the extension of atherosclerotic lesions (32). In addition, crosses of ApoE KO mice with different types of B- or T-cell-deficient mice also decreased atherosclerotic lesions (2, 22, 40). Inflammatory cytokines and chemokines, such as gamma interferon (IFN-γ), tumor necrosis factor alpha (TNF-α), TNF-β, interleukin-12 (IL-12), IL-1β, and monocyte chemoattractant protein 1 (MCP-1), increase the influx of monocyte into the endothelium, an important step in fatty streak formation (21, 24, 39). Therefore, atherosclerosis is now considered a chronic inflammatory disease, and systemic infections are thought to play an important role in initiating and/or perpetuating the pathophysiology of aortic lesions. In fact, some studies indicate that bacterial and viral pathogens could be responsible for atherosclerotic development (35). Sunnemark et al. (37) have shown that the combination of an infection of the protozoan parasite *Trypanosoma cruzi* with an atherogenic diet induced atherosclerotic lesions in the aorta of atherosclerosis-resistant CBA/J mice.

Toxoplasma gondii is an obligate intracellular protozoan parasite which is spread worldwide, infecting as many as one billion people. In the acute phase of infection, *T. gondii* tachyzoites trigger the synthesis of IL-12 and other costimulatory cytokines, which initiate the synthesis of IFN-γ by NK cells and $CD4^+$ $CD8^-$ αβ T lymphocytes (16, 17, 20, 23). In the chronic phase, levels of proinflammatory cytokines decrease, but highly polarized Th1 $CD4^+$ T lymphocytes as well as $CD8^+$ T lymphocytes are thought to maintain a robust IFN-γ production, a main component of the host resistance to infection with *T. gondii* (14, 18). The parallels between the inflammatory processes elicited during *T. gondii* infection and those involved in the pathogenesis of atherosclerosis led us to ask whether *T. gondii* infection could interfere with the development and progression of aortic lesions in the ApoE KO mice. ApoE is a major constituent of very low density lipoproteins (VLDL) and chylomicrons and is an important ligand for the receptor that mediates the uptake of these lipoproteins. ApoE KO mice have impaired clearance of VLDL and chylomicrons from the blood, which results in hypercholesterolemia and favors the

* Corresponding author. Mailing address: Laboratório de Bioquímica Nutricional, Departamento of Bioquímica e Imunologia, ICB—UFMG, Caixa Postal 486, 30161-970, Belo Horizonte, MG, Brazil. Phone: 55-31-34992652. Fax: 55-31-34415963. E-mail: alvarez@ufmg.br.

development of atherosclerotic lesions (28, 29). The atherosclerotic lesions of ApoE KO mice exhibit a similar distribution, microscopic appearance, and cellular composition to those found in humans (19).

Our findings show that toxoplasmosis promotes atherosclerotic development, despite a reduction in serum cholesterol and atherogenic lipoproteins. We observed an increase in the inflammatory infiltration, inducible nitric oxide synthase (iNOS), and MCP-1 mRNA expression in the lesion area in the aortas of animals infected with *T. gondii*. We conclude that the stimulation of the proinflammatory response, including systemic production of IFN-γ as well as enhancement of oxidative stress, could be responsible for accelerated atherosclerosis during toxoplasmosis infection in ApoE KO mice.

MATERIALS AND METHODS

Animal and diet. Ten-week-old, male, ApoE-deficient KO mice were distributed, taking into consideration initial body weight (20 g) and total cholesterol (380 mg/dl). Body weight and food intake were evaluated daily and weekly during the 5 experimental weeks. The protocol was approved by the Animal Care Committee of Universidade Federal de Minas Gerais (UFMG process 039/03). As *Toxoplasma* infection induced anorexia in infected mice, food offered to the control mice was adjusted to be the same amount as that consumed by experimental animals.

Experimental infection. The low-virulence ME-49 strain of *T. gondii* was used to infect animals in this experiment. For experimental infections, mice received 10 ME-49 cysts in a volume of 0.1 ml by the intraperitoneal route.

Blood, liver, and feces samples. Serum lipoproteins were separated by fast protein liquid chromatography, as previously described (12, 31). Liver was removed, washed in saline solution, dried in filter paper, weighed, and frozen at −20°C. Feces from the last experimental day were collected, homogenized, and frozen at −20°C. The hepatic and fecal lipids were extracted as previously described (27). Total cholesterol levels in fast protein liquid chromatography fractions and hepatic and fecal extracts were determined by using commercial kits (KATAL, Belo Horizonte, Brazil).

Quantification of serum IFN-γ and nitrite/nitrate levels in the sera. Nitrate was reduced to nitrite in lipid-free serum with nitrate reductase and measured according to the Griess colorimetric reaction (9). Serum IFN-γ was assayed in a two-site enzyme-linked immunosorbent assay with the rat anti-IFN-γ monoclonal antibody R46A2 (American Type Culture Collection, Rockville, Md.) and a polyclonal rabbit serum specific for the cytokine, as previously described (4). IFN-γ levels were calculated by reference to the standard curve constructed with recombinant cytokine (Genzyme, Cambridge, Mass.).

Histological analysis. The heart and proximal section of the aorta were removed from animals and cleaned of adventitial tissue. The top halves of the hearts were obtained under stereoscopic observation and fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate-buffered saline at room temperature. The specimens were routinely processed for paraffin embedding and analyzed according to the methods described by Paigen et al. (28) with modifications as briefly described below. Every consecutive section (10-μm thick) throughout the aortic root area (300 μm) was stained with hematoxylin and eosin. The aortic root area was recognized by the proximal presence of aortic valve leaflets. One of every three sections (in a total of 10 sections per mouse) was kept for morphometric analysis with an image analyzer (Kontron Eletronic 300 software) to process images obtained from a microscope coupled to a digital camera. The total lesion area of each animal was the sum of lesion areas obtained from the selected 10 sections. The results were expressed as averages of the total lesion area per group of 11 noninfected and 12 infected animals. To determine the cellularity of the lesion, the number of inflammatory cells per field was counted. The area of each field was determined automatically by the software ($n = 6$ mice, 3 nonconsecutive sections per animal). Extracellular matrix staining was performed as previously described (7) on 4 nonconsecutive sections per animal ($n = 6$ animals per group) of the ascending aorta adjacent to those stained with hematoxylin and eosin. Extracellular collagen was visualized with Gomori trichrome stain. All microscopic analyses were performed by a scientist blinded to the groups.

Measurement of MCP-1 and iNOS expression by RT-PCR. Fresh samples of the aortic root (as described for histological analysis) were used for RNA extraction. The total RNA was extracted with Trizol reagent (GIBCO BRL, Rockville, Md.) (controls, $n = 4$; *Toxoplasma*-infected mice, $n = 4$) and cDNA prepared by reverse transcription (RT). cDNA was amplified by PCR with the MCP-1, iNOS, and hypoxanthine phosphoribosyltransferase (HPRT) primers. After initial incubation for 3 min at 95°C, samples were submitted to denaturing at 94°C for 1 min, annealing for 1 min, and extension for 2 min at 72°C. After the designated cycle numbers for each primer, the program executed a final extension of 7 min at 72°C. Nucleotide sequences, PCR product sizes, annealing temperatures, and numbers of cycles for each primer are listed as follows: MCP-1 sense, CCGGAATTCCACTCACCTGCTGCTACTCATTCAC, and antisense, CGCGGATCCCTGGTTTCTTGGGTTTGCTGTG, 505 bp, 62°C, 27 cycles; HPRT sense, GTTGGATACAGGCCAGACTTTGTTG, and antisense, GATTCAACTTGCGCTCATCTTAGGC, 162 bp, 58°C, 31 cycles; INOS sense, CATGGCTTGCCCCTGGAAGTTTCTCTTCA, and antisense, GCAGCATCCCCTCTGATGGTGCCATCG, 827 bp, 54°C, 36 cycles. The PCR products were separated by acrylamide gel electrophoresis and stained with silver nitrate as previously described (1, 31).

Heart and aorta *T. gondii* parasitism. Tissue parasitism was evaluated by immunocytochemistry in deparaffinized aorta sections subjected to antigenic unmasking in a microwave oven as previously described (36).

Immunohistochemistry studies. Samples of the perilesional area (aortic root and adjacent cardiac muscle) were cryopreserved for the study of the presence of $CD4^+$, $CD8^+$, and $F4/80^+$ cells. Serial 5-μm-thick sections starting from the root of aorta were prepared, fixed in cold acetone, and stained with immunoperoxidase as previously described (11). Cells from three sections were counted for each animal.

Statistical analysis. The data were analyzed by the Komogorov-Smirnov test to verify normal distributions. Student *t* tests were used to compare control and infected groups with a level of significance set at a P value of <0.05.

TABLE 1. Body weight, food intake, hepatic lipids, and cholesterol, fecal lipids, and 3-alpha-OH-sterols of noninfected and *T. gondii*-infected ApoE KO mice for 5 weeks

Parameter	Result for[d]:	
	Control mice	*T. gondii*-infected mice
Food intake (g/mouse/wk)[a]	14.5 ± 1.5	15.0 ± 1.4
Initial body weight (g)[a]	21.5 ± 1.0	21.0 ± 1.0
Final body weight (g)[a]	18.4 ± 0.7	18.6 ± 0.9
Liver lipids (mg/g of liver)[b]	67.5 ± 5.0	56.1 ± 6.0
Liver cholesterol (mg/g of liver)[b]	4.27 ± 0.43	3.95 ± 0.19
Fecal lipids (mg/g of feces)[c]	24.0 ± 6.8	21.7 ± 4.0
Fecal 3-alpha-OH-sterols (mg/g of feces)[c]	2.09 ± 0.08	2.48 ± 0.22
Anti-*T. gondii* immunoglobulin G (absorbancy at 495 nm)[a]	0.05 ± 0.009	0.549 ± 0.039[e]

[a] $n = 14$ for control group; $n = 17$ for *T. gondii* group.
[b] $n = 8$ to 10 animals per group.
[c] $n = 6$ animals per group.
[d] Results are means ± standard errors.
[e] $P < 0.001$.

RESULTS

Toxoplasmosis reduces the serum cholesterol and atherogenic lipoproteins. There were no differences in initial and final body weight and food intake between the experimental and control groups (Table 1). However, there was a reduction in body weight in both groups during the experiment. This reduction, seen after the first week of infection, was probably due to high rates of catabolism and anorexia caused by the systemic Th1 response elicited by the infection with *T. gondii*. As food offered to the noninfected mice was adjusted to the amount consumed by infected animals, mice in the control group lost weight at the same rate as *T. gondii*-infected group.

The *Toxoplasma* infection significantly reduced the serum cholesterol concentration in both the fourth and fifth weeks of

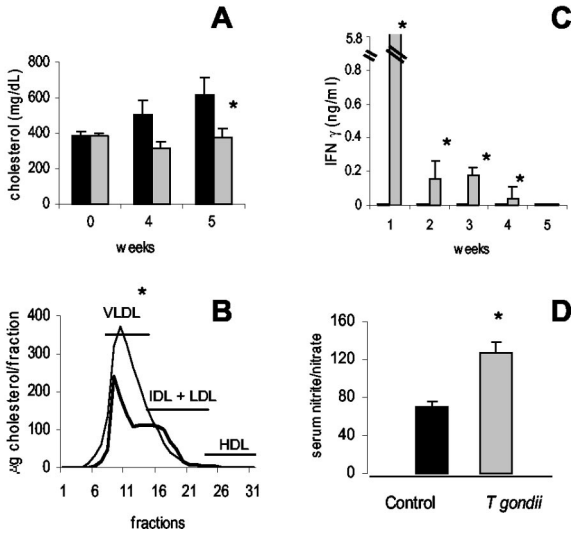

FIG. 1. (A) Total serum cholesterol of noninfected ($n = 14$, black bars) or *T. gondii*-infected ApoE KO mice ($n = 17$, gray bars) after 0, 4, and 5 weeks postinfection. (B) Lipoprotein profile of noninfected ($n = 5$, fine line) and *T. gondii*-infected ($n = 5$, bold line) ApoE KO mice after 5 weeks of experiment (VLDL, fractions 5 to 10; IDL + LDL, fractions 11 to 19; HDL, fractions 21 to 26). The averages ± standard errors of the VLDL, IDL + LDL, and HDL fractions were 355 ± 51, 99 ± 15, and 1.2 ± 15 for the control group and 175 ± 14, 109 ± 7, and 5.2 ± 2.1 for the *T. gondii* group, respectively. (C) IFN-γ concentration in serum of noninfected ($n = 6$, black bars) or *T. gondii*-infected ApoE KO mice ($n = 6$, gray bars) at the beginning of and during the experiment. (D) Serum nitrite/nitrate concentration (micromoles/liter) of noninfected ($n = 9$, black bar) and *T. gondii*-infected ($n = 12$, gray bar) ApoE KO mice after 5 weeks of experiment. *$P < 0.05$.

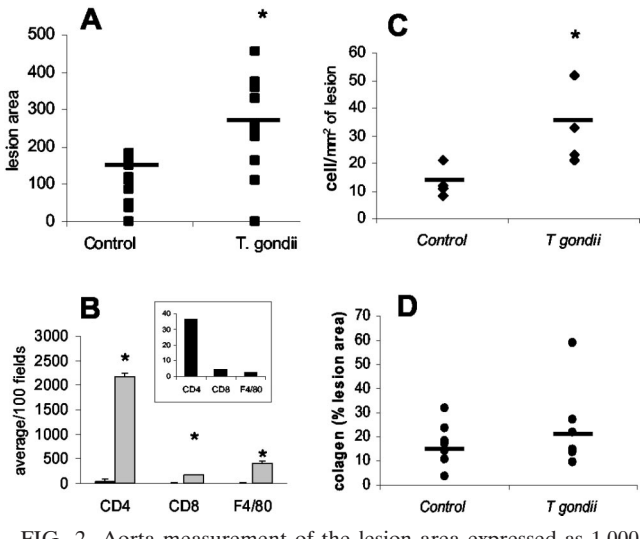

FIG. 2. Aorta measurement of the lesion area expressed as 1,000 micrometer^2 ($n = 11$ noninfected mice and 12 *T. gondii*-infected mice) (A), inflammatory infiltrate ($n = 6$ per group); phenotype of cells from control group (B), phenotype of inflammatory cells ($n = 6$ per group) (C), and percentage of collagen ($n = 6$ per group) (D) in noninfected and *T. gondii*-infected ApoE KO mice after 5 weeks of experiment. Squares (A), diamonds (C), and circles (D) represent individual measurements. Horizontal lines represent the averages of groups. (B) Hatched and black bars represent the average of number of cells per 100 fields in 7 infected and 3 control animals, respectively. *, $P < 0.05$.

the experiment (Fig. 1A). The reduction was mainly due to a reduction in atherogenic VLDL fraction (Fig. 1B). No significant difference was seen in high-density lipoprotein (HDL) fraction. When hepatic and fecal total lipids and cholesterol were analyzed, no differences were seen between the groups (Table 1), suggesting that the cholesterol reduction was not due to differences in liver concentration or intestinal excretion of lipids.

***T. gondii* infection increases serum levels of IFN-γ and nitrite during the 5 weeks of experimentation.** As acute *T. gondii* infection induces a Th1 immune response, we evaluated the serum concentration of IFN-γ at different times postinfection. IFN-γ levels were elevated in the first week postinfection and were significantly higher than controls until the fourth week postinfection (Fig. 1C). Consistent with the higher levels of IFN-γ, *Toxoplasma*-infected mice had serum nitrate/nitrite levels (127.6 ± 11.0 μM) which were 83% higher than those of the control mice (70 ± 5.7 μM) (Fig. 1D).

Increased aortic lesions in *T. gondii*-infected mice. Despite the reduction in the serum atherogenic fractions, histological analysis showed that the lesion area of *T. gondii*-infected animals was augmented compared to noninfected mice (Fig. 2A). *Toxoplasma*-infected mice also presented significantly increased inflammatory infiltration in atherosclerotic lesions (3.2 + 0.4 inflammatory cells/mm^2 of lesion versus 1.3 + 0.2 inflammatory cells/mm^2 of lesion, respectively) (Fig. 2B). Small mononuclear inflammatory cells, interposed between macrophages, characterized the cellular infiltration. Analysis of the cellular phenotype in the lesion area and the adjacent cardiac muscle revealed a predominance of CD4$^+$ cells in both uninfected and infected groups (Fig. 2C). However, the total number of cells was higher in the infected animals than in the controls. In *T. gondii*-infected animals, the percentage of F4/80-positive cells was about 16% of total cells, more than double the percentage of positive cells in the control group (6%). In addition, the total numbers of CD4$^+$, CD8$^+$, and F4/80-positive cells were 60, 42, and 161 times higher in *T. gondii*-infected mice than in control mice. No differences in collagen deposition in the lesion area were observed, since the percentage of stained area was similar between groups (17% ± 4% and 22% ± 7.7% of the total area, $P = 0.56$) (Fig. 2D).

After 5 weeks of infection, infected animals presented larger aortic lesions than the control group ([275.93 ± 22.9] × 10^3 μm^2 versus [129.49 ± 28.66] × 10^3 μm^2, respectively). A well-defined fatty streak composed of lipid-loaded macrophages and sparse inflammatory infiltrate was seen in control mice (Fig. 3A and C). In *T. gondii*-infected animals, the lesion was more extensive, with prominent degenerative alterations and inflammatory infiltration, characterizing a more advanced stage (Fig. 3B and D). Despite the influence of *T. gondii* on atherogenesis, no parasite was evident in the lesion site after 5 weeks of infection. However, parasites were found in cardiac muscle adjacent to the aortic valve insertion in 3 of the 11 mice that were studied (Fig. 3E and F). The right panels of Fig. 3 (CD4$^+$, CD8$^+$, and F4/80$^+$ cells) illustrate the leukocyte infiltrate in the perilesional area from mice infected with *T. gondii*, as detected by immunocytochemistry.

RT-PCR analysis of iNOS and MCP-1 showed a statistical

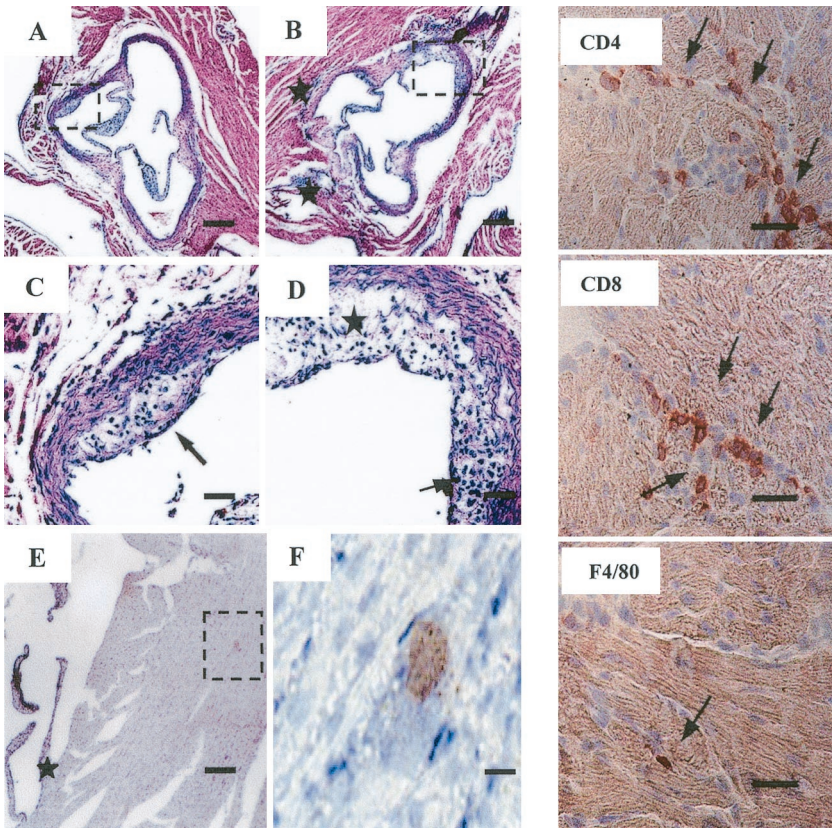

FIG. 3. Histological aspect of the aorta of noninfected and *T. gondii*-infected ApoE KO mice after 5 weeks of experiment; typical lesions of control (A) and infected (B) mice are within the dashed square. (B) Presence of inflammatory focus (star) in *Toxoplasma*-infected group but not in control group. (C) Presence of well-defined fatty streak in control group, constituted by xantomized macrophages (arrow). (D) In *T. gondii*-infected animals, the lesions are more developed, with areas of cellular proliferation (arrow) and degenerative alterations (star), characterizing a more advanced stage of lesion. (E and F) Presence of cyst (within dashed square) in cardiac muscle near the aortic valve insertion (star). Note that there is no infiltration around the cyst. (CD4, CD8, and F4/80) Immunohistochemistry of atherosclerosis lesions in *Toxoplasma*-infected animals. Arrows in indicate cells stained with CD4-, CD8-, and F4/80-specific monoclonal antibodies, respectively. Bars, 200 μm (A and B), 35 μm (C and D), 70 μm (E), 11 μm (F), and 25 μm (CD4, CD8, and F4/80).

increase in the expression of these transcripts in the aortas of *T. gondii*-infected mice compared to control mice (Fig. 4). MCP-1 is related to the recruitment of monocytes to the site of lesion and its expression can be stimulated by many factors, including oxidized LDL. In the same manner, high levels of iNOS are associated with an increase in LDL oxidation. Taken together, these data suggest that *T. gondii* infection can stimulate the migration of inflammatory cells and expression of proinflammatory factors to the site of atherosclerotic lesion, accelerating atherogenesis in infected mice.

DISCUSSION

The importance of concomitant infections in the development of aortic lesions is part of an ongoing debate concerning the pathogenesis of atherosclerosis. To the best of our knowledge, this is the first study correlating *T. gondii* infection with the development of atherosclerosis. Our results showed that *T. gondii* increased the size of aortic lesions in ApoE KO mice. Although serum cholesterol is a risk factor of atherosclerosis, we did not find a positive relationship between cholesterolemia and atherosclerosis in *T. gondii*-infected mice. Infected animals presented a reduction in the serum VLDL cholesterol fraction that represents an important proatherogenic lipoprotein in mice. These results agree with those obtained by Paigen et al. (29), Dansky et al. (6), and Grimsditch et al. (19), who suggested that the correlation between blood cholesterol and atherosclerosis is not evident in cases of concomitant infection.

Specifically, a decrease in serum cholesterol was also described by Sunnemark et al. (37), who fed CBA/J mice infected with *T. cruzi* a high-fat diet, and Doenhoff et al. (10), who infected ApoE KO mice with *Schistosoma mansoni*. Further, systemic chronic infections and inflammations are associated with low cholesterolemia and HDL cholesterol (25). However, in the examples provided above, the reduction of total cholesterol reflects an unspecific reduction of all lipoprotein fractions. Our results demonstrate a reduction in the VLDL fraction specifically. The reduction in this proatherogenic lipoprotein associated with unchanged levels of HDL cholesterol could suggest a reduction of atherogenesis in those mice. Contrarily, in the present study, this lipoprotein profile was associated with the enhancement of plaque formation in infected mice.

The mechanism for lowering the levels of VLDL during *T. gondii* infection is unknown. Nevertheless, it is known that the

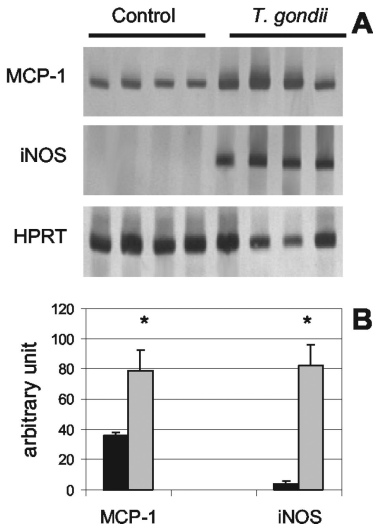

FIG. 4. (A) Expression of the MCP-1 and iNOS endogenous mRNA in aortic tissue of noninfected ($n = 4$) and *T. gondii*-infected ($n = 4$) ApoE KO mice. (B) Densitometric analysis of control (black bar) and *T. gondii*-infected (gray bar) bands. The quantification was normalized to the levels of HPRT expression. Bars represent means and vertical lines represent standard errors for each group. *, statistical difference ($P < 0.05$) compared to the noninfected group.

cholesterol is concentrated in the cell membrane and apical organelles of the tachyzoites (13) and that *T. gondii* is dependent on cholesterol derived from the host (3, 5). It is well known that lower intracellular levels of cholesterol stimulate the endogenous synthesis of cholesterol and enhance expression of LDL (B/E) receptor expression and, consequently, lipoprotein endocytosis and internalization of blood VLDL and LDL cholesterol. Indeed, in vitro studies demonstrated that LDL cholesterol uptake by infected host cells is two to three times higher than that of noninfected cells (5). Thus, the enhanced VLDL cholesterol uptake by infected host cells could be one factor determining the lower serum levels of VLDL cholesterol in infected animals. In addition, the hypocholesterolemic effects of cytokine production elicited during *T. gondii* infection could further contribute to the reduction of the total VLDL fraction and, consequently, of total cholesterol in infected animals.

High levels of proinflammatory cytokines produced in response to *T. gondii* infection have been found to promote atherosclerosis (24). TNF-α, IL-12, and IL-1β, for example, increase monocyte influx, a possible mechanism of atherosclerosis development. IFN-γ, TNF-α, and IL-12 are associated with the progression of early lesions, as demonstrated in experiments comparing immunodeficient and immunocompetent ApoE KO mice (32). Gupta et al. (21) found a 60% reduction in the atherosclerotic lesion size of the aorta when IFN-γ receptor-deficient ApoE KO mice were compared to control ApoE KO mice. Similarly, exogenous IFN-γ administration enhanced the atherosclerotic progression in ApoE KO mice (39).

The mechanism by which IFN-γ accelerates the plaque formation is not totally clear. This effect appears to be mediated at least in part by molecules induced by IFN-γ. The results presented here demonstrated that *Toxoplasma* infection is associated with higher serum concentrations of nitrite/nitrate and the expression of iNOS and MCP-1 mRNA in aortic lesions. The parasite elicited IFN-γ to trigger iNOS to degrade arginine and produce high levels of NO (8). Although NO is an important vasodilator component, high levels of NO contribute to the increase of oxidative stress via peroxynitrite formation and LDL oxidation (22, 26). The latter induces MCP-1 expression and the consequent migration of the monocyte toward the lesion site and lipoprotein uptake (22, 26). Oxidized LDL is also able to induce IL-12 production from macrophages and favors differentiation of naive T cells to a type 1 immune response (38), inducing more NO production and LDL oxidation. It is also possible that other immunological factors induced by IFN-γ are involved in the process of enlargement of atherosclerosis lesions in mice infected with *T. gondii.* This is an important question that we are currently addressing in our ongoing studies.

Histological analysis indicated that the lesions of *T. gondii*-infected mice are larger and contain more-intense inflammatory infiltrate than noninfected ApoE KO mice. The aortic lesions from control and *T. gondii*-infected animals were in the early to intermediate stages. In these stages, a pronounced plaque progression associated with Th1 immune responses, oxidative stress, macrophage migration, and lymphocyte influx occurs (2). Because *T. gondii* tachyzoites can invade any nucleated cells from their vertebrate host, one important question raised by our studies is whether local infection was initiating a process of tissue damage, consequent recruitment of inflammatory cells, and development of atherosclerosis. In the present study, we found parasite cysts in the heart tissue close to the aorta in 3 of 11 mice. However, immunocytochemistry studies did not reveal parasite antigens associated with aortic lesions. It is well established that infection with *T. gondii* causes a switch from a Th2 response to a Th1 immune response specific to nonrelated antigens (15, 34). Therefore, we hypothesize that *T. gondii* infection accelerates the development of atherosclerosis by promoting Th1 immune response to the components of ongoing aortic lesions.

Our results suggest that *T. gondii* infection provides a proatherogenic stimulus, inducing Th1 cytokine production and oxidative stress, which culminates in a rapidly progressing atherosclerotic lesion. Given the large population of people infected with *T. gondii* both in developed and developing countries, we believe *T. gondii* is a potential risk factor for the development of atherosclerosis in humans and deserves further investigation.

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