Absence of *Mycobacterium tuberculosis* in Arterial Lesions from Patients with Takayasu's Arteritis

LAURENT ARNAUD, EMMANUELLE CAMBAU, ISABELLE BROCHERIOU, FABIEN KOSKAS, EDOUARD KIEFFER, JEAN-CHARLES PIETTE, and ZAHIR AMOURA

ABSTRACT. ***Objective.*** Previous studies have suggested that *Mycobacterium tuberculosis* (MT) could be involved in the pathogenesis of Takayasu's arteritis (TA). The search for MT in arterial lesions of TA has never been assessed directly by sensitive methods. Our aim was to assess the presence of MT in arterial samples obtained in patients with TA.

Methods. Fresh arterial samples were collected from 10 consecutive patients (9 women and 1 man, median age 42 yrs, range 19–67 yrs) with a diagnosis of TA according to the American College of Rheumatology criteria who underwent vascular surgical procedures for their disease. Three patients had recent onset of TA and 7 had longstanding disease. No patient had evidence of active tuberculosis. Arterial biopsies were collected during vascular surgical procedures, and were systematically studied by a pathologist specializing in vascular diseases. Presence of MT was assessed in the biopsies by acid-fast and auramine-fluorochrome stainings, mycobacterial cultures, and direct amplification test (DAT) for MT.

Results. Histological examination showed active (n = 5) and inactive (n = 5) arterial lesions. MT was not detected in arterial lesions of either active or inactive TA, by acid-fast and auramine-fluorochrome staining, mycobacterial cultures, or DAT. No DAT inhibitors were found.

Conclusion. Our study does not support a direct role of MT in the pathogenesis of arterial lesions in either recent or longstanding TA, but does not exclude the possibility of a cross-reaction between mycobacterial and arterial antigens. (First Release June 15 2009; J Rheumatol 2009;36:1682–5; doi:10.3899/jrheum.080953)

Key Indexing Terms:
TAKAYASU'S ARTERITIS — TUBERCULOSIS
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Takayasu's arteritis (TA) is a granulomatous arteritis predominantly affecting the elastic arteries such as the aorta, its major division branches, and the pulmonary arteries. The etiology of TA is still unclear more than 100 years after its first description by Takayasu[1]. However, most available data suggest that both cellular and humoral immune responses are involved in pathogenesis[2].

A link between TA and tuberculosis has been suggested for a long time, as TA is more common in individuals originating from Asia, Africa, and South America, where the incidence of tuberculosis is high, and because granulomatous lesions may be observed in both diseases[3,4]. Interestingly, Serratrice *et al*[5] and Duzova, *et al*[6] have reported cases of patients with simultaneous occurrence of TA and tuberculosis, further supporting a possible relationship between the 2 diseases.

Several laboratory findings support the idea of an antigen-specific immune reaction against *M. tuberculosis* in TA. Both humoral and cell-mediated immune responses directed towards *M. tuberculosis* antigens have been reported in patients with TA[7-11]. Aggarwal, *et al*[7] and Moraes, *et al*[8] have reported the existence of an increased humoral immune response directed towards the mycobacterial 65-kDa heat-shock protein (mHSP65) in patients with TA. Kumar Chauhan, *et al*[10] have demonstrated a significant correlation between T cells reactive against mHSP65 and its human homolog (hHSP60), as well as between anti-mHSP65 and anti-hHSP60 IgG antibodies, suggesting that *M. tuberculosis* may have a role in the immunopathogenesis of TA through a molecular mimicry mechanism.

Surprisingly, the presence of *M. tuberculosis* in arterial lesions of patients with TA has not been assessed directly using sensitive microbial assays[12]. The aim of our study was to assess the presence of *M. tuberculosis* in arterial lesions

From the Internal Medicine Department and French National Reference Center for Rare Systemic Auto-immune Diseases, National Reference Center for Mycobacteria, Department of Pathology, and Department of Vascular Surgery, Hôpital Pitié-Salpêtrière, Université Paris 6, Assistance Publique-Hôpitaux de Paris, Paris, France.

L. Arnaud, MD, Internal Medicine Department and French National Reference Center for Rare Systemic Auto-immune Diseases; E. Cambau, MD, PhD, National Reference Center for Mycobacteria; I. Brocheriou, MD, PhD, Department of Pathology; F. Koskas, MD, PhD; E. Kieffer, MD, Department of Vascular Surgery; J-C. Piette, MD; Z. Amoura, MD, Internal Medicine Department and French National Reference Center for Rare Systemic Auto-immune Diseases, Hôpital Pitié-Salpêtrière.

Address reprint requests to Prof. Z. Amoura, Service de Médecine Interne, Hôpital Pitié Salpêtrière, 47-83 Bd de l'Hôpital, Paris, France. E-mail: zahir.amoura@psl.aphp.fr

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of patients with TA by 3 different methods: acid-fast and auramine-fluorochrome stainings, mycobacterial cultures, and nucleic acid amplification, which is a sensitive method that may detect nonviable bacteria[13]. Presence of *M. tuberculosis* in arterial lesions of patients with TA would provide a clue to direct involvement of this infectious agent in the pathogenesis of TA.

MATERIALS AND METHODS

Patients. Our case series is based on the prospective analysis of arterial biopsies obtained in 10 consecutive patients with TA followed in the internal medicine department of our tertiary care center (Pitié-Salpêtrière Hospital, Paris, France). The diagnosis of TA was made according to the American College of Rheumatology (ACR) criteria[14]. Presence of a temporal artery abnormality, new-onset headache, visual loss, jaw claudication, or polymyalgia rheumatica was carefully excluded in the oldest patients, who had negative temporal artery biopsies and did not fulfill ACR criteria for giant cell arteritis[15]. These 10 consecutive patients were operated on in the vascular surgery department of our center, which specializes in surgical care of patients with TA[16]. All these patients underwent a vascular surgical procedure for either a clinically and/or hemodynamically significant arterial stenosis, or for an aortic aneurysm or stenosis. Fresh arterial biopsies were obtained during surgery, and samples of these biopsies were systematically analyzed by a pathologist with experience in vascular pathology (IB) and for presence of *M. tuberculosis* as a routine diagnosis procedure performed in our center. Local ethics commitee approval and patient informed consent were obtained before the study began.

Microbiological processing. Preparation of arterial samples was performed using the technique described by Shepard for leprosy biopsies[17]. Fresh arterial biopsies were washed in sterile phosphate buffered saline to remove the remaining blood. These samples were minced, crushed on stainless steel mesh, and mixed with 1 ml of sterile water under sterile conditions. The total volume was split into 3 aliquots. The first was used to prepare smears for acid-fast and auramine-fluorochrome stainings, and cultures on Lowenstein-Jensen culture media according to the usual procedures. The second and third aliquots were used for direct amplification testing (DAT), which is a very sensitive method to detect *M. tuberculosis*.

Amplification method. Nucleic acid amplification was performed using the sensitive Amplified *Mycobacterium tuberculosis* direct assay (AMTD; Gen-Probe Inc., San Diego, CA, USA), following the manufacturer's instructions. This test amplifies the 16S ribosomal RNA of the *M. tuberculosis* complex by a transcription-mediated amplification. The resulting amplicons are detected by a hybridization protection assay using a specific probe for *M. tuberculosis*[13].

Briefly, 50 μl of the second aliquot were added to a tube containing 25 μl of amplification reagent and 200 μl of oil. The tube was incubated at 42°C for 5 min in a heating block. The amplification enzyme (25 μl) was added, and the tube was incubated at 42°C for 2 h. Then, 20 μl of termination reagent were added, and the sample was incubated at 60°C for 10 min. After addition of the detection reagent (300 μl), the tube was incubated at 60°C for 11 min, and then remained at room temperature for 5 min. The samples were read in a Gen-Probe luminometer (Gen-Probe Inc.) and results were recorded in relative light units (RLU). According to the manufacturer's instructions, a positive test result was defined as a luminescence reading of 30,000 RLU or greater.

Positive and negative controls. Each test was run in duplicate and included positive (5×10^3 CFU/ml *M. tuberculosis*) and negative (5×10^3 CFU/ml *M. terrae*) extraction and amplification controls, as well as positive and negative hybridization controls provided by Gen-Probe. To rule out the presence of polymerase chain reaction inhibitors in the prepared arterial samples, additional positive controls were obtained by adding 100 bacilli of *M. tuberculosis* into the third aliquots, which were further submitted to the amplification procedure.

RESULTS

Patient characteristics. Arterial samples were obtained from 10 patients (9 women and 1 man, median age 42 yrs, range 19–67 yrs). All these patients were treated by corticosteroids and/or other immunosuppressive agents. Median delay between TA diagnosis and surgical procedures was 5.5 years (range 0–22 yrs). No patient had evidence of active tuberculosis at the time of surgery, as assessed by Mantoux test, chest radiograph, mycobacterial cultures of urine samples, bacilloscopy, and sputum cultures. Patient characteristics and surgical data are presented in Table 1.

Pathological analysis. Histological examination of arterial samples revealed active granulomatous arteritis in 4 patients, active nonspecific arteritis in 1 patient, and inactive arterial lesions with intimal and adventitial fibrosis highly suggestive of past arteritis in 5 patients (Table 2). Thus, 5 (50%) of 10 patients were classified as having pathologically active TA, and 5 patients (50%) as having pathologically inactive TA.

Microbiological analysis. Smears for acid-fast and auramine-fluorochrome staining, as well as mycobacterial cultures on Lowenstein-Jensen culture media were negative for all arterial samples (Table 2). *M. tuberculosis* was not detected by the AMTD DAT assay in patients with either pathologically active or inactive TA (Table 2).

DISCUSSION

Previous clinical and laboratory studies have suggested that *M. tuberculosis* could be involved in the pathogenesis of TA[3-11]. However, the search for *M. tuberculosis* in arterial lesions of patients with TA has never been examined directly by sensitive methods[12]. In this study, we have shown that *M. tuberculosis* could not be detected in arterial samples from 10 consecutive patients with TA of both Caucasian and non-Caucasian origins, with both pathologically active and inactive arteritis, by acid-fast and auramine-fluorochrome staining, mycobacterial cultures, or the sensitive AMTD amplification testing.

Increased prevalence of tuberculosis and of positive tuberculosis skin tests has been reported in Asian, African, and South American series of patients with TA[3,4]. However, these findings may be only fortuitous, as tuberculosis is endemic in these regions.

One interesting aspect of our study is that the search for *M. tuberculosis* was performed using microbiological techniques that may detect not only live *M. tuberculosis* bacilli, as the reference culture method, but also nonviable or dormant bacilli, and even nucleic acid that might have remained in the tissue. Arterial samples were prepared using Shepard's method, known to be one of the most sensitive methods to prepare slow-growing mycobacteria-containing samples[17]. Although acid-fast microscopy of smears followed by cultural confirmation remains the cornerstone of the diagnosis of active tuberculosis[13], sensitivity of smears

Table 1. Patient characteristics and pathological features.

Patient	Sex	Age at TA Diagnosis, yrs	Age at Biopsy, yrs	Ethnicity	Immunosuppressive Treatments	Site of Arterial Biopsy	Indication for Surgery	Evidence of Active Tuberculosis*
1	F	35	36	Caucasian	Yes	R carotid artery	Arterial stenosis	No
2	F	31	31	North African	Yes	L carotid artery	Arterial stenosis	No
3	M	17	19	Caucasian	Yes	L carotid artery	Arterial stenosis	No
4	F	16	20	North African	Yes	R carotid artery	Arterial stenosis	No
5	F	45	48	North African	Yes	Aorta	Aortic stenosis	No
6	F	36	49	Caucasian	Yes	Aorta	Aortic aneurysm	No
7	F	49	56	Caucasian	Yes	Aorta	Aortic stenosis	No
8	F	12	21	Hispanic	Yes	L carotid artery	Arterial stenosis	No
9	F	38	50	North African	Yes	Aorta	Aortic aneurysm	No
10	F	45	67	North African	Yes	Aorta	Aortic aneurysm	No

* Assessed by Mantoux test, chest radiograph, mycobacterial cultures of urine samples, bacilloscopy and sputum cultures. TA: Takayasu's arteritis.

Table 2. Results of pathological and microbiological analyses.

Patient	Pathology	Auramine and Acid-fast Stainings	Mycobacterial Cultures	DAT Results*	Search for Inhibitors	DAT Positive Controls**
1	Granulomatous arteritis	Negative	Negative	Negative	Absent	Positive
2	Granulomatous arteritis	Negative	Negative	Negative	Absent	Positive
3	Granulomatous arteritis	Negative	Negative	Negative	Absent	Positive
4	Granulomatous arteritis	Negative	Negative	Negative	Absent	Positive
5	Active non-specific arteritis	Negative	Negative	Negative	Absent	Positive
6	Intimal and adventitial fibrosis Patchy lymphocyte infiltrates	Negative	Negative	Negative	Absent	Positive
7	Intimal and adventitial fibrosis Patchy lymphocyte infiltrates	Negative	Negative	Negative	Absent	Positive
8	Intimal and adventitial fibrosis Scarring of media Patchy lymphocyte infiltrates	Negative	Negative	Negative	Absent	Positive
9	Intimal and adventitial fibrosis Patchy lymphocyte infiltrates	Negative	Negative	Negative	Absent	Positive
10	Intimal and adventitial fibrosis Scarring of media	Negative	Negative	Negative	Absent	Positive

* Amplified *M. tuberculosis* direct test. Results expressed in relative light units (RLU). Positive test result defined as luminescence reading ≥ 30,000 RLU.
** Assessed by adding 100 bacilli of *M. tuberculosis* to the third aliquots.

ranges only from 22% to 78%[18], and culture is dependent on the viability of the bacilli[19]. All the tests used for active tuberculosis diagnosis yielded negative results in our study, which was expected as all the patients had undergone immunosuppressive treatments and showed no tuberculosis symptoms. Any cryptic but active mycobacterial infection would have probably resulted in an active and patent tuberculosis in these patients.

We did not detect the presence of *M. tuberculosis* complex RNA. Since this method was shown to be 97% sensitive and up to 100% specific[20], we could reasonably conclude that arterial samples from patients with TA did not and had not contained tuberculous bacilli.

Interestingly, the search for *M. tuberculosis* yielded negative results in patients with both recent and active arteritis and in patients with longstanding and pathologically inactive TA. Vascular surgical procedures were performed within 2 years from the diagnosis of TA in Patients 1, 2, and 3, suggesting that *M. tuberculosis* is not present in the vascular wall even at an early and active stage of the arterial lesions.

Among the limitations of our study was the relatively small number of biopsies analyzed. However, taking into account the very low prevalence of the disease in European and North American populations[21,22], and the limited indications for vascular surgery in TA (clinical and/or hemodynamically significant arterial stenoses, and large aneurysms), our series may be considered as a valuable exploratory work.

Because we did not find any evidence of *M. tuberculosis* in the arterial samples from patients with TA, a direct role of this infectious agent in the pathogenesis of TA seems unlikely. Our study excludes neither the possibility of another

infectious etiology of the disease, nor the presence of an extraarterial antimycobacterial immune response triggering a cross-reaction directed towards an antigen of the arterial wall[10]. Future studies focused on the identification of cross-reacting arterial antigens would be important to delineate the exact pathogenesis of the disease.

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