Antimonocyte Antibodies in Takayasu's Arteritis: Prevalence of and Relation to Disease Activity

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ABSTRACT. ***Objective.*** To investigate the prevalence of antimonocyte antibodies (AMA) in Takayasu's arteritis (TA) and their relationship with disease activity.

Methods. IgG-AMA were studied in the sera of 60 patients with TA (29 active disease, 31 inactive) and 43 controls by a cellular ELISA using glutaraldehyde fixed U-937 cells or peripheral blood monocytes as antigen. Relationship of AMA with disease activity was evaluated by measuring titers of these antibodies in followup sera of 15 AMA positive patients with active TA undergoing immunosuppressive therapy.

Results. Twenty-six of 60 TA patients (43%) compared to 4 of 43 controls (9%) ($p < 0.001$) and 20 of 29 patients with active disease (69%) compared to 6 of 31 patients with inactive disease (19%) ($p < 0.001$) were positive for AMA. The antibody titers were significantly higher in patients with active disease than those with inactive disease (0.396 ± 0.172 vs 0.232 ± 0.096; $p < 0.001$). In the followup study of 15 patients with active disease who received immunosuppressive therapy, we observed normalization of AMA titers in 6 of the 7 patients who became inactive, compared to only one of the 8 patients whose disease remained active during followup ($p < 0.01$).

Conclusion. AMA are present in a significant proportion of patients with TA and correlate with disease activity, suggesting a possible pathogenic role of these antibodies in TA. (J Rheumatol 2003;30:2023–6)

Key Indexing Terms:
ANTIMONOCYTE ANTIBODIES TAKAYASU'S ARTERITIS DISEASE ACTIVITY

Takayasu's arteritis (TA) is a large vessel vasculitis characterized by granulomatous inflammation of large elastic arteries, mainly the aorta and its major branches, and is considered to have an autoimmune etiology[1,2].

Endothelial injury is a key event in the development of TA[3]. We recently observed antiendothelial cell antibodies (AECA) in the disease that exhibit complement dependent cytotoxicity to endothelial cells[4]. In addition to AECA, a number of cellular and humoral immune mechanisms have been implicated in the pathogenesis of the disease[5]. Vascular infiltration of monocytes, granulomatous inflammation in the arterial wall during the early/active stage of the disease, and high serum concentrations of interleukin 6 correlating with disease activity[2,6] together suggest that monocytes in TA are activated and may have a central role in mediating vascular injury in TA.

The factor(s) that can cause activation of monocytes/macrophages may be important in understanding the role of these cells in the pathophysiology of the disease. Development of antimonocyte antibodies (AMA) in TA may be one such factor. Recently, AMA have been described in Wegener's granulomatosis (WG)[7] and peripheral atherosclerosis[8], which like TA have activated monocytes/macrophages in the arterial lesions. Thus it is plausible that patients with TA may have AMA.

We investigated the prevalence of these antibodies in TA and their relationship with disease activity.

MATERIALS AND METHODS

Subjects. The subjects consisted of 60 patients (37 women, 23 men; mean age 27 ± 13 yrs) fulfilling the American College of Rheumatology criteria for TA[9]. Twenty-nine patients were in active stage of the disease and 31 had inactive disease as described[10]. Forty-three age and sex matched healthy individuals served as controls.

Sera from each individual were obtained with informed consent and stored at –80°C until use. AMA titers were also studied at 3-monthly intervals in the followup sera of 15 AMA positive patients with active TA undergoing immunosuppressive therapy (median period of followup 8.5 mo; range 2–24 mo). Remission of disease was established by regression of clinical features as well as decline in concentrations of erythrocyte sedimentation rate and/or C-reactive protein, the laboratory measures used as markers of disease activity.

Detection of AMA. IgG-AMA were investigated in the sera of patients and controls by a cellular ELISA using U-937 cells (ATCC no. CRL-1593.2, American Type Culture Collection, Rockville, MD, USA)[11] as described[12], with the modification that cells were immobilized to solid phase by glutaraldehyde fixation as we have described for endothelial cells[10].

Polystyrene flat bottom microtiter plates (Nunc, Roskilde, Denmark) were coated with U-937 cells (1×10^5 cells/well) and after centrifugation (300 g, 10 min) the monolayer of cells was immobilized to solid phase by

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fixing with 0.25% glutaraldehyde (SRL, Mumbai, India) for 20 min at room temperature. Washing between the steps was performed with phosphate buffered saline (PBS, pH 7.4) containing 0.2% bovine serum albumin (BSA; wash buffer). Nonspecific binding sites were blocked by incubating the plate with 2% BSA in PBS for 1 h at 37°C. Test and reference sera were diluted 1:1000 with wash buffer or wash buffer alone were added 100 µl/well in triplicates and the plate was incubated 2 h at 37°C. The bound antibodies were detected by incubating the plate with 100 µl/well alkaline phosphatase conjugated anti-human IgG (Fab_2-specific, 1:5000; Sigma, St. Louis, MO, USA). The color reaction was developed by adding 100 µl/well of p-nitrophenyl phosphate (1 mg/ml; Sigma) and the absorbance was read in an automated ELISA reader (Tecan Spectra, Austria) at 405 nm. The cutoff value for confirming a sample was AMA positive was taken as mean + 2 SD of optical densities (OD) of the controls. The intra and interassay coefficients of variation were < 5% and 15%, respectively.

The sera that were reactive against U-937 cells were tested using the same cellular ELISA for their reactivity to normal human peripheral blood monocytes isolated by plastic adherence (purity > 80% as determined by flow cytometry using anti-CD14 staining).

Statistical analysis. Mann-Whitney U-test was used to compare the levels of AMA in patients and controls as well as in different groups of patients, i.e., with active and inactive TA. Z statistics was used for comparing the prevalence of these antibodies in different groups.

RESULTS

Twenty-six out of 60 patients with TA (43%) and 4 of 43 controls (9%) were positive for AMA ($p < 0.001$). The reactivity of AMA positive sera to U-937 cells and blood monocytes was found to be equivalent.

The prevalence of AMA was significantly higher in patients with active disease (20/29, 69%) compared to those with inactive disease (6/31, 19%) ($p < 0.001$). Titers of these antibodies were also significantly higher in the patient group (0.302 ± 0.147) compared to controls (0.167 ± 0.062) ($p < 0.001$) and in patients with active disease (0.396 ± 0.172) compared to those with inactive disease (0.232 ± 0.096) ($p < 0.001$) (Figure 1).

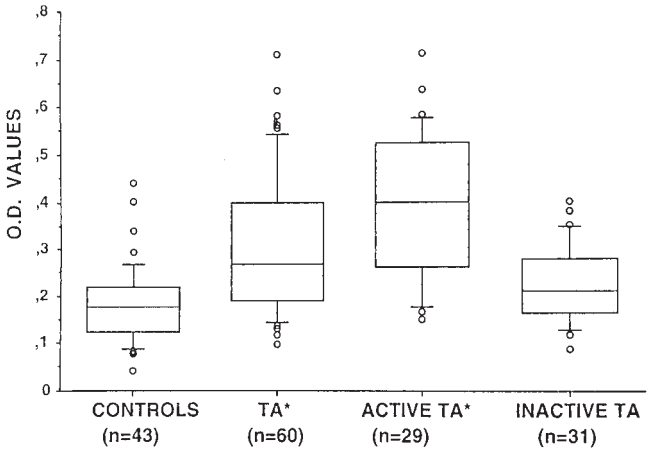

Figure 1. Concentrations of antimonocyte antibodies (AMA) expressed in optical density values in the 4 groups: controls, all patients with TA, patients with active disease, and patients with inactive disease. Horizontal line represents the 50th percentile and the vertical line extends from the 10th to the 90th percentile. *$p < 0.001$ comparing patient group versus controls and active disease group versus the inactive disease group.

Of the 15 AMA positive patients with active disease in whom a followup study was done, 7 were observed to become inactive and 8 remained active during the followup period. Six of the 7 (85%) patients who became inactive during followup showed normalization of AMA titers, compared to only one of the 8 (12%) patients who continued to be active ($p < 0.01$) (Table 1).

DISCUSSION

To our knowledge this is the first report on AMA in TA. Previously, a panel of monoclonal AECA derived from a single patient with TA were tested for their reactivity to U-937 cells by the same method, using unfixed cells immobilized to solid phase with poly-L-lysine, but no significant reactivity was observed[12].

We observed an overall prevalence of 43% of these antibodies in patients with TA. AMA with distinct specificity for monocytes have been observed in 79% of patients with WG, 30% of patients with sarcoidosis, and 20% of patients with Crohn's disease[7]. In another study these were observed in 65% of patients with peripheral atherosclerosis[8]. We have observed them in 70% of young patients with myocardial infarction (unpublished data). Thus these antibodies appear to be present in disorders that have a granulomatous inflammation and/or in which monocytes/macrophages play an active role in pathogenesis.

Studies on AMA have detected these antibodies by indirect immunofluorescence using ethanol fixed monocytes or by ELISA using monocyte extract as antigen[7] or by cytotoxic techniques against monocytes[8]. We detected these antibodies by cellular ELISA using glutaraldehyde fixed U-937 cells as antigenic substrate, with no difference in the results using U-937 cells or blood monocytes. This indicates that U-937 is a suitable cell line for the study of AMA, and our method may serve as a simple screening test for the detection of these antibodies without using monocytes, which are less numerous and technically difficult to isolate from peripheral blood.

The relation of AMA to disease activity has not been described previously[7,8]. We observed significantly higher prevalence as well as titers of these antibodies in patients with active disease as compared to those with inactive disease. Further, we also observed that most of the patients who showed regression in disease activity also showed normalization of AMA titers during followup. These observations suggest that AMA may serve as a marker of disease activity and may also have a pathogenic role in TA.

One of the mechanisms by which AMA may be pathogenic is by the activation of monocytes that can directly or indirectly cause vascular damage by secreting lytic proteases, reactive oxygen species, chemoattractants, and inflammatory cytokines[13]. Recently, antibodies from patients with heparin induced thrombocytopenia were observed to bind monocytes and trigger cellular activation,

Table 1. Antimonocyte antibody (AMA) titers in followup sera of 15 patients with TA with active disease treated with immunosuppressive therapy. Cutoff value for AMA positivity was 0.291, calculated as mean + 2 SD of controls.

Patient	Age/Sex	AMA Titers (pretreatment)	AMA Titers (last followup)	Followup Period, mo	Disease Status (last followup)
1	40 F	1.077	0.166	3	Inactive
2	12 F	0.415	0.280	10	Inactive
3	24 F	0.380	0.126	6	Inactive
4	28 F	1.012	0.288	18	Inactive
5	45 F	0.443	0.194	23	Inactive
6	31 F	0.396	0.209	19	Inactive
7	33 F	0.350	0.380	10	Inactive
8	40 F	0.296	0.180	2	Active
9	16 F	1.735	0.575	7	Active
10	35 F	0.882	0.653	3	Active
11	28 F	1.232	0.498	10	Active
12	26 F	0.878	1.097	8	Active
13	40 M	1.077	1.113	5	Active
14	20 F	1.247	2.055	24	Active
15	38 F	1.033	0.794	11	Active

leading to production of the proinflammatory cytokine interleukin 8[14].

The exact mechanism for the generation of AMA in TA is presently not known, but activated monocytes/macrophages present in the disease appear to be responsible for generation of these antibodies. The activation or activation induced apoptosis of monocytes/macrophages can cause expression of constitutive antigens above threshold levels or appearance of some neoantigens, which could lead to a breakdown of the tolerance, resulting in the formation of AMA[15]. Once formed, these antibodies could amplify the inflammatory response by activating monocytes/macrophages. However, the initial event that induces activation of these cell types in TA remains to be determined. The presence of activated monocytes/macrophages in other vasculopathies such as WG[16] and peripheral atherosclerosis[17] where AMA have been detected, demonstration of activation induced apoptosis of monocytes *in vitro*[18], and animal studies showing production of autoantibodies immunized with apoptotic cells[19,20] strongly favor this hypothesis.

We observed AMA in a significant proportion of patients with TA. Significantly higher prevalence as well as titers of these antibodies in patients with active disease than in those with inactive disease and normalization of AMA titers in most of the patients who became inactive during followup together point toward a pathogenic role of these antibodies in TA. Further studies on antigenic target(s), pathogenic mechanism(s), and the mechanism of formation of these antibodies may shed light on our understanding of the immunopathogenesis of TA.

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