

Dendritic cells co-localize with activated CD4+ T cells in giant cell arteritis

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Abstract

Objective

Giant cell arteritis (GCA) is a vasculitis predominantly affecting medium- and large-sized arteries. Recent data show the co-localization of dendritic cells and *Chlamydia pneumoniae* in vascular biopsies from GCA patients. Here we define the topographical relation of dendritic cells and these activated T-cells to determine the antigen presenting cell in GCA, and to examine several auxiliary biochemical and genetic aspects relating to the role of bacteria such as *C. pneumoniae* in eliciting GCA.

Methods

18 paraffin-embedded temporal artery biopsy specimens from 14 patients with GCA that were PCR-positive for *C. pneumoniae* were examined by two-color immunohistochemistry for the topographical relationship between dendritic cells and activated T-cells. In addition the presence of GTP-binding proteins, Tumor necrosis factor alpha (TNF α), and Toll-like receptor 4 (TLR4) was investigated. 15 temporal artery specimens from 10 patients without GCA served as controls.

Results

In all GCA specimens, dendritic cells co-localized in the immediate vicinity of activated CD4+ Talin-expressing T cells, and these were predominantly found in granulomatous infiltrates. Confocal microscopy confirmed the cell-cell contact of dendritic cells with activated T cells. Results further showed that RhoA and Rac1 were predominantly present in the region of granulomatous infiltrates. TNF α production and expression was found in dendritic cells and macrophages, predominantly in granulomatous infiltrates and in endothelial cells of the vasa vasorum dispersed in the adventitial and medial layers of the temporal artery. No control specimens showed TNF α expression. More than 95% of dendritic cells were positive for TLR4; macrophages and endothelial cells localized in the adventitia showed TLR4 production.

Conclusions

The immediate co-localization of dendritic cells and activated T cells indicate a high probability that the former represent the antigen presenting cells in GCA. In addition, because of the presence of Rho A and Rac1 in the granulomatous infiltrates, we speculate that they provide the right environment for cell-cell contact and adhesion, and that they may promote the internalization of bacteria. TNF α is expressed at high levels in the granulomatous infiltrates of temporal artery specimens from patients with GCA. Since TLR4 is produced in the same cell types, and predominantly in the adventitial layer of the temporal artery, we suggest that these receptors are coupled to signal transduction pathways that control TNF α expression.

Key words

Giant cell arteritis, dendritic cells, *Chlamydia pneumoniae*, antigen presentation.

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Introduction

GCA is a vasculitis that predominantly affects medium- and large-sized arteries. Recent studies suggest that GCA may be an antigen-driven disease (1). The clustering of GCA cases in specific time periods and in particular geographic locations indicates that, in addition to genetic predispositions for the disease, environmental or seasonal factors may play a significant role (2). Importantly, upper respiratory tract symptoms are often recognised as part of the syndrome in both GCA and polymyalgia rheumatica (PMR). In a previous work, we identified *C. pneumoniae* in temporal artery specimens from 8/9 patients with GCA predominantly in granulomatous infiltrates in the adventitial layer of the temporal artery (3); one of 4 samples from PMR patients was immunopositive, but PCR-negative, for *C. pneumoniae*, but all other control samples were negative for *C. pneumoniae*. A case report from another laboratory supports the possibility of an association between *C. pneumoniae* infection and GCA (4). In addition, it has been suggested that reinfection with this organism may induce isolated and systemic vasculitis (5). Other data further show the co-localisation of dendritic cells and *C. pneumoniae*, predominantly in the adventitial layer of the temporal artery. Thus, we have suggested that dendritic cells (DC) represent the antigen-presenting cells in this disease. However, not all published data support the presence of *C. pneumoniae* in temporal artery specimens from GCA patients (6, 7).

The central role of DC in antigen-specific responses has been proposed (8, 9), and studies of DC isolated from non-lymphoid tissues suggest that cells in these sites are specialized for the uptake and processing of foreign antigens (10). Most DC in peripheral tissues *in situ* are of the immature phenotype. After detecting microbial products or proinflammatory cytokines, immature DC transform into the mature form. At this stage, these cells demonstrate exceptional capacity for T cell stimulation. Granulomatous infiltrates represent to some extent tertiary lymphoid-like structures, which sup-

ports the interaction between CD4⁺ T cells and DC in the wall of temporal arteries. Disease-relevant tissue-infiltrating, activated, Interferon (IFN)-producing T cells represent only a subset of 2–4% (11). Previous studies showed that more than 90% of these IFN-producing cells were CD4⁺/CD45RO⁺. 60–80% of disease-relevant CD4⁺/CD45RO⁺ IFN-secreting T cells had reorganized the cytoskeletal protein Talin, indicating an interaction of the T cell receptor and an antigen-presenting cell. Given that putative disease-relevant CD4⁺ T cells are specifically enriched in the adventitia, we hypothesized that this is the area where antigen recognition occurs. Thus, it is of major interest to investigate the topographic relation of DC to the activated T cells.

Rho, Rac and Cdc42 are members of the GTPase family implicated in actin-mediated cytoskeletal rearrangements that occur concomitantly with particle uptake. Cdc42 and Rac1 play important roles in regulating macropinocytosis and phagocytosis in DC (12, 13) and mediate FcR phagocytosis (14), while complement receptor-mediated phagocytosis is dependent upon Rho (15). Thus, if *C. pneumoniae* is involved in eliciting and/or maintaining GCA, activation of GTPase family members would be expected in arterial specimens.

Toll-like receptors (TLRs) are cell surface receptors that signal activation of cells in response to microbial products via the innate immune system (16). A constitutively active mutant of the first human Toll-family protein described (TLR4) was shown to induce the expression of cytokines and co-stimulatory molecules on antigen-presenting cells. DC express TLR4, which plays a major role in LPS-induced DC maturation. Pattern recognition receptors such as TLRs expressed by immature DC allow these cells to recognize microbial antigens and undergo maturation into immunogenic forms (17). Thus, expression of TLR4 on DC would be expected if *C. pneumoniae* is the source of antigens eliciting the inflammation in GCA. Moreover, a few hours after bacterial infection, DC synthesize several cytokines and chemokines, including

TNF α (18,19), which mediate leukocyte migration to sites of infection (20). In this report, we present evidence that activated T cells and DC are in contact in the granulomatous infiltrates of temporal arteritis specimens. Moreover, we show the presence of RhoA and Rac1, production of TNF α , and expression of TLR4 in the adventitia of the temporal arteries of GCA patients. Together, data from all these studies support the contention that DC indeed are the antigen presenting cells in GCA.

Materials and methods

Study population

Temporal artery biopsy specimens were procured from 14 individuals with demonstrated GCA. Specimens from 9 patients in this group had already been studied (3). For 5 patients, samples from both the left and right arteries were available. Samples from 10 patients without GCA served as controls. This control group is composed of two subgroups, one of 3 patients with PMR and the second of 7 patients with other diseases. The diagnoses of these 7 were: anterior ischemic optic neuropathy and myocardial insufficiency in 2 patients respectively; and transitory ischemic attack, stroke, and systemic lupus erythematosus in one patient respectively. In 1/3 PMR patients and 4/7 controls, biopsy specimens were available from both the left and right temporal arteries. 9/14 patients with GCA were female, 5 were male. In comparison, 5/10 control patients were female, 5 were male. The mean age in the GCA group was 76 years, that for the control group 71 years. GCA was diagnosed according to the American College of Rheumatology (ACR) classification criteria, from temporal artery biopsies showing the typical relevant histopathology. The 10 control patients fulfilled two of the ACR criteria for GCA and were biopsied because of persisting bilateral headaches. Inclusion was limited to patients 50 yrs and older. According to the pathology reports, 80% of patients with GCA fulfilled the McDonnell classification for Grade 1, while the remaining 20% fulfilled the classification for Grade 2. No GCA patient fit into classification

Grade 3 or showed significant arteriosclerosis. In 8/14 GCA patients, the onset of disease was preceded by upper respiratory tract symptoms. Patient I had had several bouts of pneumonia, and Patients II, IV, VIII, and X had had chronic bronchitis; Patients II and XII also had a documented cough. Patient V had been diagnosed with pleuritis sicca. Two other GCA patients (III and IX) had low grade fever prior to onset of the disease. Only one patient with PMR had upper respiratory tract symptoms prior to the onset of disease.

Tissue sampling

From the paraffin-embedded tissue sections comprising the complete panel of 20 samples/patient, we selected 4 consecutive samples to use for *in situ* hybridisation. For the immunohistochemical studies, we used the remaining 16 consecutive sample sections.

Antibodies and *in situ* hybridization reagents

The following reagents were used: Rabbit anti-S-100 Ab (Dako, Glostrup, Denmark), murine anti-CD68 mAb (KP1; Dako Corp., Carpinteria, CA), mouse anti-Talin mAb (SIGMA, Saint Louis, Missouri, USA), mouse anti-CD4 mAb (Becton Dickinson, Mountain View, CA), mouse anti-TNF α mAb (DPC Biermann, Nauheim, Germany), rabbit polyclonal anti-human Cdc42 mAb (Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-human Rac Ab (Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-human Rho A Ab (Santa Cruz Biotechnology, Inc.), rabbit polyclonal anti-human TLR4 Ab (Torrey Pines Biolabs, Houston, TX), TRIC-labelled secondary mouse anti-rabbit Ab (dianova; Hamburg, Germany), FITC-conjugated goat anti-mouse IgG (dianova, Hamburg, Germany), Novostain Super ABC Reagent (Medac, Wedel, Germany), TNF α *in situ* hybridization kit (Maxim Biotech, Inc., San Francisco, CA).

Double fluorescence staining

The original rabbit anti-S-100 Ab (1:1000) combined with a TRIC-labelled (red fluorescence) secondary mouse anti-rabbit Ab was used. Sections were

then microwave heated and again stained either with the mouse anti-CD4 mAb in combination with the FITC-conjugated secondary antibody. On consecutive sections the mouse anti-Talin mAb in combination with an FITC-conjugated goat anti-mouse IgG (green fluorescence) with the mouse anti-CD4 mAb in combination with the TRIC-conjugated secondary antibody. For double immunolabelling, paraffin-embedded tissue samples were deparaffinized, blocked for endogenous peroxidase activity, and further blocked with horse-serum as above. Sections were first stained with the rabbit anti-human S-100 Ab (1:1000) and developed by subsequent incubations with biotinylated secondary Ab, the Novostain Super ABC Reagent, and finally 3-amino-9-ethylcarbazole solution. Sections were then microwave heated in citrate buffer, retreated for peroxidase activity, blocked with horse serum for 30 min at room temperature, stained with mouse anti-CD4 mAb (1:50), mouse anti-Talin mAb (1:40) and mouse anti-TNF α mAb (1:5) (21), and developed by incubation with biotinylated secondary Ab, Novostain Super ABC Reagent, and DAB solution.

Immunolabelling of tissue sections

Paraffin-embedded temporal artery biopsy samples were deparaffinized, then microwave heated in citrate buffer. After heating, endogenous peroxidase activity was blocked with hydrogen peroxide. Tissue sections were then blocked with horse serum for 30 min at room temperature and stained either with rabbit polyclonal anti-human Cdc42 mAb (dilution 1:50), rabbit polyclonal anti-human Rac Ab (1:100), rabbit polyclonal anti-human Rho A Ab (1:100), or with a rabbit polyclonal anti-human TLR4 Ab (1:200). Sections labelled with either Ab were developed by subsequent incubation with biotinylated secondary Ab, the Novostain Super ABC Reagent, and finally with diaminobenzidine tetrahydrochloride (DAB) solution. Immunohistochemical assays were done twice, and positive and negative biopsies were run together and processed at the same time. The specificity and sensitivity of all Ab

titrations was confirmed by staining of lymph node tissue. Non-specific binding of secondary Abs was excluded by omitting the primary Ab. The technician performing the assays was blinded to the nature of the biopsy. The results of the test were read before the nature of the biopsy was known by the reader. Two readers (ADW and AP) independently read all test results.

In situ hybridization

Paraffin-embedded tissue samples were deparaffinized, blocked for endogenous peroxidase activity, and further blocked with horse-serum/DEPC as above. Sections were stained with the rabbit anti-human S-100 Ab (1:1000) or with the mouse anti-human CD68 mAb (1:100) and developed by subsequent incubations with biotinylated secondary Ab, the Novostain Super ABC Reagent, and finally 3-amino-9-ethylcarbazole (AEC) solution. Sections were then incubated with 2 mg/ml proteinase K for 10 min at 37°C. *In situ* hybridization with sense and anti-sense probes for TNF α was performed using a commercially available kit. Slides were counterstained with haematoxylin.

Results

Co-localization of dendritic cells and activated CD4+ T cells in granulomatous infiltrates

We first investigated the geographic relationship of infiltrating T cells and DC in temporal artery specimens from patients with GCA. Immunohistochemical staining with an anti-CD4+ mAb showed that infiltrating T cells in such specimens were to be found in all three layers of the arterial wall. Approximately 35% of the CD4+/CD45 RO+ T cells accumulated in the adventitial tissue, 50% in the media, and 15% in the intima.

Importantly, the majority of DC identified by immunohistochemical studies using anti-S100 Ab were located in the adventitial layer. 5-70 S-100-positive cells were clearly identifiable in each specimen from GCA patients. DC were localized to the inner adventitia and were arranged in a circular array along the external elastic lamina. In different

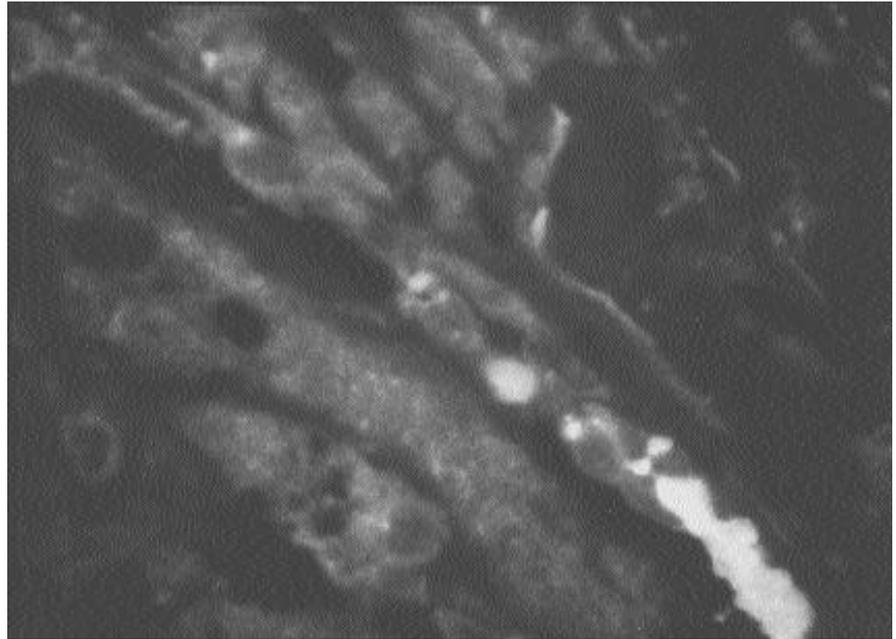


Fig. 1. The immediate relationship of dendritic cells and CD4+ T cells as demonstrated via confocal microscopy using double immunofluorescence staining in a temporal artery tissue specimen from a patient with GCA. Positive staining with the rabbit anti-human S-100 Ab combined with a tetramethylrhodamine isothiocyanate-labeled (red fluorescence) secondary mouse anti-rabbit Ab identified dendritic cells (red stain). Using the mouse human anti-CD4+ Ab in combination with FITC-conjugated secondary goat anti-mouse Ab identified CD4+ T cells (green stain). Original magnification x100.

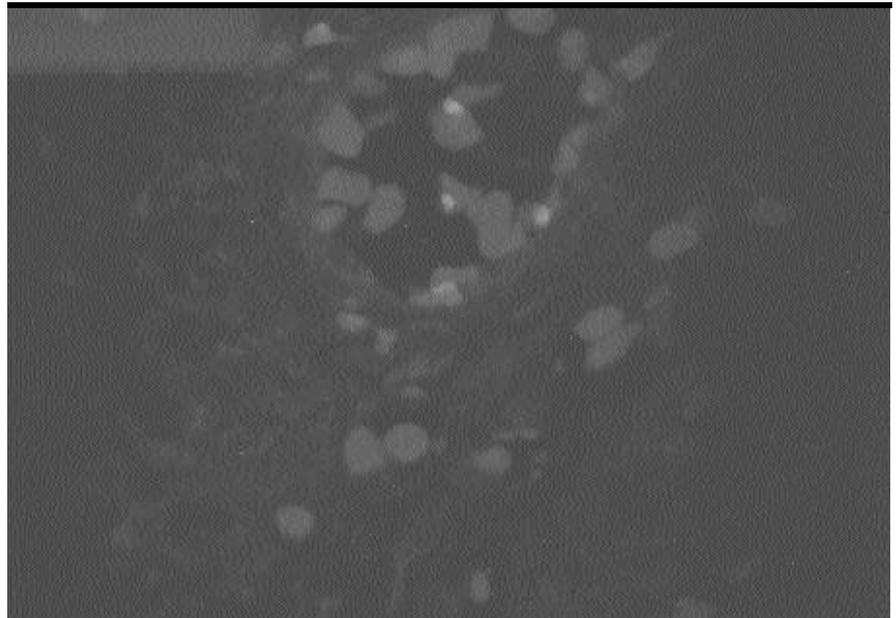


Fig. 2. Co-localization of CD4+ T cells and Talin-expression as demonstrated via confocal microscopy using double immunofluorescence staining in a temporal artery tissue specimen from a patient with GCA. Positive staining with the CD4 T cell Ab combined with a tetramethylrhodamine isothiocyanate-labeled (red fluorescence) secondary Ab identified CD4+ T cells (red stain). Using the human anti-Talin Ab in combination with fluorescein isothiocyanate-conjugated (green fluorescence) secondary Ab identified Talin-expression (green stain). Original magnification x100.

temporal artery specimens, DC appeared in clusters. Confocal microscopic observation of double staining of these samples with the anti-CD4+

and an anti-S100 mAb showed that DC localized in the immediate vicinity of CD4+ T cells (Fig. 1). In contrast, 70 - 80% fewer S-100-positive cells were

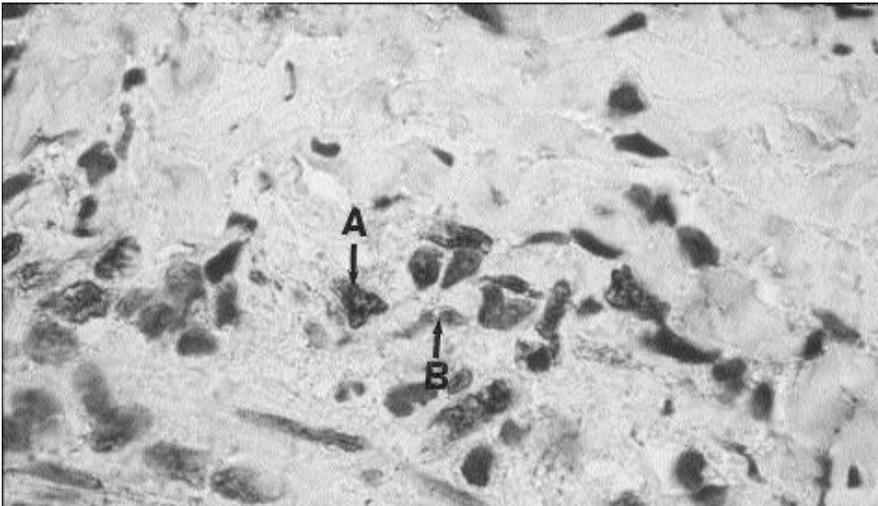


Fig. 3. Localization of Talin-expressing T cells and dendritic cells in temporal arteries in patients with GCA using double-immunohistochemistry. (A) Talin-expressing T cells (Talin; dark staining) in the adventitial layer of the artery. Anti-Talin immunoperoxidase. (B) Immediate vicinity of Talin-expressing T cells and dendritic cells (S-100; red staining) at the adventitia-media junction. Anti-S-100 immunoperoxidase; oil immersion magnification, x 63.

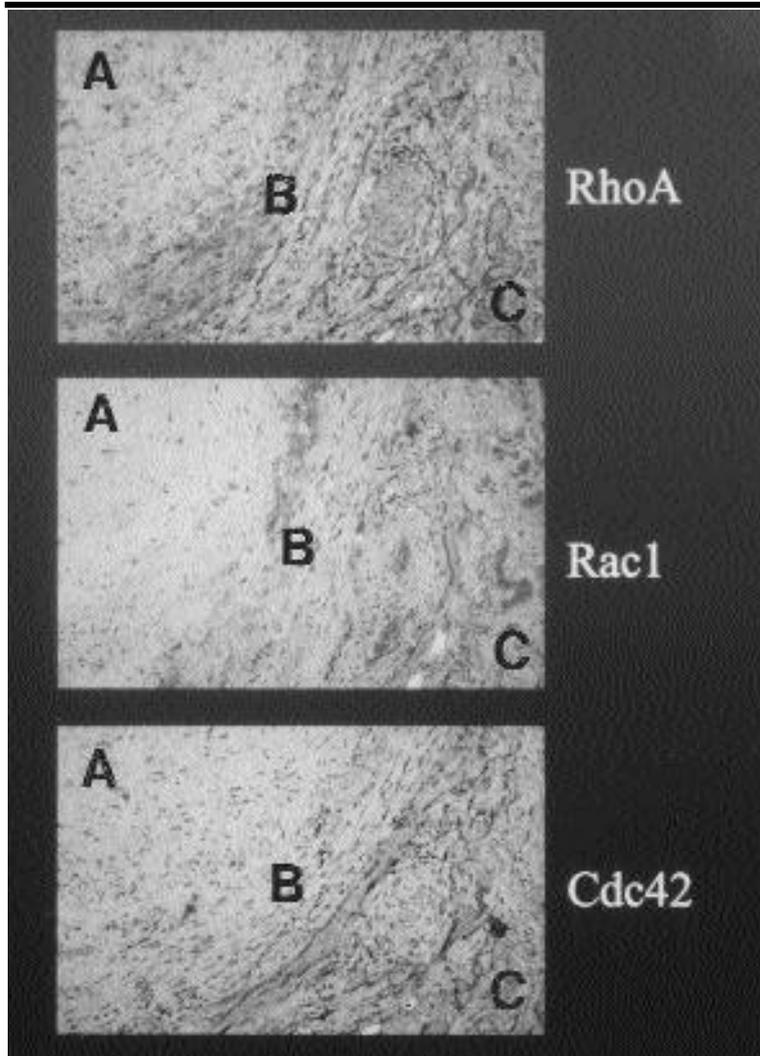


Fig. 4. Topography of RhoA, Rac1 and Cdc42 in consecutive temporal artery paraffin sections from a patient with giant cell arteritis (red staining). (A) represents the intima, (B) the media, and (C) the adventitia of the arterial wall; immunoperoxidase, magnification x 20.

identified in temporal artery sections from control patients.

Consecutive confocal microscopic pictures confirmed that these CD4⁺ T cells in the same areas express Talin, a cytoskeletal protein indicating recent cell activation due to antigen-presentation (Fig. 2). The interaction of DC and CD4⁺ T cell was found predominantly in granulomatous infiltrates. Confocal microscopy again confirmed the immediate cell-cell contact of DC with the activated Talin-expressing CD4⁺ T cells (Fig. 3). To identify T cells with clustering of Talin molecules, immunohistochemistry with an anti-Talin Ab was used. The concentration of Talin at one pole of the T cell was found to be infrequent. 2-5% of all tissue-infiltrating T cells expressing the CD4/CD45 RO marker had clustering of Talin (data not shown). Earlier studies (11) indicated that more than 95% of disease-relevant activated T cells lack the CD8 molecule.

Rho A and Rac1 in C. pneumoniae-positive temporal artery specimens from GCA patients

We examined the possibility that one or more members of the Rho family of GTPases are involved in the CD4⁺/CD45RO⁺ T cell interaction. Double immunohistochemical staining was applied using the anti-Talin mAb with either anti-Rho A Ab, anti-Rac1 Ab or anti-Cdc42 Ab. The results showed that RhoA and Rac1 primarily were found in temporal artery specimens from GCA patients. Cdc42 was not detected in 90% of GCA tissue specimens. The remaining 10% of tissue specimens showed only a few Cdc42-positive cells. The two GTPases, RhoA and Rac1, were mainly present in the region of granulomatous infiltrates (Fig. 4) in the immediate vicinity of Talin-expressing cells. The concentration of Rho A in temporal artery tissue specimens of GCA patients was much higher than that of Rac1.

TNF α production and expression in dendritic cells, macrophages and endothelial cells

The distribution pattern of TNF revealed two types of arrangements. In one pattern, TNF production was

found in DC and macrophages predominantly in granulomatous infiltrates (Fig. 5a). TNF α -production was identified in the adventitia, along the external elastic lamina and in the medial layer of the artery. Generally, TNF α -producing cells appeared in clusters. The second pattern showed TNF α -synthesizing endothelial cells of the vasa vasorum (Fig. 5b) dispersed in the temporal artery adventitial layer. TNF α expression in DC, macrophages, and endothelial cells was confirmed by *in situ* hybridization (Fig. 5c). TNF α production was not observed in T cells. No control specimens showed TNF α production or expression.

Detection of TLR4 in C. pneumoniae-positive temporal artery biopsies from GCA patients

TLR4 is involved in cellular activation by microbial products, and it can lead to functional maturation of DC. Double immunohistochemical staining was performed using an anti-TLR4 polyclonal Ab in combination with anti-S-100 mAb to determine the presence and the frequency of TLR4-positive DC. More than 95% of DC were TLR4-positive (Fig. 6). TLR4-positive macrophages and endothelial cells localized almost exclusively to the adventitia of the temporal artery. Only about 25% of CD68+ cells were TLR4-positive. No control specimens showed TLR4 expression.

Discussion

GCA is a disease elicited by an inflammatory infiltrate composed of T lymphocytes, macrophages, and DC. While much evidence indicates that the disease is antigen-driven, the source of those antigens has not yet been unequivocally identified. In an earlier study from this group, we identified the intracellular bacterial pathogen *C. pneumoniae* in temporal artery specimens from GCA patients; control specimens were negative for the organism. On the basis of those and other observations, we suggested that *C. pneumoniae* might be the antigen source in GCA patients, and that DC would serve as the antigen presenting cells in that context. The present study was undertaken to pro-

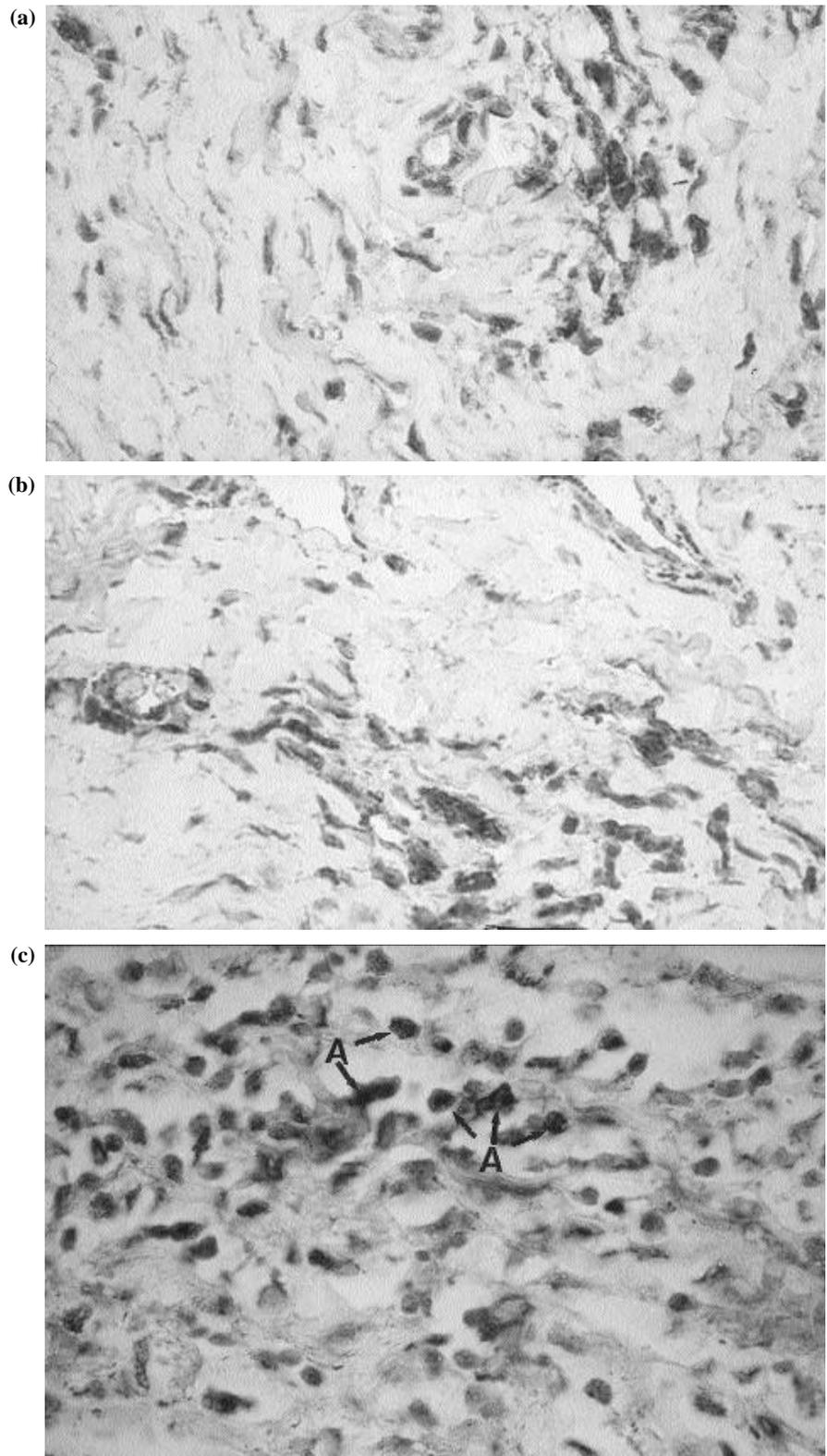


Fig. 5. TNF α production and expression in the vascular lesions of patients with GCA. Paraffin sections of temporal artery specimens were analysed by immunohistochemistry (a, b) and *in situ* hybridization with an TNF α -specific DNA oligonucleotide probe (c). (a) and (b) show representative experiments using anti-TNF α monoclonal Ab for immunohistochemical studies. (a) TNF α production (red stain) is shown in granulomatous infiltrates, (b) shows TNF α production (red stain) in endothelial cells of the vasa vasorum, predominantly in the adventitia of the temporal artery. *In situ* hybridization with antisense DNA-probe in (c) also confirms the expression of TNF α , here indicated by A (dark blue stain). In (a-c) magnification x 40.

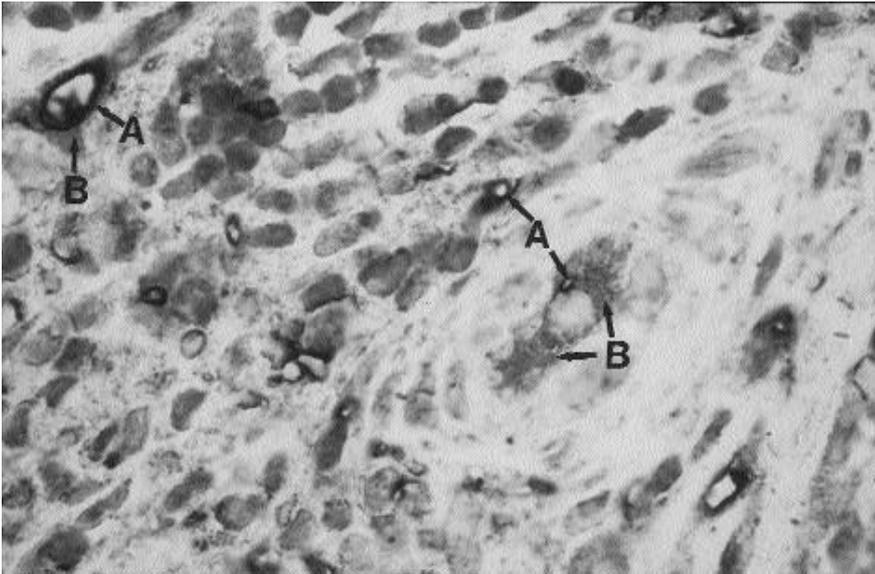


Fig. 6. Localization of TLR4 and dendritic cells in temporal arteries in patients with GCA using double-immunohistochemistry. **A:** TLR4 expression (dark staining) in granulomatous infiltrates of the temporal artery. Anti-TLR4 immunoperoxidase. **B:** Co-localization of dendritic cells (S-100; red staining) and TLR4 in the adventitia. Anti-S-100 immunoperoxidase; oil immersion magnification, x 63.

vide evidence that DC are indeed antigen presenting cells in GCA, and to investigate relevant factors which together would provide the proper microenvironment for activated T cell and DC to interact in *C. pneumoniae*-positive temporal artery specimens. Temporal artery specimens in the present study were procured from 14 individuals with demonstrated GCA. Specimens from 9 patients in this group had already been studied (3). In the present study we investigated 5 additional GCA patients for the presence of *C. pneumoniae*. All 5 additional GCA patients were *C. pneumoniae* positive. The data presented here are consistent with this organism being the antigen source in GCA, and with the contention that DC are presenters of those antigens in this disease context.

The presence of activated CD4⁺ T cells in the adventitia of the temporal artery in *C. pneumoniae*-positive GCA patient samples studied here is compatible with the model of GCA as an antigen-driven disease. Moreover, the immediate co-localization of DC and those activated T cells demonstrated in these studies indicate a high probability that DC represent the antigen presenting cells in these specimens. We therefore suggest that DC have a central role in the induction of adaptive antigen-

specific immune responses towards *C. pneumoniae* in GCA. We further speculate that these cells play a critical sentinel function for incoming *C. pneumoniae* in the temporal arteries of GCA patients.

In mammalian cells, major rearrangements of the actin cytoskeleton upon receptor stimulation or other stimulation lead to membrane ruffling, filopodia formation, or actin stress fiber formation. These rearrangements are controlled by specific GTPases belonging to the Ras superfamily, namely, Rac, Rho, and Cdc42. *Salmonella* is known to require Cdc42 but not Rac or Rho for invasion, whereas *Shigella* needs Rho but not Rac or Cdc42 (22). We thus anticipated that invading *C. pneumoniae* would require these molecules to mediate their uptake. In the analyses presented here, RhoA and Rac1 primarily were present in granulomatous regions of the *C. pneumoniae*-positive temporal artery tissue specimens. The presence of these two Rho GTPases in such infiltrates strongly suggests that they provide the right environment for cell-cell-contact and adhesion, and that they thereby promote the internalisation of *C. pneumoniae*. GCA is not the only primary systemic vasculitis where the formation of granulomatous lesions is seen in the presence of TNF α sugges-

tive of an antigen-driven process (23, 24). Since only a few Cdc42-positive cells were detected in 10% of all specimens from GCA patients, Cdc42 appears not to play a role in GCA.

Because the Toll signaling pathways are key mediators of the response to bacteria, it was of major interest in this study to examine the presence of TLR4 on DC in *C. pneumoniae*-positive temporal artery specimens from GCA patients. The data presented demonstrate that TLR4 is highly expressed on DC, macrophages and endothelial cells in the *C. pneumoniae*-positive temporal artery specimens examined. Thus, this may represent the transducing receptor for specific constituents of the bacterial cell wall of *C. pneumoniae*. TLR4 also may induce cytokines and co-stimulatory molecules on antigen-presenting cells in GCA. Other studies indicate that TLR2, rather than TLR4, seems to be more important (25). Future genetic and biochemical studies will reveal which Toll-like receptor specifically provides the most potent immune response system to microbe-specific determinants in GCA.

A few hours after bacterial infection, DC synthesize a number of important cytokines and chemokines. Production of TNF α has been detected readily in DC infected with either gram-positive or gram-negative bacteria. Moreover, the process of leukocyte migration to sites of infection is stimulated by TNF α , which is produced in response to infection (26). Many investigators have speculated as to whether the phenotypic and functional maturation that occurs in DC within 24 hours of bacterial uptake is the result of cytokine amplification during this response. In this context, an investigation of expression and production of TNF α in *C. pneumoniae*-positive temporal artery biopsies from GCA patients was of interest. The results presented show that TNF α is expressed in granulomatous infiltrates of *C. pneumoniae*-positive temporal artery specimens from patients with GCA. In the specimens examined, TNF α was expressed by DC, macrophages and endothelial cells. We therefore contend that TNF α promotes DC survival, phenotypic and

functional maturation, and that it enhances the stimulatory capacity of DC in temporal artery tissue from GCA patients. In addition, TNF α modulates DC migration to T cell areas in inflammatory infiltrates. The general capacity of DC to present bacterial antigens with high efficiency on both MHC I and II molecules can be exploited to induce strong, long-lasting immunity towards bacteria. An example of a partial protective immune response achieved by *in vivo* injection of DC loaded with *Chlamydia trachomatis* has been described (27). Thus, the results presented here regarding high level production of TNF α in *C. pneumoniae*-positive temporal artery specimens is consistent with this organism playing a primary role in elicitation of the disease.

Because a large amount of TNF α is expressed in GCA, anti-TNF α therapy may be successful in treating GCA. Indeed, recently published data reveal a potential positive response to Infliximab in four cases (28). In spite of these encouraging preliminary results, controlled studies with a greater number of patients are needed.

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