Tissue-Destructive Macrophages in Giant Cell Arteritis


Abstract—Giant cell arteritis (GCA) is an inflammatory vasculopathy in which T cells and macrophages infiltrate the wall of medium and large arteries. Clinical consequences such as blindness and stroke are related to arterial occlusion. Formation of aortic aneurysms may result from necrosis of smooth muscle cells and fragmentation of elastic membranes. The molecular mechanisms of arterial wall injury in GCA are not understood. To identify mechanisms of arterial damage, gene expression in inflamed and uninvolved temporal artery specimens was compared by differential display polymerase chain reaction. Genes differentially expressed in arterial lesions included 3 products encoded by the mitochondrial genome. Immunohistochemistry with antibodies specific for a 65-kDa mitochondrial antigen revealed that increased expression of mitochondrial products was characteristic of multinucleated giant cells and of CD68+ macrophages that cluster in the media and at the media-intima junction. 4-Hydroxy-2-nonenal adducts, products of lipid peroxidation, were detected on smooth muscle cells and on tissue infiltrating cells, in close proximity to multinucleated giant cells and CD68+ macrophages. Also, giant cells and macrophages with overexpression of mitochondrial products were able to synthesize metalloproteinase-2. Our data suggest that in the vascular lesions characteristic for GCA, a subset of macrophages has the potential to support several pathways of arterial injury, including the release of reactive oxygen species and the production of metalloproteinase-2. This macrophage subset is topographically defined and is also identified by overexpression of mitochondrial genes. Because these macrophages have a high potential to promote several mechanisms of arterial wall damage, they should be therapeutically targeted to prevent blood vessel destruction.

Key Words: reactive oxygen species • vasculitis • metalloproteinase • macrophage

Giant cell arteritis (GCA) is an inflammatory vasculopathy that affects mainly the upper extremity and extracranial branches of the aorta.1,2 The disease preferentially involves medium-sized arteries, but aortitis may occur more frequently than previously thought.3 Emerging data support the concept that GCA represents the sequelae of an immune response directed against antigens that reside in the arterial wall. Evidence has been collected that T lymphocytes play a critical role in the disease process.4 Curiously, IFN-γ as a key T-cell product accumulates in the adventitia of affected blood vessels.5 T cells that produce IFN-γ intermingle with a functionally defined subset of macrophages that produces IL-1β, IL-6, and TGF-β.6,7 The topographical arrangement of activated T cells and macrophages that produce cytokines indicates that the adventitia is the site of the initial immunologic injury. However, tissue-destructive events in this arteritis are focused on the media, media-intima junction, and intima. The arterial injury either results in intimal hyperplasia with subsequent luminal occlusion or in wall fragmentation with aneurysm formation. The relationship between the events that occur in the adventitia and the tissue destruction that affects the media and intima has not been addressed. Presently, little information exists on the molecular mechanisms that culminate in arterial damage. Proteolytic enzymes have been detected in the infiltrate. Matrix metalloproteinase (MMP)-2 and MMP-9 have both been described to be expressed in tissue-infiltrating inflammatory cells and possibly in cellular components of the arterial wall.6,8 However, MMPs are produced as proenzymes, and how they are activated in the arterial wall to exhibit proteolytic and injurious activity is not clear.9 Evidence also exists that nitric oxide (NO) is produced in the arterial tissue. Macrophages that reside in the intima express high levels of inducible nitric oxide synthase (iNOS) and are likely to produce this toxic mediator.6 The presence of NO has been associated with proinflammatory and tissue-damaging effects.10 However, the spatial restriction of macrophages that express iNOS in the intima suggests that NO does not contribute to media destruction.

To identify mechanisms involved in the destruction of the medial layer and the elastic membranes of the artery, we compared gene expression in inflamed and nonaffected temporal arteries by differential display polymerase chain reaction (DD-PCR).11 The power of this technology relates to its

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ability to provide information on the upregulation of gene transcripts derived from either infiltrating or residing cells, which allows for the detection and characterization of novel pathways involved in the disease process. Three of 19 gene products overexpressed in affected arterial tissue were derived from the mitochondrial genome. Increased transcription of mitochondrial gene products led to the recognition of mitochondrial activation in some but not all cells in the vascular lesions. Multinucleated giant cells and macrophages accumulated in the media, clustered along the elastic laminae, and situated in the center of tissue damage overexpressed mitochondrial genes. To examine the destructive potential of these particular macrophages, evidence was sought for the action of reactive oxygen species (ROS) and the expression of MMP in the arterial wall. Because free radicals have a limited life span in the tissue, they are difficult to identify directly. The most susceptible components of oxidative damage are polyunsaturated fatty acids, and the major products of lipid peroxidation are 4-hydroxy-2-nonenal (HNE) adducts. HNE adducts could be readily detected in temporal arteries with vasculitic lesions. Macrophages with upregulated mitochondrial activity and the formation of HNE adducts spatially coincided around the elastic membranes and in the media. Additional characteristic of this macrophage subset revealed that the upregulation of mitochondrial activity and the formation of HNE adducts closely correlated with the expression of MMP-2. We propose that this macrophage subpopulation, which includes multinucleated giant cells, is a major effector of tissue injury in GCA.

Materials and Methods

Samples

Temporal artery specimens were obtained during routine diagnostic biopsies. Ten patients with histologic signs of GCA were enrolled in the study after written, informed consent was obtained. Temporal artery samples that were histomorphologically negative and were derived from patients that lacked clinical evidence of systemic vasculitis served as controls. These patients underwent temporal artery biopsy as part of an evaluation for fever of unknown origin or other systemic symptoms. Five-micrometer paraffin sections were dewaxed, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Tissue embedded in OCT or paraffin from 15 patients with histologic signs of GCA, control patients, and activated PBMCs was analyzed by DD-PCR. Empirically, 4 oligo dT primers and 20 random primers can amplify different genomic DNA. For the present study, 4 oligo dT primers (T, T, A, C, G, or T) and 10 random primers (random A 5'-ACG TCA TGA C-3'; random B 5'-GTA CTC AGC 3'; random C 5'-CGT TCC GGT G-3'; random D 5'-CCA TGG TGA A3'; random E 5'-TCC ATG AGT G-3'; random F 5'-ACG TCA TGA C-3'; random G 5'-GTA CTC AGA C-3'; random H 5'-TGG AGT ATC A-3'; random I 5'-GTC ATC ATC G-3'; and random J 5'-CAT GTG AAG C-3') were arbitrarily chosen.

RNA was extracted with Trizol (Life Technologies), and contaminating genomic DNA was removed by DNase treatment (GenHunter Corporation). cDNA was synthesized with 1 of the oligo dT primers, 40 µmol/L dNTP, and avian myeloblastosis virus–reverse transcriptase (Roche Molecular Biochemicals–Boehringer Mannheim) at 37°C. cDNA was amplified by PCR (94°C, 2 minutes; 40°C, 2 minutes; 72°C, 30 seconds; 40 cycles) with [32P-α] dATP (Dupont NEN), 2 µmol/L dNTP, 1 random primer (0.5 µmol/L), 1 oligo dT primer (2.5 µmol/L), and Taq DNA polymerase (Roche Molecular Biochemicals–Boehringer Mannheim). Amplified products were separated on a 6% denaturing polyacrylamide gel (Roche Molecular Biochemicals–Boehringer Mannheim). Bands shared between GCA temporal artery specimens and not present in control specimens or in polyclonally activated PBMCs in 2 independent reactions were eluted and reamplified. PCR products were cloned into the pCR 2.1 vector with the TA cloning kit according to manufacturer’s instructions (Invitrogen). Plasmids grown in transformed INVaF cells were isolated with the Qiagen plasmid kit (Qiagen). Inserts of the plasmids were analyzed with the use of automated sequencing. The GCG program (Genetics Computer Group) was used for homology searches to match obtained sequences with known genes.

RNase Protection Assay

Templates for riboprobes were generated by amplifying the inserts in the pCR 2.1 vector with T7-linked vector universal primers (T-7 antisense 5'-GAT TAC TAA GCA ACA GAG AGG GGT AAC GGC CGC CAG TGT GCT G-3'; sense 5'-TAA CCG CCG CCA GTG TGC TG-3'). The templates for the GAPDH probe were generated by PCR with GAPDH-specific primers (T-7 antisense 5'-GAT TAC TAA GCA ACA GAG AGG GGT AAC GGC CGC CAG TGT GCT G-3'; sense 5'-GTC TTC ACC ATG ACC ATG-3') to retrieve a 150-nt fragment. Riboprobes were obtained by T7 polymerase in vitro transcription with MaxScript (Ambion) in the presence of [32P-α] UTP (Dupont NEN) and were purified by gel electrophoresis with an 8-mol/L urea denaturing 5% polyacrylamide gel and subsequent elution in 0.2% SDS, 1 mmol/L EDTA, and 0.5 mol/L ammonium acetate.

The RNAse protection assays were performed with Direct Protect according to manufacturer’s instructions (Ambion). All samples were adjusted for GAPDH mRNA content. In brief, specimens were lysed in lysis buffer that contained guanidine thiocyanate and hybridized overnight at 37°C in the presence of molar excess of radioactively labeled riboprobe. Single-stranded RNA was digested with RNaseA. mRNA-riboprobe hybrids were alcohol-precipitated, separated on a native 5% polyacrylamide gel, and semi-quantified with the Molecular Imager for 32P (Bio-Rad Laboratories). A serial dilution of RNA that contained the appropriate sequence was included in every RNAse protection assay for internal standardization.

Antibodies

Cells with upregulated mitochondrial proteins were identified with mouse mAb against 65-kDa mitochondrial antigen (1:100; Biogenesis) and cytochrome oxidase subunit 1 (1:100; Molecular Probes). Mouse mAb against CD3 (1:100), CD68 (1:150; PG-M1 or KP-1), and human α-smooth muscle actin (1:50) as well as all secondary and horseradish peroxidase–conjugated antibodies were obtained from Dako. Rabbit polyclonal Ab against MMP-2 (1:2000) was kindly provided by Dr. W. Stetler-Stevenson, NIH, Washington, DC. HNE-modified amino acid residues (lysine, histidine, and cysteine) on protein were detected by a rabbit polyclonal Ab (1:1000). Streptavidin-conjugated alkaline phosphatase and Vector Red substrate kit were purchased from Vector Laboratories. Goat anti-rabbit Ig Cy2-conjugated Ab (1:200) was obtained from Jackson ImmunoResearch.

Immunohistochemistry

Five-micrometer paraffin sections were dewaxed, and endogenous peroxidase was blocked. After antigen recovery with steam, nonspecific binding was blocked with 5% rabbit serum (Life Technologies). To stain mitochondrial sections were incubated with mouse mAb against 65-kDa mitochondrial antigen overnight or mouse mAb
against cytochrome oxidase subunit 1 for 30 minutes. Subsequently, the slides were developed with biotinylated secondary Ab, streptavidin-coupled alkaline phosphatase, and the Vector Red staining kit. For 2-color immunohistochemistry, sections were blocked with 5% normal goat serum (Life Technologies) and incubated with mAb against CD68 (PG-M1), α-smooth muscle actin, CD3, or MMP-2 for 30 minutes and developed with horseradish peroxidase and 3,3′-diaminobenzidine (Sigma Chemical) as chromagen. Nuclei were counterstained with hematoxylin. Negative controls without primary Ab were included in every series. Single- and double-positive cells were counted with the use of an Axioshot microscope (Zeiss). For double immunofluorescence, sections were incubated with Ab against MMP-2 for 30 minutes and subsequently stained with fluorescent goat anti-rabbit Ig Cy2-labeled Ab. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma).

To stain HNE adducts in proteins, 5-μm frozen sections from temporal artery specimens were fixed in acetone and 1% paraformaldehyde. After the slides were blocked with 5% normal goat serum, they were labeled with rabbit polyclonal Ab against HNE-modified amino acids by overnight incubation at 4°C. Sections were developed with secondary Ab, alkaline phosphatase, and Vector Red. Adjacent sections were stained with mAb against 65-kDa mitochondrial antigen (1:100) in identical fashion.

Computer Imaging
The temporal artery sections stained for HNE and 65-kDa mitochondrial antigen were scanned with a confocal immunofluorescent laser-scanning microscope (Axioskop, Zeiss) and analyzed with the KS400 image analysis system (Kotron Electronics). The stained area was quantified as the percentage of the total tissue area and separately measured for the different layers of the arterial wall. Double immunofluorescence sections stained for 65-kDa mitochondrial antigen and MMP-2 were scanned and analyzed for single- and double-positive areas with the same imaging system.

Results
Upregulation of Mitochondrial-Encoded Genes in Temporal Arteries Affected by GCA
To search for mechanisms that contribute to vascular damage in GCA, DD-PCR was used to identify genes differentially expressed in the inflamed arterial tissue. Temporal artery samples from 5 patients with GCA were compared with specimens from 5 patients without histomorphological evidence of arteritis and without clinical evidence of polymyalgia rheumatica. To exclude genes commonly expressed in activated mononuclear cells, RNA was also isolated from polyclonally stimulated PBMCs. Bands were considered to be differentially expressed if they were reproducibly upregulated in at least 2 independent DD-PCR reactions and if they were found in at least 4 of the 5 patient samples and none of the controls. Within the arbitrarily chosen window of 10 random primers and 4 oligo dT primers, 19 bands were found that fulfilled these criteria. Sequence analysis of the differentially expressed amplification products and a homology search revealed matches with known gene products for 9 sequences; 10 sequences were unknown. Three of the predicted gene products were encoded by mitochondrial DNA. Sequences highly homologous to 12S rRNA, NADH dehydrogenase subunit 4, and cytochrome oxidase subunit 1 were found to be specifically overexpressed in the inflamed arteries compared with noninflamed arteries and in vitro–activated PBMCs. The 2 enzymes, NADH dehydrogenase subunit 4 and cytochrome oxidase subunit 1, belong to complexes I and IV of the respiratory chain, respectively.

To confirm overrepresentation in GCA arterial tissue, the expression levels of the mitochondrial-encoded gene transcripts were measured with RNase protection assays (Figure 1). Quantification of mRNA levels revealed a 2-fold upregulation of 12S rRNA in temporal artery specimens from GCA patients. Cytochrome oxidase subunit 1 expression increased 1.5-fold.

Mitochondrial Activation Is a Characteristic Feature of CD68+ Macrophages That Reside Along the Elastic Laminae and in the Media
The mitochondrial origin of 3 gene products identified by DD-PCR suggested a special role for mitochondrial function in the inflammatory response of GCA. To identify the cell of origin of upregulated mitochondrial genes in the vascular lesions, sections from temporal arteries of patients with GCA were stained with 2 mitochondria-specific antibodies that recognize a 65-kDa mitochondrial antigen or cytochrome oxidase subunit 1 (Figure 2A and 2C, respectively). Both antibodies displayed the same differential staining pattern in the arterial wall. They preferentially stained cells located around the internal elastic lamina and in the media, which suggests that this subpopulation of cells had upregulated expression of mitochondrial proteins (Figures 2 and 3). No immunostaining with either antibody was found in temporal arteries negative for arteritis (Figure 2E). The lack of staining with antibodies specific for mitochondrial antigens in normal arteries and the intimal and adventitial tissue of inflamed arteries was consistent with previous reports that indicated that immunocytochemical demonstration of mitochondrial products is restricted to selected tissues and cells.15 Immunocytochemical staining of mitochondrial products was restricted to selected tissues and cells.15 Immunocytochemical staining of mitochondrial products was restricted to selected tissues and cells.15
Figure 2. Immunohistochemical detection of mitochondrial antigens in temporal arteries from GCA patients. Sections of temporal arteries were stained with mAb specific for the 65-kDa mitochondrial antigen, developed with Vector Red substrate, and visualized with immunofluorescence. High levels of the 65-kDa mitochondrial antigen (A) and cytochrome oxidase subunit 1 (C) were expressed by cells in the media and in the vicinity of the internal elastic lamina. Noninflamed normal arteries (E) did not contain cells with upregulated expression of mitochondrial products. The internal elastic lamina is indicated by arrows. Appropriate specificity controls on adjacent sections are shown in B and D. Magnification ×100.
localization of mitochondrial products in GCA arteries demonstrated a layer-specific expression of mitochondrial products in the arterial wall and suggested an altered functional state of a cell population localized in the media and at the media-intima junction. Two-color immunohistochemistry with T cell, macrophage, and smooth muscle cell (SMC) markers revealed that nearly all cells with enhanced transcription of mitochondrial genes expressed the CD68 phenotype (Figure 3A). Cells characterized by high levels of mitochondrial antigens included multinucleated giant cells (Figure 3B). In addition, intense staining was a characteristic finding for CD68\(^{+}\) macrophages arranged in granulomata. CD3\(^{+}\) lymphocytes with mitochondrial activation were rare (<2%) and few cells that expressed smooth muscle actin had upregulated transcription of mitochondrial genes.

We have previously described that tissue-infiltrating CD68\(^{+}\) cells can be subdivided into distinct subsets on the basis of their topography and their functional profile.\(^6\) To explore whether mitochondrial activation was associated with a certain topographical organization and functional commitment, the frequencies of CD68\(^{+}\) cells staining with 65-kDa mitochondrial antigen specific antibodies were determined in the different layers of the arterial wall in a series of patients (Figure 4). Upregulation of mitochondrial genes was infrequent among CD68\(^{+}\) cells in the adventitia (median, 5%) although these macrophages are known to be activated and produce monokines.\(^6\) Also, only \(\approx 15\%\) of CD68\(^{+}\) macrophages in the intima were characterized by increased mitochondrial transcription. In contrast, between 38% to 68% of macrophages around the internal and external elastic laminae stained positive for the antibodies. In summary, upregulation of mitochondrial gene transcription was a typical finding for a subpopulation of macrophages that infiltrate tissue and for multinucleated giant cells, which suggested that these cells had unique functional capabilities.

**Figure 3.** Immunolocalization of mitochondrial antigens in CD68\(^{+}\) macrophages. Sections of inflamed temporal arteries were immunostained with anti-CD68 (brown) and anti-65-kDa mitochondrial antigen (red). A. A region of the arterial media is shown (magnification \(\times 400\)). High levels of mitochondrial antigens were expressed in a subset of CD68\(^{+}\) macrophages (A). Temporal artery specimens were immunostained as described in Figure 2A. Multinucleated giant cells stained intensely with anti-65-kDa mitochondrial antigen as shown here at a higher magnification (B). Red color depicts staining for 65-kDa mitochondrial antigen; blue identifies nuclei stained with DAPI (magnification \(\times 1000\)). Arrows indicate a fragment of the internal elastic lamina. Negative controls showed no autofluorescence of giant cells (see Figure 2B).

**Figure 4.** Topographical arrangement of CD68\(^{+}\) macrophages with upregulated mitochondrial products. Temporal artery specimens from 5 patients with GCA were double-stained with mouse mAb against 65-kDa mitochondrial antigen and CD68. The percentage of double-positive cells was determined for each histologic layer: intima, media, and adventitia, and the area directly adjacent to the internal elastic lamina. Frequencies of double-positive cells are displayed as box plots with median, 25th, and 75th percentiles as boxes and the 10th and 90th percentiles as whiskers.

**Cells With Upregulated Mitochondrial Gene Expression Colocalize With Membrane Destruction Caused by Oxidizing Free Radicals**

The topographical restriction of CD68\(^{+}\) cells with upregulated mitochondrial transcription to the center of tissue damage in the blood vessel wall suggested that these cells were specialized to inflict arterial injury. Possible mechanisms include the release of ROS and the production of MMP. One of the stable sequelae of oxidative damage in tissue is lipid peroxidation of fatty acids in membrane bilayers. ROS readily attack \(\omega-3\)-polyunsaturated fatty acids of membrane phospholipids that results in the release of toxic aliphatic aldehydes such as malondialdehyde bis-(diethyl acetal) and HNE.\(^12,16\) Malondialdehyde and HNE destructively interact with proteins or nucleotides to form adducts. HNE protein adducts were readily detectable on multinucleated giant cells and on macrophages that surrounded the elastic laminae and on macrophages in the media (Figure 5).
Diffuse tissue staining with HNE-specific antibodies was typical for granulomata in which mononuclear cells as well as surrounding tissue were strongly positive. In addition, confluent stains of SMC membranes were found in selected areas of the media. Lipid peroxidation of cell membranes was rarely spread throughout the entire medial layer but instead displayed a patchy distribution.

To establish whether mitochondrial activation and oxidative injury of cells coincided, the tissue distribution of both phenomena was correlated in sections from 5 patients with GCA (Table 2). There was a close anatomic relationship between CD68+ cells with upregulated mitochondrial proteins and tissue regions altered by ROS-mediated peroxidation. Not only were the CD68+ cells with mitochondrial activation targets for oxidative damage, they were also surrounded by ROS-damaged cells (Figure 5A and 5B). The colocalization of both phenomena strongly suggests that ROS are generated in these specialized macrophages in which they cause autodamage and are involved in widespread cytopathic events that target the SMC layer.

Co-occurrence of ROS and MMP-2 Production in CD68+ Macrophages Accumulating in Areas of Tissue Destruction

We have previously described that a fraction of CD68+ cells arranged along the elastic laminae and infiltrating the media are characterized by their ability to produce MMP-2. Recent reports have emphasized that MMPs are produced as proenzymes and that ROS but not NO have an active role in releasing the functional enzyme. To assess whether MMP-2 and ROS production could be assigned to identical or distinct cells and could be correlated with the upregulation of mitochondrial gene transcription, 2-color immunofluorescence was used (Figure 6A). Positivity for both markers, anti-MMP-2 and anti–65-kDa mitochondrial antigen, was frequently encountered. Multinucleated giant cells were consistently positive for MMP-2 production and mitochondrial activation. Sixty percent to 95% of MMP-2+ cells in the media and the adjacent elastic laminae also stained with anti–65-kDa mitochondrial antigen Ab (Figure 6B). A small proportion of cells had the ability to secrete MMP-2 in the absence of increased mitochondrial activity. Mitochondrial activation without MMP-2 synthesis was distinctly infrequent. Thus, the characteristic functional profile of CD68+ cells that reside in the SMC layer and around the elastic membranes that define the media-adventitia and media-intima junctions includes the production of proteolytic enzymes, mitochondrial activation, and the release of ROS. Findings identify these macrophages as cells with a high potential for tissue injury.

Discussion

GCA is a systemic vasculitis that causes a spectrum of clinical symptoms. Manifestations of systemic inflammation, such as a brisk, acute-phase response; fever; anemia; and weight loss can dominate the clinical presentation. However, the most serious complications arise from inflammation-mediated damage of the extracranial arteries and the aorta. Arterial wall destruction of the aorta that results in aneurysm formation and rupture is a disastrous consequence. Arterial occlusion can cause blindness and stroke. Although the inflammation appears to be a sequelae of antigen recognition by T cells in the adventitia, the actual mechanisms of tissue injury are unclear. The present study provides strong, albeit indirect, evidence that specialized macrophages and giant cells mediate media destruction in this disease through their
ability to release ROS and secrete MMP-2. The highly destructive potential of this defined subset of macrophages and giant cells was associated with the overexpression of mitochondrial products. Because these cells can be molecularly and functionally differentiated from other tissue-infiltrating macrophages and from resident cells, it might be possible to target them specifically.

The release of toxic oxygen products has not been considered as a disease component in GCA. Conversely, the formation of ROS has been accepted as a critical pathway of pathology in other vasculitic syndromes. A role of oxidizing free radicals has been discussed in Wegener’s granulomatosis, a vasculitis characterized by the emergence of autoantibodies to enzymes in neutrophils. It is possible that these autoantibodies can recognize their target antigens on the surface of neutrophils and directly induce respiratory burst. In contrast to Wegener’s granulomatosis, neutrophils are virtually absent from the inflammatory infiltrate in GCA. The histologic hallmark of GCA is granulomas with the accumulation of macrophages, lymphocytes, epithelioid cells, and multinucleated giant cells. This histomorphology is highly suggestive of a central role of macrophages in the arterial injury induced by GCA.

We explored whether oxidative stress and oxygen radical-mediated tissue damage has a role in GCA. ROS-related pathology was suggested by the typical granuloma formation in GCA. In experimental pulmonary granulomatosis, high quantities of superoxide anions are released synchronously with the development of granulomata. Treatment of experimental animals with an oxygen scavenger, d-α-tocopherol, inhibited granuloma development. Also, enzymatic suppression of oxygen metabolites could prevent granuloma formation. Granulomatous reactions are followed by significant damage to the underlying tissue structures. Extensive fibrosis usually accompanies resolution of the granuloma and results in irreversible disruption of tissue architecture and physiology. To provide evidence for a direct action of oxygen-derived free radicals in the arterial wall, we searched

Figure 6. Expression of MMP-2 (72-kDa collagenase) in macrophages with upregulated mitochondrial products. Paraffin-embedded sections of temporal arteries affected by GCA were stained with a polyclonal rabbit anti–MMP-2 Ab and with the anti-65-kDa mitochondrial antigen mAb. Anti–MMP-2 binding was detected with a Cy2-labeled secondary antibody (B), and anti–65-kDa mitochondrial antigen mAb was developed with Vector red (C). A negative control staining is shown in panel A. Immunostains were scanned with a confocal laser-scanning microscope. MMP-2 production was a feature of giant cells arranged as a garland along the intimal elastic lamina and of a subpopulation of macrophages with upregulated mitochondrial proteins in the arterial media. In giant cells, metalloproteinase and mitochondrial staining polarized to different regions of each cell (magnification ×100). D, Tissue sections from 5 patients were evaluated for the co-occurrence of mitochondrial activation and MMP-2 expression in macrophages with the use of 2-color immunohistochemistry. The number of single- and double-positive cells was determined in the media and at the media-intima junction. Results are shown as the mean of 5 sections of each specimen. Most of the macrophages with upregulated mitochondrial protein expression produced MMP-2. Double-positive cells (MMP-2 and 65-kDa mitochondrial antigen) are indicated by the gray bar; single-positive cells (65-kDa mitochondrial antigen), solid bar; and single-positive cells (MMP-2), open bar.
for adduct formation on cell membranes. One of the toxic mechanisms of ROS is lipid peroxidation. HNE production and subsequent formation of Michael adducts with amino acids is a marker of lipid peroxidation. HNE was detected through the use of specific antibodies that recognize these adducts on the amino acids lysine, histidine, and cysteine. This approach has been shown to be a highly sensitive tool to measure the action of toxic ROS in vivo. The staining pattern obtained by the anti-HNE Ab demonstrated peroxidized lipids on the surface of CD68+ cells and also on surrounding CD68− cells. Preferred targets for lipid peroxidation were SMCs and the media. ROS-induced damage to cell membranes was absent in the intimal and adventitial layers, which emphasized that direct tissue injury focuses on the media and the bordering elastic lamina. The congruent distribution of cells with mitochondrial activation and ROS-induced tissue damage indicated a causal relationship between these 2 phenomena. The respiratory burst of phagocytes is usually mediated by NADPH oxidase translocated to the cell membrane, and cells may need to be metabolically hyperactive to sustain this activity. However, it has been reported that this enzyme is downregulated as monocytes differentiate; thus, this pathway may no longer be dominant in the tissue lesions. Alternatively, it could be hypothesized that mitochondrial activation is directly involved. Increased enzymatic reactions of the electron transport chain in mitochondria are associated with a leakage of ROS that may be sufficient to induce lipid peroxidation.

The generation of ROS in the arterial wall may not only inflict direct tissue damage but may also be involved in the regulation of the activity of proteolytic enzymes. We have previously reported that a specialized population of CD68+ cells synthesizes MMP-2. MMP-2+ CD68+ cells can be defined by their localization between the medial SMC and in close vicinity to the internal and external elastic laminae. MMPs are synthesized as proenzymes and need further processing to release active enzymes. A recent study by Rajagopalan et al has linked the action of ROS with the regulation of activation of MMPs in foam cells isolated from atherosclerotic plaques. To investigate the relationship between the formation of oxidants and the synthesis of MMP-2, we examined the co-occurrence of these 2 macrophage functions in the vascular lesions. Mitochondrial activation overlapped closely with MMP-2 formation and indicated that the production of ROS and MMP-2 identifies a defined macrophage subset. A unique subset of this macrophage population is multinucleated giant cells. These macrophages, as well as the giant cells, reside in the center of tissue injury and appear to be cell populations with the potential to be highly destructive.

The present study has raised several questions that must still be studied. Curiously, upregulation of mitochondrial proteins was not a feature of all tissue-infiltrating cells, although that would have been expected. Also, medial SMCs were not among the cells that overexpressed cytochrome oxidase or 65-kDa mitochondrial antigen. The phenotype of increased production of mitochondrial products was strictly limited to a subset of cells: CD68+ macrophages and giant cells in a defined topographical localization. It would be most interesting to explore the specific signals that induce MMP production and upregulation of mitochondrial transcription with subsequent increase of mitochondrial activity. Previous studies have shown that IFN-γ produced by T cells in the adventitia is the key cytokine of the inflammatory response in GCA. IFN-γ is known to be a potent macrophage activator; however, additional stimuli may determine the activation of these medial macrophages and their pattern of gene expression. More importantly, macrophage differentiation and activation appears to vary among GCA patients, and these differences correlate with the extent of arterial wall injury and remodeling. GCA patients with minimal intimal hyperplasia have a largely preserved arterial architecture with minimal destruction of the elastic lamina, whereas patients with marked intimal hyperplasia are characterized by a disrupted internal elastic lamina. These histologic differences do not appear to be different stages of the disease process but instead reflect heterogeneity in macrophage function. Medial macrophages in patients with intimal hyperplasia have a different product profile and tend to form giant cells more frequently compared with patients without intimal proliferation (M.K., J.J.G., C.M.W., unpublished data, 1998).

The understanding of disease mechanisms operative in the damage to the arterial walls in GCA has obvious implications. Molecules implicated in causing tissue injury are prime candidates for therapeutic inhibition. Currently, there is only 1 treatment that has been shown to be efficacious: the use of corticosteroids given in large doses over several years. This treatment is complicated by a high rate of serious side effects that must be weighed against the risk for blindness, stroke, and aneurysm development. Constraints in the therapeutic options reflect the paucity of information on mechanisms operative in arterial injury. If proteolytic destruction by selected MMPs constitutes an important attack on the medial layer, therapeutic inhibition of proteases should be considered. Approaches could also be directed toward the inhibition of ROS. The tissue-damaging effects of toxic ROS may be reduced through the use of oxygen scavengers such as d-a-tocopherol. Because some of these approaches are rather nontoxic, direct application in clinical trials could be considered. Other interventions that are of a more experimental nature should be tested in a model system. We have recently succeeded in establishing a novel “animal model” for GCA. Granulomatous arteritis can be maintained in temporal artery specimens collected from patients and engrafted into severe combined immunodeficiency mice. We have used these artery-mouse chimeras to investigate the mechanism of action of corticosteroids in vivo. Therapeutic interventions directed at tissue injurious pathways could be rapidly explored in the engrafted arteries. Finally, it is likely that tissue-injurious reactions in the temporal artery of patients with GCA are not unique to vasculitis. It can be predicted that the mechanisms of arterial injury are shared in a variety of inflammatory and noninflammatory diseases that affect muscular arteries. The spectrum of diseases may include atherosclerotic disease that affects coronary arteries that have many structural features in common with temporal arteries. Understanding the disease mechanisms in GCA may encourage the search for therapeutic interventions in atherosclerotic disease.
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