Cytokine gene expression in aortic adventitial inflammation associated with advanced atherosclerosis (chronic periaortitis)

A L Ramshaw, D E Roskell, D V Parums

Abstract

Aims—To determine whether aortic adventitial chronic inflammation associated with advanced atherosclerosis ("chronic periaortitis") is associated with any detectable cytokine gene expression.

Methods—RNA was extracted from six fresh surgical specimens of atherosclerotic aortic aneurysm wall showing a spectrum of chronic periaortitis. Controls included four normal aortas and an HUT 78 T cell line. Reverse transcriptase and the polymerase chain reaction (PCR) were used to amplify mRNA for interleukins-1 α (IL-1 α), -2 (IL-2), -4 (IL-4), IL-2 receptor- α (IL-2R- α), tumour necrosis factor α (TNF- α) and gamma interferon (IFN- γ) with β -actin as an internal control.

Results—No TNF- α mRNA was detected in any of the inflamed aortic tissue samples, in contrast to the aortic T lymphocytes propagated in culture in IL-2 conditioned medium (aortic cultured T cells) and peripheral blood mononuclear cells from these patients. In contrast, IFN- γ , IL-1 α , IL-2, IL-2 receptor and IL-4 PCR products were detected for each inflamed aortic tissue RNA sample with IFN- γ mRNA expression increasing with increasing degrees of adventitial inflammation. Only β -actin mRNA was present in the normal aorta.

Conclusions—These findings indicate the active nature of aortic adventitial chronic inflammation associated with human advanced atherosclerosis ("chronic periaortitis") and show its possible progressive potential to the clinically important diseases termed "idiopathic retroperitoneal fibrosis" and "inflammatory aneurysm".

(J Clin Pathol 1994;47:721-727)

Chronic inflammatory cells are present in the aortic and arterial intima at all stages of atherogenesis. As the lesion becomes more advanced and the media thins, a spectrum of chronic inflammation is seen in the adventitia.^{1,2} The inflammatory cells consist of lymphocytes and plasma cells and lymphoid follicles are common. A varying degree of fibrosis is seen. When present in the aorta, the condition is termed "chronic periaortitis".^{1,2} Although the degree of inflammation seen usually produces no aortic wall thickening or

clinical consequences, in its most severe form the condition may represent "inflammatory aneurysm" or "idiopathic retroperitoneal fibrosis" in dilated and undilated aortas, respectively.²

Chronic periaortitis is thought to arise as a local immune response to oxidised lipids elaborated by macrophages in the atheromatous plaque.³ Immunohistochemistry has shown that, whereas the chronic inflammatory cells in the intimal atheroma consist of macrophages and T lymphocytes, in the inflamed adventitia B lymphocytes predominate, including plasma cells, surrounded by CD4 positive T lymphocytes and macrophages.⁴ MHC class II molecule expression is abundant, particularly on endothelial cells (CD31 positive). Interleukin (IL)-2 and IL-4 receptor expression is seen throughout the tissue and B cell proliferation is seen within the lymphoid follicles.⁵

Immunohistochemical analysis of cytokine dependent adhesion molecules in atherosclerosis and chronic periaortitis has shown strong anti-E-selectin (formerly endothelial leucocyte adhesion molecule-1 (ELAM-1)) staining confined to endothelial cells in the aortic media and adventitia, extensive anti-intercellular adhesion molecule-1 (ICAM-1) staining on endothelial cells in the intima and adventitia, and anti-vascular cell adhesion molecule-1 (VCAM-1) staining associated with B cell aggregates with moderate staining of vessels in lymphoid follicles.^{6,7}

Cytokines are small soluble protein mediators with specialised functions that have a central role in the regulation of all immune responses.^{8,9} These molecules are fundamental in the initiation, maintenance, and progression of inflammation, and are required for recruitment of cells, for activation and proliferation.⁸⁻¹⁰ There has been recent interest in the role of cytokines in atherogenesis.¹¹⁻¹⁵ The predominant cytokine mediators thought to be involved include platelet derived growth factor (PDGF), IL-1, monocyte chemoattractant protein-1 (MCP-1), tumour necrosis factor (TNF)- α , interferon (IFN)- γ and granulocyte-macrophage colony stimulating factor (GM-CSF). These mediators are thought to control the intimal inflammation as well as the altered functions of smooth muscle cells. While cytokines are clearly important in the inflammatory component of plaque formation, they must also have a role in chronic periaortitis where the inflammation is much more intense than that present in the intima.

To determine the presence and relative

Nuffield Department of Pathology and Bacteriology, John Radcliffe Hospital, Oxford
A L Ramshaw
D E Roskell

Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0NN
D V Parums

Correspondence to:
Dr Dinah Parums

Accepted for publication
9 February 1994

abundance of cytokines, the protein product can be measured by western blot analysis, by immunohistochemistry, and by immunoassays such as the enzyme linked immunosorbent assay (ELISA).⁸ Cytokine mRNA can be detected by northern blotting, by RNA dot blotting, and by *in situ* hybridisation, or by the polymerase chain reaction (PCR) following reverse transcription.⁸ It is often preferable to study mRNA expression, which in many instances is a reflection of the protein product.¹⁶ Of the techniques for measuring cytokines, PCR is the most reproducible as well as being extremely sensitive, requiring only small amounts of cellular RNA.¹⁷

PCR was first described for indirect amplification of RNA transcripts by Veres *et al.*¹⁸ Specific mRNA is amplified following the synthesis of copy DNA (cDNA) using the reverse transcriptase enzyme. cDNA is subsequently amplified by PCR using sequence specific primers. This method has been applied to the analysis of cytokine mRNA expression by a process called message amplification phenotyping (MAPPING).^{16,17} Most work using PCR assisted RNA amplification has been carried out for cultured cells.^{16,19} Recently, workers have begun to investigate the presence of cytokines in aortic atherosclerosis using immunohistochemistry and ELISA techniques.^{20,21}

To determine which cytokines may be modulating and controlling this B cell dominant, active inflammatory response seen in chronic periaortitis, we chose to screen tissues for mRNA expression using PCR assisted amplification of RNA. Due to the large number of known cytokines, this investigation was limited to cytokines which have been shown to be particularly important in chronic inflammation and which may be relevant to T and B lymphocyte responses. These include the pro-inflammatory cytokines TNF- α , IFN- γ , IL-1 α , as well as the cytokines IL-2 and IL-4, and the IL-2 receptor, involved in lymphocyte activation and proliferation. The resultant PCR products were taken to indicate which cytokines were present *in vivo* in chronic periaortitis and which might therefore play a part in the maintenance and progression of this chronic inflammatory process, which we believe is secondary to aortic advanced atherosclerosis.^{1,2}

Table 1 Tissue samples for PCR analysis of mRNA

Tissue no	Degree of inflammation	Wall thickness (mm)
<i>Chronic periaortitis</i> (fresh surgical abdominal aortic aneurysm tissue)		
I	Mild	3
II	Severe	8
III	Severe	30
IV	Moderate	4
V	Mild	3
VI	Severe	7
<i>Normal aorta</i> (Post mortem tissue <18 hours)		
A	Absent	2
B	Absent	2
<i>(Surgical tissue <1 hour)</i>		
C	Absent	2
D	Absent	2

Methods

Samples included six abdominal aortic tissues with chronic periaortitis (I to VI), two post mortem "normal" aortas (A and B), and two "normal" aortas received as surgical pathology specimens (C and D) (table 1). The histology was observed on paraffin wax sections from blocks of tissue taken adjacent to the tissue for RNA extraction.

Peripheral blood was collected 24 hours preoperatively from three patients with aneurysm (cases I, III, and V (table 1)) and from three laboratory volunteers (a 27 year old woman; a 29 year old man; and a 28 year old man). Mononuclear cells were isolated by Ficoll-Paque (Pharmacia) density gradient centrifugation.

CONTROL CELL LINES

Two cell lines were used (HUT 78 and MCF-7). The HUT 78 human lymphoma T cell line is highly inducible for cytokines when stimulated with phytohaemagglutinin-P (PHA).¹⁶ HUT 78 cells were therefore used as a positive control for the analysis of cytokine mRNA expression. The MCF-7 breast cell line (a largely non-secretory cell) was used as a negative control.

HUT 78 cells were stimulated to increase the synthesis of specific cytokine mRNA. Phytohaemagglutinin (10 μ g/ml) (PHA) (Sigma) in sterile water was added to HUT 78 cells in exponential growth. 10⁷ PHA stimulated HUT 78 cells, 10⁷ unstimulated HUT 78 cells, and 10⁷ MCF-7 cells were harvested and prepared for RNA extraction.

AORTIC CULTURED T LYMPHOCYTES

To compare the *in vivo* and *in vitro* expression of cytokines, two cases of abdominal aortic aneurysm tissue (IV and V (table 1)) were cultured *in vitro* in IL-2 conditioned medium.²² Biopsy samples from abdominal aortic aneurysms were cut into small pieces (1 mm³) and placed in 2 ml of media (RPMI 1640 (Flow), 2 mM glutamine (Flow), 10% heat inactivated normal human serum, 10 μ g/ml gentamycin (Flow), 10 units/ml recombinant human IL-2 (Cetus)) per well in a 24 well plate (Flow). The cultures were placed at 37°C, 5% CO₂ in a humid incubator. Controls were carried out by placing tissue pieces in wells without IL-2. Media was replenished every three to four days.

About 3 $\times$ 10⁷ cells were harvested after four to six weeks of growth. Cytospins were prepared and immunocytochemistry was performed using the alkaline phosphatase anti-alkaline phosphatase (APAAP) method.²³ Monoclonal antibodies included 3D4 (anti-CD3, T cell associated),²⁴ T3-10 (anti-CD4, T helper cell associated),²⁵ Tü102 (anti-CD8, T cytotoxic/suppressor cell associated),²⁵ HD37/4KB128 (anti-CD19/22, B cell associated)^{26,27} and EBM/11 (anti-CD68, macrophage-associated).²⁸ Immunocytochemistry showed that the aortic tissue cultured cells were predominantly T cells. They were 80% CD4 positive (T helper cell associated), 15% CD8 positive (T cytotoxic/suppressor cell

associated), 5% CD19/22 positive (B cell associated) and less than 1% CD68 positive (macrophage associated).

TISSUE AND CELL PREPARATION

Biopsy and necropsy samples were minced (1 cm² of tissue was cut into 1–2 mm³ pieces) with a sterile scalpel. After rinsing briefly in sterile phosphate buffered saline (PBS) to remove excess red blood cells, the tissue was transferred to a 50 ml centrifuge tube (Falcon UK) containing 2 ml of 4 M guanidinium-thiocyanate solution (Sigma UK). Tissues were homogenised at top speed for 20–40 seconds using a Polytron (Kinematica, Switzerland). Samples were stored at –70°C until required for RNA extraction.

Cultured cells and blood mononuclear cells (about 1–3 × 10⁷ cells) were centrifuged at 200 × g for five minutes in a 15 ml centrifuge tube and then washed in sterile phosphate buffered saline (PBS). Cells were vortexed for one minute in 2 ml of 4M guanidinium-thiocyanate solution and stored at –20°C.

RNA ISOLATION AND DETECTION

RNA extraction from cell and tissue samples was based on the acid-guanidinium-thiocyanate-phenol-chloroform method of Chomczynski and Sacchi.²⁹ The integrity of the RNA was assessed by electrophoresis following glyoxal treatment in a 1% agarose gel stained with ethidium bromide.

REVERSE TRANSCRIPTION

First-strand cDNA was synthesised from each sample of RNA in DEPC-water using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus). Reverse transcription was carried out in a 750 µl microfuge tube in a total volume of 20 µl reaction mix (1 mM each of deoxynucleotide (d)ATP, dCTP, dGTP, dTTP (Gibco, BRL); 2.5 mM MgCl₂; PCR buffer containing 50 mM KCl, 10 mM TRIS-HCl, pH 8.5; 5 µM oligo d(T)16 (Pharmacia LKB); 20 U/ml RNAase inhibitor (Pharmacia LKB); 50 U/ml reverse transcriptase (cloned Moloney Murine Leukemia Virus (MMLV) reverse transcriptase)(Gibco BRL)) and 0.5 µg of RNA. Reactions were carried out at 42°C for 30 minutes, heat denatured at 99°C

for five minutes, and placed on ice. Volumes of 10 µl were then removed for amplification of cDNA using specific primers.

Oligonucleotide primers (100 ng) specific for β-actin, IFN-γ, TNF-α, IL-1α, IL-2, IL-2 receptor-α and IL-4 were used in this study and are described in table 2. These primers, which span introns to control for amplification of contaminating DNA, were based on published sequences. IFN-γ and TNF-α primers were obtained from Clontech, USA. IL-2, IL-2 receptor-α, IL-4 and β-actin primers were synthesised in the Department of Biochemistry and Molecular Biology, University of Leeds. IL-1α primers were obtained from the GeneAmp RNA PCR kit (Cetus, USA).

AMPLIFICATION BY PCR

Polymerase chain reaction (PCR) assisted amplification of RNA was carried out as described by Veres *et al*¹⁸ and Brenner *et al*¹⁶ directly in the reverse transcription reaction tubes by adding 80 µl reaction mix (2 mM MgCl₂, PCR buffer, 2.5 U AmpliTaq DNA polymerase from Thermophilus Aquaticus (Amersham), 0.2 µM sense and anti-sense oligonucleotide primers) to each sample. Tubes were mixed and centrifuged at 10 000 × g for 30 seconds; 100 µl of light mineral oil was overlaid onto each sample to prevent evaporation during incubation.

PCR was carried out in a thermal cycler (Ericomp, Lazer Ltd UK). Conditions were optimised and standardised for each primer pair using PHA-stimulated HUT 78 T cell samples. Optimal temperatures (for denaturation, for annealing, and for primer extension) were determined for each primer pair, and carried out over 35 cycles. There was about one minute ramp time between temperatures to allow the samples to equilibrate. PCR products were stored at 4°C.

PCR assisted mRNA amplification was repeated at least once for each sample type. Controls for false positive results were carried out for each set of primers by substituting water for RNA. RNA specificity was tested by omitting reverse transcriptase for each primer pair. PCR primer sequences and the expected molecular weight of each cDNA product is shown in table 2.

Table 2 Oligonucleotide primer pair sequences

cDNA	5' sense primer 3' antisense primer	Amplified fragment size (base pairs)	Reference for cDNA sequence
β-actin	5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' 5'-CTAGAAGCATTGCGGTGGACGATGGAGGG-3'	661	30
IFN-γ	5'-ATGAAATATACAAGTTATATCTTGGCTTT-3' 5'-GATGCTCTTCGACCTCGAAACAGCAT-3'	485	31
TNF-α	5'-ATGAGCACTGAAAGCATGATC-3' 5'-TCACAGGGCAATGATCCCAAAGTAGACCTGCCC-3'	702	32
IL-1α	5'-GTCTCTGAATCAGAAATCCTTCTATC-3' 5'-CATGTCAAATTTCACTGCTTCATCC-3'	420	33
IL-2	5'-ATGTACAGGATGCAACTCCTG-3' 5'-TCAAGTCAGTGTGAGATGATGCTTTGACAAAA-3'	462	34
IL-2R-α	5'-TTATCATTTTCGTGGTGGGGCAGATGGTTTA-3' 5'-TCTACTCTTCTCTGTCTCCGCTGCCAGGT-3'	391	35
IL-4	5'-ATGGGTCTCACCTCCCACTGCT-3' 5'-TCAGCTCGAACACTTTGAATATTTCTCTCTCAT3'	462	36

Figure 1 Low power view of an atherosclerotic aortic biopsy specimen (case IV, aortic wall thickness 4 mm) showing the moderate infiltrates of mononuclear cells in the aortic adventitia (left), media (centre) and in the base of the intimal atheroma (right) (haematoxylin and eosin).

ANALYSIS OF PCR PRODUCTS

PCR products were size fractionated by electrophoresis through 2% agarose gels to determine the presence, size, and degree of amplification of a particular transcript. Specificities of the resultant bands were validated by their predicted size in comparison with standard molecular weight markers (pHC624-Taql and pMJ3-Nci1). Semi-quantitative analysis was carried out by determining the relative PCR band intensity for a

given primer pair. Band intensity was graded as absent (no detectable product) (–), low (+), moderate (++) or strong (+++) according to the fluorescence intensity of the ethidium bromide staining. Gels were photographed using ultraviolet transillumination.

Results

Normal aortic tissues (specimens A-D) showed no evidence of atheroma or inflammation. The atheromatous aortic aneurysm samples (specimens I to VI) all showed atherosclerosis with a varying degree of adventitial inflammation and aortic wall thickness as described in table 1. The histology and grading of inflammation (fig 1) was as described before.²⁴

A strong signal for β actin cDNA was detected in all samples, confirming the integrity of the RNA. Cytokine mRNA profiles are shown in table 3 and fig 2. To confirm that all fragments were derived from amplification of cDNA products, duplicate tests were analysed without the addition of reverse transcriptase. No PCR products were detected in these tests.

RNA isolated from PHA-stimulated HUT 78 cells resulted in strong PCR product bands for each transcript, while the unstimulated HUT 78 RNA resulted in weaker products for each cytokine. For the MCF-7 RNA, strong β -actin and weak IL-1 α PCR products were detected. PCR products from the aortic cultured T cell RNA (from cases IV and V) were detected for all of the primer pairs, including TNF- α . Only PCR products corresponding to β -actin were detected from the “normal” aortic

Table 3 Relative gene expression detected in cell lines, aortic tissues, and cultured cells

RNA sample	β -actin	IFN- γ	TNF- α	IL-1 α	IL-2	IL-2R	IL-4
<i>Cell lines</i>							
HUT 78(+PHA)	+++	+++	++	++	+++	++	+++
HUT 78 cell line	+++	+	+	+	+	+	++
MCF-7 cell line	+++	–	–	+	–	–	–
<i>Inflamed aorta (inflammation)</i>							
AAA tissue I (mild)	+++	+	–	+++	+	++	++
AAA tissue II (severe)	+++	+	–	+++	++	++	++
AAA tissue III (severe)	+++	+++	–	++	++	++	++
AAA tissue IV (moderate)	+++	+	–	++	++	++	++
AAA tissue V (mild)	+++	+	–	++	+	++	++
AAA tissue VI (severe)	+++	++	–	+	+	++	++
<i>Normal aorta</i>							
Tissue A	+++	–	–	–	–	–	–
Tissue B	+++	–	–	–	–	–	–
Tissue C	+++	–	–	–	–	–	–
Tissue D	+++	–	–	–	–	–	–
<i>Patient blood</i>							
PBM I	+++	+	+	++	+	++	++
PBM III	+++	++	+++	+++	+	++	++
PBM V	+++	–	++	+++	+	+	+
<i>Volunteer blood</i>							
PBM J	+++	++	++	+	–	+	++
PBM L	+++	++	+	–	+	+	++
PBM R	+++	++	+	–	+	+	++
<i>Cultured T cells</i>							
T cells tissue IV	+++	++	++	++	+	++	++
T cells tissue V	+++	++	++	++	+	++	+
<i>Controls</i>							
Water	–	–	–	–	–	–	–
No reverse transcriptase	+	–	–	–	–	–	–

The relative PCR band intensity for a given primer pair was graded as; absent (no detectable product) (–), low (+), moderate (++) or strong (+++).

Figure 2 Representative patterns of cytokine gene expression in; Hut78 stimulated T cell line; AAA tissue (case III); normal aorta (case A), and negative control for β -actin; IFN- γ ; TNF- α ; IL-1 α ; IL-2; IL-2 receptor; IL-4 and IL4 receptor. Molecular weight marker lanes are included.

wall RNA samples (specimens A-D). The variation in the blood samples from cases I, II and V is shown in table 3. All blood specimens revealed a band for TNF- α . Aneurysm patient blood (from cases I, II and V) seemed to contain more IL-1 α transcripts than did the volunteer "normal" (J, L, R) blood.

PCR products were not detected for TNF- α in any of the inflamed aortic tissue samples (I to VI). (This was in contrast to the aortic cultured T cell RNA (from cases IV and V), which did result in TNF- α PCR products.) In contrast, IFN- γ and IL-1 α bands were detected for each inflamed aortic tissue RNA sample (I to VI), although to varying degrees. The PCR product for IFN- γ was very strong from inflamed aortic tissue III, which had extreme fibrosis. IFN- γ was also relatively strong in aortic tissue VI with severe inflammation and many germinal centres. PCR products corresponding to IL-1 α were strong in all of the inflamed aortic cases, except tissue VI, where only a very weak band was detected. PCR products for IL-2, IL-2R, and IL-4 were detected in all of the inflamed aortic RNA samples and were much more uniform in intensity than the other cytokine products. All blood specimens indicated bands for IL-2, IL-2R, and IL-4. Aneurysm patient blood (I, II and V) seemed to contain more IL-2 and IL-2R transcripts than did the volunteer "normal" blood (J, L, R).

Discussion

We have detected cytokine gene expression in chronic periaortitis and compared the results with non-diseased aortic tissue and cell controls. PCR assisted mRNA analysis permitted a rapid, highly sensitive, and reproducible semi-quantitative initial screening of RNA samples for the presence of cytokines. The *in vivo* presence of cytokine and cytokine receptor mRNA transcripts was investigated for six cases of inflamed atherosclerotic aortic tissue. TNF- α was not detected in chronic periaortitis by this method, but the other pro-inflammatory cytokines IL-1 α , IL-2, IL-4 and the IL-2 receptor were detected in all six cases (I to VI) but were not detected in normal aorta (A-D); mRNA expression of IFN- γ increased with increasing severity of the aortic adventitial inflammation. Only β -actin mRNA was present in the normal aorta.

IL-1 is important in acute and chronic inflammation, in activating cells and in cell recruitment.^{37,38} High concentrations of IL-1 α have also been found in synovial tissue in rheumatoid arthritis and in thyroid gland in Grave's disease.³⁹ The concentrations of IL-1 in the tissue may reflect the state of chronic inflammation—either active and developing, or resolving. Of course, abundance does not necessarily imply importance, and the *in vivo* role of any of these cytokines remains to be elucidated.

The finding of IL-2 mRNA in the inflamed aortic wall is consistent with the presence of activated and proliferating T cells in chronic periaortitis. IL-2 and IL-4 are important in T and B cell differentiation and proliferation and the IL-2 receptor is upregulated during inflammation and activation states.³⁸ Furthermore, consistent with the mRNA results, immunohistochemistry revealed IL-2 receptor- α protein expression in tissue with chronic periaortitis, but not in the non-diseased aortas.⁵

The presence of the cytokine transcripts in the blood is probably due to the natural killer and T cell populations. These cells are known to synthesise and secrete cytokines such as IFN- γ , IL-1, IL-2 and IL-4.^{8,40} While the detection of cytokines in the tissue is not thought to be artefactual through blood contamination, the absence of TNF- α in the tissue samples, contrasting with its presence in the blood samples, indicates that the level of sensitivity of this assay was too low to detect such contamination.

Further differences in mRNA expression were demonstrated by comparing *in vivo* and *in vitro* results. TNF- α was not detected in the tissue with chronic periaortitis but it was detected in the aortic cultured T cells after three weeks' propagation *in vitro*. Many cytokine investigations have been carried out on mRNA isolated from tissue cells propagated *in vitro*.^{39,41} While these *in vitro* studies may indicate the potential cytokine synthesis and secretion, they may be due to non-specific activation in cell culture.

We have previously documented the presence of cytokine inducible molecules,

HLA-DR,⁴⁵ VCAM-1, E-selectin and ICAM-1⁶⁷ in chronic periaortitis, which may provide circumstantial evidence for the presence of cytokine. TNF- α is of importance in the induction of ICAM-1 and E-selectin and it acts synergistically with IL-4 to increase VCAM-1 mediated T cell adhesion to cultured endothelial cells.⁴² TNF- α has been detected in macrophages in carotid artery atheroma using immunohistochemistry¹⁴ but not in tissue minces of whole atherosclerotic aorta using ELISA assays.²⁰

The absence of TNF- α in chronic periaortitis may be due to a lack of stimulatory signals, or perhaps to the presence of inhibitory signals. Oxidised low density lipoprotein (LDL), which is abundant in the atherotic plaque, inhibits the cellular expression of TNF- α in vitro.⁴³ It remains to be seen if this is the mechanism responsible for the possible absence of TNF- α in the inflamed aortic wall. Culturing the aortic tissue cells in vitro in the presence of oxidised LDL might provide further insight into its effects on cytokine expression.

TNF- α is believed to be an important cytokine due to its abundance in chronic inflammatory diseases such as rheumatoid arthritis.³⁹ TNF- α has, in fact, been implicated as one of the most important regulators in chronic inflammation.³⁹ The lack of TNF- α in chronic periaortitis indicates that a "full-blown" progressive chronic inflammatory response can, however, develop in the absence of this mediator. Other cytokines may compensate for the apparent absence of TNF- α in chronic periaortitis.⁴⁴ In addition, the absence of TNF- α , which is chemotactic for polymorphonuclear cells, may be responsible for the lack of these cells in chronic periaortitis. In contrast, polymorphonuclear cells are seen in rheumatoid arthritis where TNF- α is also abundant.⁴⁵

These studies confirm the view that chronic periaortitis is an active, progressive, local immune response associated with advanced atherosclerosis. Further research needs to be directed towards understanding the similarities and the differences in the genesis and perpetuation of the inflammatory processes occurring in the intima as part of atherosclerosis and in the adventitia in chronic periaortitis. Immunohistology and *in situ* hybridisation techniques would permit the identification of cells responsible for the production of cytokines that influence fibrosis, such as acidic and basic fibroblast growth factor (FGF), and for platelet derived growth factor (PDGF), transforming growth factor (TGF) α and β , monocyte chemotactic protein 1 (MCP-1) and macrophage colony stimulating factor (M-CSF), IL-6 and IL-8 as well as IFN- γ , IL-1, IL-2 and IL-4.

These further studies on the cytokine network in atherosclerosis may lead to a greater understanding of how the cellular (and non-cellular) elements of the lesion interact at all stages. We may then hope for scientifically based therapeutic strategies for disease prevention and diagnosis. It may be possible to

identify sites in the cytokine network that are susceptible to therapeutic intervention using antibodies, recombinant cytokines, cytokine receptor antagonists or by using gene transfection to inhibit cytokine mediated inflammation in atherogenesis.^{15 46}

This work was supported by the British Heart Foundation and the Oxford Medical Research Fund.

- Mitchinson MJ. Chronic periaortitis and periarthritis. *Histopathology* 1984;8:589-600.
- Parums DV. The spectrum of chronic periaortitis. *Histopathology* 1990;16:423-31.
- Parums DV, Brown DL, Mitchinson MJ. Serum antibodies to oxidized LDL and ceroid in chronic periaortitis. *Arch Pathol Lab Med* 1990;114:383-7.
- Ramshaw AL, Parums DV. Immunohistochemical characterization of inflammatory cells associated with advanced atherosclerosis. *Histopathology* 1990;17:543-52.
- Ramshaw AL, Parums DV. Inflammatory cells in chronic periaortitis are activated and proliferating. *J Pathol* 1991;61:61A.
- Wood KM, Cadogan MD, Ramshaw AL, Parums DV. The distribution of adhesion molecules in human atherosclerosis. *Histopathology* 1993;22:437-44.
- Ramshaw AL, Parums DV. The distribution of adhesion molecules in chronic periaortitis. *Histopathology* 1994;24:23-32.
- Balkwill FR. *Cytokines. A Practical Approach*. Oxford: IRL Press, 1991.
- Feldmann M, Brennan FM, Chantry D, Haworth C, Turner M, Katsikis P, et al. Cytokine assays: role in evaluation of the pathogenesis of autoimmunity. *Immunol Rev* 1991;119:105-23.
- Nathan C, Sporn M. Cytokines in context. *J Cell Biol* 1991;11:981-6.
- Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atherosclerotic plaques. *J Clin Invest* 1991;88:1121-7.
- Ross R, Masuda J, Raines EW, Gown AM, Katsuda S, Sahara M, et al. Localization of PDGF-B protein in macrophages in all stages of atherogenesis. *Science* 1990;248:1009-12.
- Takeya M, Yoshimura T, Leonard EJ, Takahashi K. Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monocyte chemoattractant protein-1 monoclonal antibody. *Hum Pathol* 1993;24:534-9.
- Tipping PG, Hancock WW. Production of tumour necrosis factor and interleukin-1 by macrophages from human atheromatous plaques. *Am J Pathol* 1993;142:1721-8.
- Clinton SK, Libby P. Cytokines and growth factors in atherogenesis. *Arch Pathol Lab Med* 1992;116:1292-300.
- Brenner CA, Tam AW, Nelson PA, Engleman EG, Suzuki N, Fry KE, et al. Message amplification phenotyping (MAPPING): A technique to simultaneously measure multiple mRNAs from small numbers of cells. *BioTechniques* 1989;7:1096-103.
- Rappolee DA, Mark D, Banda MJ, Werb Z. Wound macrophages express TGF- α and other growth factors *in vivo*: analysis by mRNA phenotyping. *Science* 1988;241:708-12.
- Vers G, Gibbs RA, Scherer SE, Caskey CT. The molecular basis of the sparse fur mouse mutation. *Science* 1987;237:415-7.
- Ehlers S, Smith KA. Differentiation of T cell lymphokine gene expression: the *in vitro* acquisition of T cell memory. *J Exp Med* 1991;173:25-36.
- Pearce WH, Sweis I, Yao JS, McCarthy WJ, Koch AE. Interleukin-1 beta and tumour necrosis factor-alpha release in normal and diseased human infrarenal aortas. *J Vasc Surg* 1992;16:784-9.
- Koch AE, Kunkel SL, Pearce WH, Shah MR, Parikh D, Evanoff HL, et al. Enhanced production of the chemotactic cytokines interleukin-8 and monocyte chemoattractant protein-1 in human abdominal aortic aneurysms. *Am J Pathol* 1983;142:1423-31.
- Kurnick JT, Gronvik KO, Kimura AK, Lindbloom JB, Skoog VT, Sjöberg O, et al. Long term growth in vitro of human T cell blasts with maintenance of specificity and function. *J Immunol* 1979;122:1255-60.
- Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984;32:219-29.
- Kung PC, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* 1979;206:347-9.
- Erber WN, Pinching AJ, Mason DY. Immunocytochemical detection of T and B cell populations in routine blood smears. *Lancet* 1984;i:1042-5.
- Pezzutto A, Dorken B, Feller A, Moldenhauer G, Schwartz K, Wernet P, et al. HD37 monoclonal antibody; a useful reagent for further characterization of "non T, non B" lymphoid malignancies. In: Reinherz EL, Haynes BF, Nadler M, Bernstein ID, eds. *Leukocyte typing II*. New York: Springer Verlag, 1986:391-402.

- 27 Stein H, Gerdes J, Mason DY. The normal and malignant germinal centre. *Clin Haematol* 1982;11:531-59.
- 28 Kelly PMA, Bliss E, Morton JA, Burns J, JO'D McGee. Monoclonal antibody EBM/11: high cellular specificity for human macrophages. *J Clin Pathol* 1988;41:510-5.
- 29 Chomezynski P, Sacchi N. Single step method of RNA isolation by acid guanidinium triocyanate-phenol-chloroform extraction. *Analyt Biochem* 1987;162:156-9.
- 30 Gunning P, Ponte P, Okayama H, Engel J, Blau H, Kedes L. Isolation and characterization of full length cDNA clones for human α -, β - and γ -actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. *Mol Cell Biol* 1983;3:787-95.
- 31 Gray PW, Leung DW, Pennica D, Yelverton E, Najaran R, Simonsen CC, *et al.* Expression of human immune interferon cDNA in E coli and monkey cells. *Nature* 1982;295:503-8.
- 32 Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R, Palladino MA, *et al.* Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984;312:724-6.
- 33 March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G, Price V, *et al.* Sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 1985;315:641-7.
- 34 Holbrook NJ, Smith KA, Fornace AJ, Comeau CM, Wiskocil RL, Crabtree GR. T-cell growth factor: complete nucleotide sequence and organization of the gene in normal and malignant cells. *Proc Natl Acad Sci USA* 1984;81:1634-8.
- 35 Nikaido T, Shimizu A, Ishida N, Sabe H, Teshigawara K, Maeda M, *et al.* Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature* 1984;311:631-5.
- 36 Yokota T, Otsuka T, Mosmann T, Banchereau J, DeFrance T, Blanchard D, *et al.* Isolation and characterization of a human interleukin cDNA clone, homologous to mouse B-cell stimulatory factor 1, that expresses B-cell- and T-cell-stimulating activities. *Proc Natl Acad Sci USA* 1986;83:5894-8.
- 37 Arai K, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: coordinators of immune and inflammatory responses. *Ann Rev Biochem* 1990;59:783-836.
- 38 Rees RC. Cytokines as biological response modifiers. *J Clin Pathol* 1992;45:93-8.
- 39 Feldmann M, Brennan FM, Chantry D, Haworth C, Turner M, Abney E, *et al.* Cytokine production in the rheumatoid joint: implications for treatment. *Ann Rheum Dis* 1990;49:480-6.
- 40 Ortaldo JR. Cytokine production by CD3- large granular lymphocytes. In: Reynolds CW, Woltrout RH, eds. *Functions of the natural immune system*. New York: Plenum Press, 1989:299-320.
- 41 Brennan FM, Field M, Chu CQ, Feldmann M, Maini RN. Cytokine expression in rheumatoid arthritis. *Br J Rheumatol* 1991;30:76-80.
- 42 Thornhill MH, Wellicome SM, Mahiouz DL, Lanchbury JSS, Kyan-Aung U, Haskard DO. Tumour necrosis factor combines with IL-4 or IFN- γ to selectively enhance endothelial cell adhesiveness for T cells. *J Immunol* 1991;146:592-8.
- 43 Hamilton TA, Gouping MA, Chisholm GM. Oxidized low density lipoprotein suppresses the expression of tumor necrosis factor- α mRNA in stimulated murine peritoneal macrophages. *J Immunol* 1990;144:2343-50.
- 44 Paul WE. Pleiotrophy and redundancy: T cell-derived lymphokines in the immune response. *Cell* 1989;57:521-4.
- 45 Brown KA. The polymorphonuclear cell in rheumatoid arthritis. *Br J Rheumatol* 1988;27:150-5.
- 46 Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-9.