

Tumor necrosis factor- α plays an important role in restenosis development

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ABSTRACT Genetic factors appear to be important in the restenotic process after percutaneous coronary intervention (PCI), as well as in inflammation, a pivotal factor in restenosis. TNF α , a key regulator of inflammatory responses, may exert critical influence on the development of restenosis after PCI. The GENetic DEterminants of Restenosis (GENDER) project included 3104 patients who underwent a successful PCI. Systematic genotyping for six polymorphisms in the TNF α gene was performed. The role of TNF α in restenosis was also assessed in ApoE*3-Leiden mice, TNF α knockout mice, and by local delivery of a TNF α biosynthesis inhibitor, thalidomide. The -238G-1031T haplotype of the TNF α gene increased clinical and angiographic risk of restenosis ($P=0.02$ and $P=0.002$, respectively). In a mouse model of reactive stenosis, arterial TNF α mRNA was significantly time-dependently up-regulated. Mice lacking TNF α or treated locally with thalidomide showed a reduction in reactive stenosis ($P=0.01$ and $P=0.005$, respectively). Clinical and preclinical data indicate that TNF α plays an important role in restenosis. Therefore, TNF α genotype may be used as a risk marker for restenosis and may contribute to individual patient screening prior to PCI in clinical practice. Inhibition of TNF α may be an anti-restenotic target strategy.—Monraats, P. S., Pires, N. M. M., Schepers, A., Agema, W. R. P., Boesten, L. S. M., de Vries, M. R., Zwinderman, A. H., de Maat, M. P. M., Doevendans, P. A. F. M., de Winter, R. J., Tio, R. A., Waltenberger, J., 't Hart, L. M., Frants, R. R., Quax, P. H. A., van Vlijmen, B. J. M., Havekes, L. M., van der Laarse, A., van der

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PERCUTANEOUS CORONARY INTERVENTION (PCI) has become the main treatment for patients with atherosclerotic lesions. However, it is limited by restenosis (1). Restenosis is not a random event, but affects selectively a subset of patients prone to develop lumen renarrowing after PCI. Inherited factors may explain part of the risk of restenosis in certain patients, which cannot be attributed to conventional clinical variables (2). Inflammatory responsiveness plays a pivotal role in restenosis. Therefore, it is plausible that differences in genetic make-up of inflammatory genes between individuals may explain part of the risk of restenosis (3–5). An important mediator in the inflammatory response is tumor necrosis factor- α (TNF α). TNF α is a key proinflammatory cytokine produced by a number of cells, including macrophages, neutrophils, endothelial cells, and VSMCs. TNF α acts locally at sites of tissue injury induced by vessel wall damage and has many biological functions (6). Therefore, we believe TNF α is a key factor in regulating restenosis development. The promoter of

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the gene encoding TNF α contains polymorphic sites that are associated with different responsiveness to regulatory signals (7, 8). The aim of our study is to examine in a large patient population whether functionally relevant polymorphisms in the promoter region of the TNF α gene are related to unfavorable outcomes after PCI. To further investigate the involvement of the TNF α gene in restenosis development, we examined an established mouse model of reactive stenosis.

MATERIALS AND METHODS

GENDER project

Study design

The GENetic DEterminants of Restenosis (GENDER) project was designed to study the association between various gene polymorphisms and clinical restenosis, defined by target vessel revascularization (TVR) (9). Patients eligible for inclusion were treated successfully for stable angina, non-ST elevation acute coronary syndromes, or silent ischemia by PCI in four interventional cardiology centers in The Netherlands. Experienced operators using a radial or femoral approach performed standard angioplasty and stent placement. Before the procedure, patients received aspirin 300 mg and heparin 7500IU. Use of intracoronary stents and additional medication was at the operator's discretion. No drug eluting stents were used.

TVR by PCI or coronary artery bypass grafting (CABG) was the primary end point. An independent events committee adjudicated clinical events.

The protocol conforms to the Declaration of Helsinki and was approved by the Medical Ethics Committees of each participating institution. Written informed consent was obtained from each participant before PCI procedure.

Genetic methodology

DNA was extracted using standard procedures from blood collected in EDTA tubes. Genotyping for four TNF α promoter polymorphisms (-238G/A, -244G/A, -308G/A, and -376G/A) was performed by a multilocus genotyping assay for genetic markers of inflammation and cardiovascular disease (Roche Molecular Systems, Alameda, CA, USA) (10, 11). The -857C/T and -1031T/C polymorphisms were genotyped using Taqman-based assays (12). Primers and probes were synthesized by Applied Biosystems (Nieuwerkerk a/d IJssel, The Netherlands).

To confirm genotype assignments, PCR analysis was randomly performed in replicate on 10% of the samples. Two independent observers carried out scoring. Disagreements (<1%) were resolved by joint reading; when necessary, a repeated genotyping reaction was performed.

Angiographic assessment

Quantitative computer-assisted angiographic analysis was performed off-line on angiograms obtained just before and immediately after stenting and at follow-up in a subpopulation of patients from the GENDER study who were scheduled for re-angiography at 6 months, according to standard procedures (13). Identical projections were used for all angiograms. Binary restenosis was defined as a stenosis diameter >50% within the stent or in the 5 mm segments proximal or distal to the stent at follow-up angiography (14). Quantitative

analysis of angiograms was performed by operators not involved in the stenting procedure and unaware of the genetic data (Heartcore, Leiden, The Netherlands).

Animal experiments

Since the association found sheds no definite light on a causal relationship between TNF α and restenosis we, in order to obtain a better understanding of the role of TNF α gene in the development of restenosis, investigated the effect of this gene in an established mouse model of reactive stenosis.

Femoral artery cuff mouse model

The institutional committee on animal welfare of TNO approved all animal experiments. For all experiments (unless stated otherwise), hyperlipidemic male ApoE*3-Leiden mice (15) were fed a high cholesterol diet (ArieBlok, Woerden, The Netherlands). Blood samples to determine plasma cholesterol were collected at time of surgery.

After 3 wk on the diet, mice were anesthetized with intraperitoneal injection of 5 mg/kg Dormitor (Roche, Basel, Switzerland), 0.5 mg/kg Dormitor (Orion, Helsinki, Finland), and 0.05 mg/kg Fentanyl (Janssen, Geel, Belgium). A nonconstricting polyethylene cuff (Portex, Kent, UK) was placed around the femoral artery (16).

Real-time (RT)-PCR TNF α mRNA analysis

Animals were killed at different times after cuff placement (6, 24, 48 h and 7 days), four mice for each time point. Both cuffed right and noncuffed sham operated left femoral arteries were isolated, harvested, and snap frozen. Femoral arteries, either cuffed or noncuffed sham operated, were pooled (two arteries/sample, two samples/time point), total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA USA), and cDNA was made using Ready-To-Go RT-PCR beads (Amersham Biosciences, Uppsala, Sweden).

Intron-spanning primers and probe were designed for mouse TNF α cDNA using Primer-ExpressTM1.5 (Applied Biosystems). Housekeeping genes (HPRT, cyclophilin, and GAPDH) were used as controls. RT-PCR was performed on an ABI-PrismTM7700 system (Perkin-Elmer Biosystems, Boston, MA, USA) using RT-PCR Mastermix (Eurogentec, Seraing, Belgium). For each time point RT-PCR was performed in duplicate and the signals were averaged and corrected using the average signal of the housekeeping gene (Δ Ct). $\Delta\Delta$ Ct was defined as the difference between Δ Ct value of healthy and cuffed femoral artery. Data are presented as fold induction, calculated as $2^{-\Delta\Delta$ Ct}.

*ApoE*3-LeidenTNF α ^{-/-} mice*

Experimental mice (ApoE*3-LeidenTNF α ^{-/-} and control littermate ApoE*3-LeidenTNF α ^{+/+} mice) were obtained by cross-breeding TNF α ^{-/-} with ApoE*3-Leiden mice (17). Six male mice underwent cuff placement.

Perivascular delivery of TNF α inhibitor

Thalidomide was purchased from Sigma (St. Louis, MO, USA). Poly(ϵ -caprolactone) (PCL) -based drug delivery cuffs were manufactured as described previously (18). Thalidomide was extracted from 1% thalidomide/PCL cuffs (w/w) ($n=4$) using DMSO (Merck, Darmstadt, Germany) and quantified spectrophotometrically before and 14 days after in vivo

placement at 295 nm, the specified wavelength for thalidomide. The total release was quantified (18).

Murine femoral artery was dissected from its surroundings and an empty or a 1% thalidomide/PCL cuff (w/w) was placed loosely around it ($n=6$) (16). The TNF α presence in the vessel wall was visualized by immunohistochemistry using antibodies against TNF α (1:100, Abcam, Cambridge, UK).

Statistical analysis

Statistical analysis was performed using SPSS-11.5. Continuous variables were expressed as mean \pm SD and compared by unpaired, 2-sided Student t test. Discrete variables are expressed as counts or percentages and were compared with the Chi-square test. Deviations of genotype distribution from that expected for a population in Hardy-Weinberg equilibrium were tested using Chi-squared tests with one degree of freedom. Allele frequencies were determined by gene counting; the 95% confidence intervals of allele frequencies were calculated from sample allele frequencies based on the approximation of binomial and normal distributions in large sample sizes.

In the first stage, association between TNF α polymorphisms and TVR was assessed using the Cox proportional regression model under a codominant genetic model without adjustments for covariates so that we could assess their possible involvement in the causal pathway. All polymorphisms were also assessed using dominant and recessive models, and the model with the lowest Akaike information criterion was used in multivariable regression analysis (19). The polymorphisms were combined into haplotypes, and the effect of haplotypes on restenosis risk was estimated according to methods developed by Tanck et al. (20). Multivariable regression analysis of the TVR risk was performed with the TNF α polymorphisms using a stepwise backward selection algorithm. In the final step, clinical variables associated with

TVR were entered into the regression model. Animal data are presented as mean \pm SE and analyzed using the Mann-Whitney U test. P values < 0.05 were regarded as statistically significant.

RESULTS

GENDER project

A total of 3146 patients had a complete follow-up (99.3%) with a median duration of 9.6 months (interquartile range 3.9). Of 3146 patients, 42 had an event in the first 30 days. These patients were excluded from further analysis according to the protocol. Baseline characteristics of the population are shown in Table 1. DNA genotyping was successful in 3012 patients (97%) for the multilocus assay and in 2727 patients (87.9%) for the Taqman procedure. Results of the remaining patients are missing, due to lack of DNA or inconclusive results.

The genotypes distributions (Table 2) were consistent with the Hardy-Weinberg equilibrium ($P>0.05$), except for the -308G/A polymorphism, which was excluded from further analysis. Allele frequencies were in concordance with described frequencies (8, 21).

During follow-up 304 patients (9.8%) had to undergo TVR. Patients with the -238A/A genotype (R.R.=0.59, 95% CI: 0.37-0.94) and patients with the -1031C/C genotype (R.R.=0.77, 95% CI: 0.59-1.00) needed TVR less frequently. The other TNF α polymorphisms did not show a significant association with TVR ($P>0.5$) (Table 2).

TABLE 1. Demographic, clinical and lesion characteristics of patients with TVR and without TVR during the follow-up

	TVR ($n = 304$)	No TVR ($n = 2800$)	Total ($n = 3104$)	P value*
Age (years)	61.7 \pm 10.1	62.2 \pm 10.8	62.1 \pm 10.7	0.56
BMI ($\text{kg} \cdot \text{m}^{-2}$)	26.9 \pm 3.7	27.0 \pm 3.9	27.0 \pm 3.9	0.73
Male sex	220 (72.4%)	1996 (71.3%)	2216 (71.4%)	0.73
Diabetes	63 (20.7%)	390 (13.9%)	453 (14.6%)	0.002
Hypercholesterolemia	188 (61.8%)	1702 (60.8%)	1890 (60.9%)	0.75
Hypertension	138 (45.4%)	1121 (40.0%)	1259 (40.6%)	0.05
Current smoker	62 (20.4%)	700 (25.0%)	762 (24.5%)	0.06
Family history of MI	121 (39.8%)	977 (34.9%)	1098 (35.4%)	0.13
Previous MI	109 (35.9%)	1130 (40.4%)	1239 (39.9%)	0.12
Previous PCI	64 (21.1%)	493 (17.6%)	557 (17.9%)	0.14
Previous CABG	36 (11.8%)	340 (12.1%)	376 (12.1%)	0.97
Stable angina	198 (65.1%)	1881 (67.2%)	2079 (67.0%)	0.46
Multivessel disease	148 (48.7%)	1284 (45.9%)	1432 (46.1%)	0.26
Peripheral vessel disease	12 (3.9%)	92 (3.3%)	104 (3.4%)	0.34
Lipid lowering medication	171 (56.3%)	1516 (54.1%)	1687 (54.3%)	0.53
Restenotic lesions	27 (8.9%)	181 (6.5%)	208 (6.7%)	0.12
Total occlusions	56 (18.4%)	372 (13.3%)	428 (13.8%)	0.05
Type C lesion	94 (30.9%)	708 (25.3%)	802 (25.8%)	0.18
Proximal LAD	70 (23.0%)	619 (22.1%)	689 (22.2%)	0.60
RCX	75 (24.7%)	764 (27.3%)	839 (27.0%)	0.35
Residual stenosis $>20\%$	51 (16.8%)	299 (10.7%)	350 (11.3%)	0.001
Stent length (mm)	10.3 (0-82)	13.0 (0-93)	15 (0-146)	0.80
Diameter stenosis pre-intervention	89% (10%)	89% (10%)	89% (10%)	0.83

BMI: body mass index; MI: myocardial infarction; LAD: left anterior descending branch of the left coronary artery; RCX: circumflex branch of the left coronary artery; MI: myocardial infarction; CABG: coronary artery bypass grafting. Age is mean \pm SD; other variables are percentage of patients. * P value determined by Cox regression analysis.

TABLE 2. Distributions and univariate analysis of TNF α polymorphisms in association with TVR

Polymorphisms	Allele freq (%)	12 month TVR-rate (%)	Best fitting genetic model	P value
-244G/A	0.99/0.01	9.8% GG 0% GA/AA	Dominant	0.37
-238G/A	0.95/0.05	10.2% GG 6.3% GA/AA	Dominant	0.03
-376G/A	0.98/0.02	9.7% GG 9.0% GA 50.0% AA ^a	Additive	0.77
-857C/A	0.91/0.09	9.6% CC/CA 19.2% AA	Recessive	0.099
-1031T/C	0.80/0.20	10.3% TT 8.3% TC/CC	Dominant	0.05

^a Only two patients were homozygous for the A allele.

Strong linkage disequilibrium was found between both associated polymorphisms. Therefore, we evaluated the four different haplotypes: -238G/-1031T, -238G/-1031C, -238A/-1031T, and -238A/-1031C; relative frequencies of these haplotypes were 79%, 16%, 1%, and 4%, respectively. Twelve month TVR rates associated with these haplotypes were 10.1%, 8.0%, 6.6%, and 0%, respectively. Haplotype analysis showed that patients with the -238G/-1031T haplotype had a higher risk for restenosis (RR: 1.33, 95% CI: 1.05-1.69, $P=0.02$) compared with the patients with the other three haplotypes.

Finally, in the regression model we included patient and intervention-related characteristics that had been found to be related to TVR risk such as diabetes, stenting, residual stenosis >20%, current smoking, hypertension, and total occlusion. When the -238G/A and -1031T/C polymorphisms were entered into the multivariable analysis, only one showed a significant association, due to their strong linkage; when analyzed separately, however, both were significantly associated (Table 3).

A 6 month follow-up angiography was performed in a predefined subpopulation (478 patients), and a significant protective association between the -238A allele and angiographic restenosis was again observed (OR: 0.08, 95% CI:0.01-0.60, $P=0.002$). Angiographic restenosis rates were 22.9% for the -238G/G genotype, 2.5% for the -238G/A genotypes, and 0% for the -238A/A genotype. No significant association for the

-1031T/C polymorphism and angiographic restenosis was found ($P=0.35$). The -238G/-1031T and -238G/-1031C haplotypes were associated with increased angiographic restenosis risk compared with the -238A/-1031T and -238A/-1031C haplotypes ($P=0.002$).

Animal experiments

TNF α mRNA analysis with RT-PCR in a mouse model of reactive stenosis

We studied TNF α mRNA expression in a mouse model of reactive stenosis. At the time of cuff placement, plasma cholesterol level was 13.9 ± 3.6 mM.

TNF α transcription was up-regulated time-dependently after induction of the stenotic process (Fig. 1). TNF α mRNA showed a peak expression 24 h after vascular injury (≈ 5000 -fold increase) compared with control arteries, after which the signal declined. Sham-operated vessels (femoral artery prepared free, but without cuff placement) showed similar results as untreated nonoperated vessels (data not shown).

Neointima formation in ApoE*3-LeidenTNF α ^{-/-} mice

To analyze the effect of TNF α on restenosis development, we generated ApoE*3-LeidenTNF α ^{-/-} and control littermate ApoE*3-LeidenTNF α ^{+/+} mice. At surgery, plasma cholesterol level was 21.3 ± 4.2 mM. No differences were seen between both groups. 14 days

TABLE 3. Multivariable analysis of TNF α polymorphisms in association with TVR, including clinical factors

	RR (95% CI) ^a	P value	RR (95% CI)	P value
Diabetes	1.62 (1.20-2.19)	0.001	1.67 (1.23-2.25)	0.001
Total occlusion	1.46 (1.07-1.99)	0.02	1.47 (1.07-2.01)	0.02
Residual stenosis >20%	1.38 (0.97-1.96)	0.08	1.41 (0.98-2.02)	0.06
Stenting	0.77 (0.58-1.03)	0.07	0.75 (0.56-1.01)	0.06
TNF α -238A/A ^b	0.60 (0.37-0.98)	0.04		
TNF α -1031C/C ^b			0.75 (0.57-0.98)	0.04

^a RR = relative risk, 95% CI = 95% confidence interval.

^b Results of the multivariable regression analyses performed with either TNF α -238G/A or TNF α -1031T/C in the analysis.

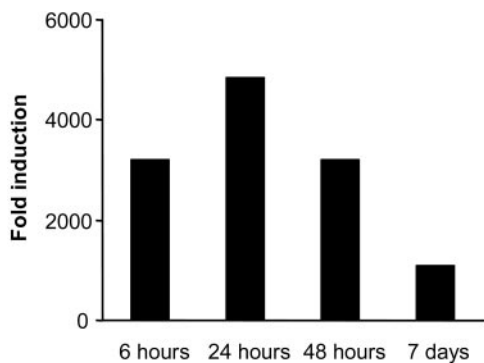


Figure 1. Fold induction of TNF α mRNA in cuff-induced reactive stenosis mouse model in time.

after cuff placement, morphometric quantification revealed significantly less neointima formation in ApoE*3-LeidenTNF α ^{-/-} than in control littermate ApoE*3-LeidenTNF α ^{+/+} mice (1927 \pm 622 vs. 8164 \pm 2803 μ m², $P=0.01$, Fig. 2). Intima/media ratio was also more reduced in TNF α knockout than in TNF α -expressing mice (0.20 \pm 0.05 vs. 0.97 \pm 0.28, $P=0.014$).

Effect of thalidomide perivascular delivery on neointima formation

To assess whether local delivery of a TNF α biosynthesis inhibitor, thalidomide, could inhibit neointima formation, PCL cuffs were loaded with 1% (w/w) thalidomide and placed around the femoral artery of ApoE*3-

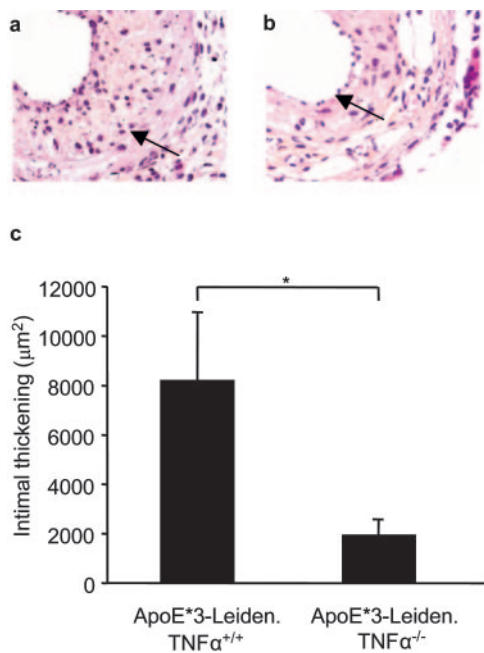


Figure 2. Representative cross sections of cuffed murine femoral arteries (HPS staining, 400 \times . Arrow indicates the inner elastic lamina). A) ApoE*3-LeidenTNF α ^{+/+} mice. B) ApoE*3-LeidenTNF α ^{-/-} mice. C) Total intimal area of cuffed murine femoral arteries 14 days after cuff placement (mean \pm SE, $n=6$). * $P < 0.05$.

Leiden mice for 14 days. At surgery, plasma cholesterol level was 11.4 \pm 0.6 mM.

Total extraction of encapsulated thalidomide of PCL cuffs before and 2 wk after placement in animals revealed a 67% release of thalidomide in the 14 day period (37.5 μ g released, i.e., 33.4% still present in the cuff).

Neointima formation in the thalidomide-treated group was profoundly decreased compared with the empty PCL cuffed arteries (1885 \pm 285 vs. 4629 \pm 625 μ m², $P=0.005$, Fig. 3). Perivascular delivery of thalidomide also resulted in a lower intima/media ratio compared with the empty counterparts (0.22 \pm 0.08 vs. 0.43 \pm 0.16, $P=0.005$).

Immunohistochemical analysis of TNF α was performed in cuffed femoral arteries of mice receiving either an empty PCL cuff or a 1% (w/w) thalidomide eluting PCL cuff to demonstrate decreased TNF α protein levels in the vessel wall. During the stenotic process, TNF α is abundantly expressed in intimal and medial tissue of cuffed femoral arteries receiving an empty PCL cuff at 14 days. Sections of cuffed femoral arteries perivascularly treated with thalidomide showed considerably less TNF α (Fig. 4).

DISCUSSION

The present study demonstrates that TNF α is involved in the process of restenosis (defined as TVR) after PCI in humans and in murine model of reactive stenosis. TNF α is a pleiotropic proinflammatory cytokine in-

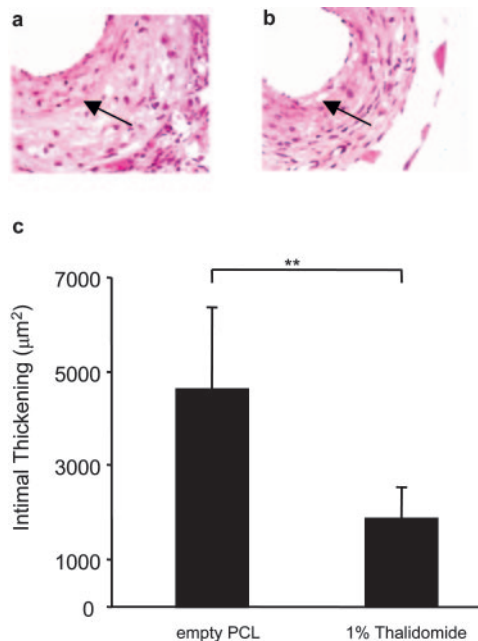


Figure 3. Representative cross sections of cuffed murine femoral arteries (HPS staining, 400 \times . Arrow indicates the inner elastic lamina). A) Empty PCL cuff. B) 1% (w/w) thalidomide eluting PCL cuff. C) Total intimal area of cuffed murine femoral arteries 14 days after cuff placement (mean \pm SE, $n=6$). ** $P < 0.01$.

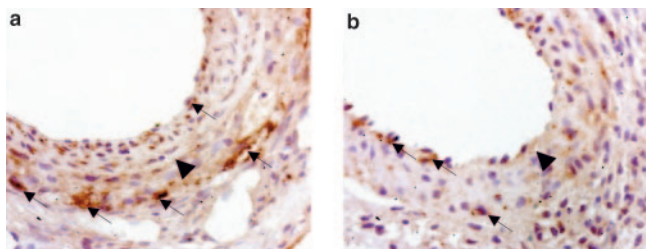


Figure 4. TNF α immunostaining of cross sections of cuffed murine femoral arteries 14 days after placement of either *A*) an empty PCL or *B*) a 1% (w/w) thalidomide eluting PCL cuff. 400 \times . Arrowheads indicate the inner elastic lamina. Arrows indicate TNF α immunostaining.

involved in many aspects of inflammation. Advanced human atherosclerotic lesions express low levels of TNF α in the basal state but high levels in response to injurious stimuli (22). In the present study we primarily assessed the role of six different TNF α polymorphisms in the development of restenosis. In our large prospective multicenter follow-up study, we demonstrated that TNF α -238A/A and the -1031C/C genotypes have a protective association with TVR after PCI. The -238A allele also showed a significant association with angiographic restenosis. The physiological significance of the -238 G/A polymorphism has not been analyzed in detail. However, one study found a significant decrease in promoter activity of patients with the -238A allele compared with patients with the -238G allele (23). Huizinga et al. found a lower TNF α production for the -238A allele compared with the -238G allele as measured in a whole blood culture (24). Fong et al. localized a repressor site to a 25 bp stretch between positions -254 and -230 in the promoter. Therefore, these authors hypothesized that -238G/A exchange could lead to increased transcriptional repression (25). For the -1031T/C polymorphism, the physiological significance has not been clearly elucidated. However, patterns of strong linkage disequilibrium between the -1031T/C and the -863C/A polymorphisms have been described (8, 21, 26). In a study of the effect of the -863C/A polymorphism and serum TNF α concentration, the rare -863A allele had significantly lower serum TNF α levels than the -863C allele in healthy middle-aged men (8).

To further explore the effect of this gene on restenosis development, we quantified TNF α transcripts in the stenotic vessel wall in a mouse model of reactive stenosis. During the stenotic process, TNF α gene transcription was time-dependently up-regulated, indicating that TNF α gene expression is activated upon vascular injury and suggesting that this cytokine is involved in the development of reactive stenosis, at least in the early stages. Furthermore, we demonstrated that mice that constitutively lack TNF α present a reduction in neointima formation. Finally, thalidomide, a compound known to enhance TNF α mRNA degradation (19, 20), caused a reduction in intimal hyperplasia. Moreover, vascular TNF α protein levels are decreased upon local thalidomide administration, suggesting that

the decrease in neointima formation is due to inhibition of TNF α biosynthesis in the injured vessel wall. Recently, Park et al. showed a significant reduction in neointima formation and proliferative activity of VSMCs by orally administered thalidomide after carotid artery denudation in Sprague-Dawley rats (27). These results are in concordance with ours. However, they used Sprague-Dawley rats, a normolipidemic model that may be less suited to test clinical relevance. Furthermore, they delivered the drug orally whereas we applied it locally, which contributes to fewer side effects and higher localized action. Thalidomide may be a suitable candidate for locally applied anti-restenosis therapy through the development of a thalidomide eluting stent. Thalidomide teratogenic side effects reported earlier on this drug may be less important since patients undergoing PCI are usually beyond reproductive age, and the drug is applied locally and in low concentration.

Limitations of the study

We made use of an atherosclerotic mouse model to study the effect of TNF α on restenosis; in this model we were not able to test the TNF α polymorphisms found in humans. However, we believe this model contributes to a better understanding of the involvement of TNF α in restenosis. Furthermore, although mice studies can be used for analysis, it should be realized that perivascular cuff placement results initially in adventitial injury whereas in patients PCI results in intimal injury. It is not certain to what extent these apparently different ways of vascular injury differ in their reaction regarding vascular activation and the resulting intimal hyperplasia.

Another possible limitation of our study is the lack of plasma TNF α data. However, we believe that plasma determinations are of little additive value since pre-PCI plasma measurements of the protein do not reflect the genetically determined differences in reaction to a trauma such as PCI. Also, it is conceivable that local differences in reactions are not represented systemically. In humans it is nearly impossible to measure gene products in the vessel wall locally in the acute phase after treatment.

Finally, the TNF α gene is localized on chromosome six and belongs to the MHC class III region. Its proximity to the MHC class I and II raises the possibility that variations within the TNF α locus are present because of linkage disequilibrium with the MHC.

CONCLUSIONS

Taken together, we demonstrated a role of TNF α on restenosis development. Genetic variants in the TNF α gene explain differences with regard to restenosis susceptibility after PCI. Therefore, when these results are confirmed in other studies, screening patients for this genotype could lead to a better risk stratification of patients at increased risk for restenosis and thereby individualize treatment, for instance by a drug eluting stent strategy. [F]

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