Genetic polymorphisms of apolipoprotein E4 and tumor necrosis factor β as predisposing factors for increased inflammatory cytokines after cardiopulmonary bypass

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Objective: Cardiopulmonary bypass induces a rise in cytokines released by activated monocytes. The apolipoprotein E and the tumor necrosis factor β polymorphisms are risk factors for atherosclerosis. The aim of the study was to investigate whether the genetic variants of apolipoprotein E (*APOE*E4*) and tumor necrosis factor β (*TNFB*A329G*) affect cytokine release after cardiopulmonary bypass.

Methods: Thirty-eight patients underwent standard coronary artery bypass grafting procedures. Genotyping for *APOE*E4* and *TNFB*A329G* was performed. Concentrations of interleukin 8 and tumor necrosis factor α were measured for 48 hours after surgery. Clinical data were collected prospectively.

Results: Fourteen patients (37%) carried the combination non-*APOE*E4*/wild-type *TNFB*A329*, 12 patients (32%) showed non-*APOE*E4*/*TNFB*A329G*, 9 patients (24%) had *APOE*E4*/*TNFB*A329G*, and 3 patients (7%) had *APOE*E4*/wild-type *TNFB*A329*. Total amount of tumor necrosis factor α was significantly higher in patients carrying the combination *APOE*E4*/*TNFB*A329* than in those carrying non-*APOE*E4*/wild-type *TNFB*A329* ($P < .0001$). Clinical data were similar except for intubation time and amount of transfusion, which were significantly increased in patients with genetic polymorphisms ($P = .022$, $P = .033$).

Conclusion: Presence of *TNFB*A329G* polymorphism in addition to *APOE*E4* variant is associated with significantly higher releases of interleukin 8 and tumor necrosis factor α , prolonged intubation, and increased transfusion relative to patients without genetic variants. Preoperative determination of *APOE/TNFB* genotypes in patients undergoing coronary artery bypass grafting may lead to additional perioperative measures to ameliorate systemic inflammatory response.

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Coronary artery disease (CAD) has a significant heritable element, falling into the category of a complex multigenetic disease.¹ A variety of candidate genes have been investigated as predisposing factors for CAD, including those involved in lipid metabolism.¹

The importance of apolipoprotein E (ApoE)-mediated increases in cholesterol level in the severity of atherosclerosis has been controversial.^{2,3} However, several studies have identified a relationship between the ApoE genotype and atherosclerosis. These studies indicate that the allele for the $\epsilon 4$ variant of ApoE, *APOE*E4*, contributes to the development of atherosclerosis and is a major factor responsible for predisposition to this disease.^{4,5}

The *APOE* gene is polymorphic, resulting in three common alleles (*E2, *E3, and *E4) and six different genotypes *APOE**E2/*E2, *APOE**E3/*E2, *APOE**E4/*E2, *APOE**E3/*E3, and *APOE**E3/*E4, *APOE**E4/*E4).² The *APOE**E4 allele is associated with high serum total and low-density lipoprotein cholesterol concentrations,⁶ which in turn are well-established risk factors for CAD.⁷

Recently, a number of genes involved in the inflammatory response have also aroused interest.² The atherosclerotic plaque undergoes many changes consistent with a chronic inflammatory process.⁸ The proinflammatory cytokine tumor necrosis factor (TNF) α has been localized in atheromatous plaques and may contribute to the progression of atheroma by augmenting the inflammatory response.⁹ TNF- α secretion shows a high degree of interindividual variability, which is at least in part genetically determined.¹⁰ A number of TNF- α and - β gene polymorphisms are known with the theoretical potential to affect either cytokine function or secretion.¹¹⁻¹³ Although as yet no functional studies of these polymorphisms have been performed, they have been investigated in inflammatory conditions.¹⁴ Because of the evidence implicating ApoE and TNF- α and - β in the pathogenesis of CAD, we have investigated whether polymorphisms in the *APOE* and *TNFB* genes are also related to the severity of proinflammatory cytokine release in patients undergoing cardiopulmonary bypass (CPB) procedures.

Patients and Methods

Patient Groups

During the 18-month period from February 2000 to August 2001, a total of 38 patients undergoing coronary artery bypass grafting (CABG) were selected for the study. Informed consent was obtained from each patient according to the protocol of the ethics committee of the University Hospital Zürich. Patients with known infection, known neoplasm, previous CABG, or a preoperatively inserted intra-aortic-balloon pump were excluded from the study.

Operative Techniques

CPB was performed with a Stöckert roller pump system (Stöckert Instrumente GmbH, Munich, Germany) and a Shiley-Dideco Maxima hollow-fiber oxygenator (Dideco, Mirandola, Italy). Cold blood cardioplegic solution with an initial dose of 15 mL/kg was applied for myocardial protection. Repeated infusions of 300 mL were given every 15 minutes or earlier if electrical activity occurred. Before aortic declamping, 500 mL warm blood cardioplegic solution (hot shot) was administered at 37°C for 2 minutes at a pressure of 50 mm Hg. Rewarming to a rectal temperature greater than 34°C was achieved with a heat-exchange oxygenator, warming blanket, and heated, humidified gases.

Blood Sampling and Analysis

For each patient peripheral venous blood samples were obtained through a central venous catheter at 10 different time points. At the first time an additional tube of ethylenediaminetetraacetic acid-

anticoagulated blood was drawn for genotyping. The first sample was taken before anesthesia, the second half an hour after reopening of coronary circulation (declamping of aorta), the third 1 hour after reopening of the coronary circulation, the fourth 2 hours after reopening of the coronary circulation, the fifth 4 hours after reopening of coronary circulation, the sixth 8 hours after reopening of the coronary circulation, the seventh 16 hours after reopening of the coronary circulation, the eighth 24 hours after reopening of the coronary circulation, the ninth 32 hours after reopening of the coronary circulation, and the tenth 48 hours after reopening of the coronary circulation.

After centrifugation, serum or plasma samples were frozen immediately at -80°C until analysis. The following parameters were measured off-line for all samples: inflammation parameters, namely TNF- α , interleukin (IL) 8, and c-reactive protein. Genotyping was performed for the following loci: *APOE4* and *TNFB*.

Concentrations of TNF α and IL-8 in the serum were determined by automated enzyme chemiluminescence immunoassay on an Immulite I system (Diagnostics Product Corporation, Los Angeles, Calif, and Buehlmann Laboratories AG, Allschwil, Switzerland). For genotyping, genomic DNA was extracted from 200 μ L of ethylenediaminetetraacetic acid-anticoagulated blood with the MagNA Pure LC (Roche Diagnostics, Rotkreuz, Switzerland) and real-time polymerase chain reaction was performed on a LightCycler (Roche Diagnostics) according to the method of Aslanidis and coworkers¹⁵ with primers and hybridization probes synthesized by TIB MolBiol (Berlin, Germany).

Clinical Variables

For each patient, medical history and demographic data as well as the postoperative course were collected prospectively. Data included length of intensive care unit stay, length of hospital stay, and occurrence of myocardial infarction, bleeding, arrhythmia, or other complications, such as infection necessitating antibiotic treatment.

Statistical Methods

The Fisher exact test and the Student *t* test with mean and SDs were used for comparison of ratios and means for normally distributed data. The Mann-Whitney test was used where applicable. All measurements between the various groups were controlled for all time points by repeated-measures analysis of variance with Bonferroni corrections.

Results

Of the 38 patients enrolled, 14 patients (37%) carried the combination non-*APOE**E4/wild-type *TNFB**A329, 12 patients (32%) showed a non-*APOE**E4/*TNFB**A329G genotype (10 heterozygous, 2 homozygous), 9 patients (24%) had the combination of *APOE**E4/*TNFB**A329G (8 heterozygous, 1 homozygous) and 3 patients (7%) had *APOE**E4/wild-type *TNFB**A329. For the sake of understanding, we first focused on the two groups of patients with wild-type *TNFB**A329 versus the *TNFB**A329G carriers regardless of the *APOE* status. Second, we compared the patients with both polymorphisms (*APOE**E4/*TNFB**A329G) with the patients with no polymorphism (non-*APOE**E4/

TABLE 1. Demographics, intraoperative data, and clinical outcome for patients with wild-type *TNFB* and patients either heterozygous or homozygous for the *TNFBA329G polymorphism**

	Wild type (n = 17)	Polymorphism (n = 21)	P value
<i>APOE</i> *E4 (No.)	3	9	NS
Age (y, mean $\pm$ SD)	66.9 $\pm$ 5.3	62.5 $\pm$ 8.3	NS
Gender (female/male)	0:17	4:17	NS
Ejection fraction (%; mean $\pm$ SD)	62 $\pm$ 10	62 $\pm$ 11	NS
Bypass time (min, mean $\pm$ SD)	88 $\pm$ 15	104 $\pm$ 25	NS
Crossclamp time (min, mean $\pm$ SD)	53 $\pm$ 10	57 $\pm$ 10	NS
Intubation (min, mean $\pm$ SD)	773 $\pm$ 234	1427 $\pm$ 841	.043
Transfusions (U, mean $\pm$ SD)	0.8 $\pm$ 0.9	2.4 $\pm$ 2.7	NS
Intensive care unit stay (d, mean $\pm$ SD)	1.5 $\pm$ 0.7	2.4 $\pm$ 1.8	NS
Infections (No.)	1	0	NS
Atrial fibrillation (No.)	4	1	NS
Hospital stay (d, mean $\pm$ SD)	10.5 $\pm$ 3.3	9 $\pm$ 1.9	NS

NS, Not significant. Statistical significance was defined as $P < .05$.

wild-type *TNFB**A329). Comparison of the preoperative profile (age, sex, diagnosis, risk factors) between the wild-type *TNFB**A329 versus the *TNFB**A329G carrier group demonstrated no statistical difference. Furthermore, the intraoperative management between the two groups of patients conferred comparability, because no statistically significant differences could be demonstrated (Table 1).

TNF- α and IL-8 plasma levels before the operation were comparable in all patients. However, in *TNFB**A329G heterozygous or homozygous carriers significantly higher amounts of TNF- α were found at 1 hour ($P = .025$), 2 hours ($P = .043$), and 24 hours ($P = .011$) after reopening of the coronary circulation. Highest levels of circulation TNF- α were found 4 hours after reopening of the coronary circulation. The total output of TNF- α (measured as areas under the curve) was significantly higher in patients carrying the *TNFB**A329G genetic variant than in the wild-type carriers, with 101.9 $\pm$ 56.1 pg/mL and 77.7 $\pm$ 29.6 pg/mL, respectively ($P < .01$; Figure 1).

Similar results were found for IL-8, with higher levels of IL-8 demonstrated in the *TNFB**A329G heterozygous and homozygous group compared with the *TNFB* wild-type group, with statistical significance at 4 hours ($P = .041$), 8 hours ($P = .032$), and 16 hours ($P = .049$) after reopening of the coronary circulation. The total apparent cytokine output of IL-8 (measured as areas under the curve) was higher in patients either heterozygous or homozygous for the *TNFB**A329G genetic variant than in those homozygous for the *TNFB* wild-type allele, with 314.0 $\pm$ 107.4 pg/mL versus 127.1 $\pm$ 76.0 pg/mL, respectively ($P < .01$; Figure 2).

With respect to patients with the combination of both polymorphisms, in *APOE**E4/*TNFB**A329G heterozygous and homozygous carriers significantly higher levels of TNF- α than in patients with no polymorphisms at all (non-*APOE**E4/*TNFB**A329) were found at 0.5 hours ($P = .007$), 1 hour ($P = .0006$), 4 hours ($P = .042$), and 24 hours

($P = .003$) after reopening of the coronary circulation. The highest levels of circulating TNF- α were found after 4 hours in both groups; mean level was statistically significantly higher in *APOE**E4/*TNFB**A329G heterozygous and homozygous carriers, with 13.2 $\pm$ 6.3 pg/mL versus 7.9 $\pm$ 3.1 pg/mL in the non-*APOE**E4/*TNFB**A329 wild-type carrier group ($P = .0002$). The total apparent cytokine output of TNF- α (measured as area under the curve) was significantly higher in patients carrying both mutations than in the non-*APOE**E4/*TNFB**A329G heterozygous and homozygous carrier group, with 132.5 $\pm$ 63.0 pg/mL versus 79.0 $\pm$ 30.9 pg/mL, respectively ($P < .0001$; Figure 3).

Clinical data were similar in all groups except for the duration of intubation and the amount of transfusions. Both were significantly increased in patients with two genetic variants versus none (Table 2).

Discussion

This study showed that the presence of the *TNFB**A329G allele is linked to an increase of cytokine release in patients after CPB. Interestingly, the combination of *APOE**E4 and *TNFB**A329G genetic variants was associated with a further increase in TNF- α plasma level.

CPB is known to cause an inflammatory response, mainly as a result of the contact of blood with artificial surfaces of the bypass circuit and the reperfusion phenomenon. This inflammatory reaction includes activation of the humoral and cellular immune system, which leads to increased cytokine release. Recent studies demonstrate that CABG without the use of CPB was associated with a reduction of the inflammatory response combined with decreased postoperative morbidity and mortality.^{16,17} Whether this inflammatory response is due solely to the use of CPB is being debated. However, a genetic background may also play a role in influencing cytokine plasma levels induced by cardiac operations. Inbal and associates¹⁸ found an *APOE**E4 allele frequency of 9.4% in young men with an

Figure 1. TNF- α at different time points for *TNFB* wild-type (*TNF beta wt*) and *TNFB**A329G heterozygous or homozygous (*TNF beta het or hom*) carriers. Time point 0 on x axis indicates sample taken immediately before start of anesthesia. Data points represent mean; error bars represent SD.

Figure 2. IL-8 at different time points for *TNFB* wild-type (*TNF beta wt*) and *TNFB**A329G heterozygous or homozygous (*TNF beta het or hom*) carriers. Time point 0 on x axis indicates sample taken immediately before start of anesthesia. Data points represent mean; error bars represent SD.

acute myocardial infarction versus 5.3% in a control group. In this study, patients with a *APOE**E4 polymorphism had a 9-fold increased estimated risk of development of an acute myocardial infarction.

In addition, evidence exists suggesting that the genetic variations of TNF locus are involved in determining susceptibility to inflammatory autoimmune diseases such as rheumatoid arthritis and asthma.¹⁹ We therefore evaluated whether the genotype *APOE**E4 variant and the *TNFB**A329G allele are connected to the release of proinflammatory cytokines during CPB surgery. Our results suggest that the *APOE**E4 variant and the *TNFB**A329G allele may be important in the production or release of TNF- α and IL-8 at a molecular level.

In an isolated rat heart model of ischemia and reperfusion injury the local production and release of TNF- α leads to significant depression of coronary flow as well as a decreased myocardial contractility and an increase of creatine kinase production,²⁰ therefore *APOE**E4 as well as

*TNFB**A329G polymorphism carriers might be at higher risk for development of more severe myocardial dysfunction during CPB surgery. TNF- α is believed to be a pivotal proinflammatory cytokine in the pathogenesis of the systemic inflammatory response syndrome. Recently it has been shown that a genomic restriction fragment length polymorphism within the TNF locus is correlated with increased TNF- α plasma levels and poor prognosis in sepsis.²¹ TNF- α is also known to be a potent inducer of the synthesis of secondary proinflammatory mediators such as IL-8. IL-8 is a crucial cytokine known to attract and activate neutrophils and is known to play a role in the pathophysiology of the capillary leak syndrome.²²

Interestingly, the combination of *APOE**E4 variance and the *TNFB**A329G polymorphism lead to an additional increase of TNF- α relative to the patients with the *TNFB**A329G genetic variant alone. These results show that the production or release of cytokines is not regulated by only one gene polymorphism but most likely is also due

Figure 3. TNF- α at different time points for non-*APOE***E4* and *TNFB* wild-type carriers (*nonE4/TNF-B wt*) vs *APOE***E4* and *TNFB***A329G* heterozygous or homozygous carriers (*E4/TNF-B het or hom*). Time point 0 on x axis indicates sample taken immediately before start of anesthesia. Data points represent mean; error bars represent SD.

TABLE 2. Demographics, intraoperative data, and clinical outcome for patients with non-*APOE***E4* and wild-type *TNFB* and patients with *APOE***E4* and either heterozygous or homozygous for the *TNFB***A329G* polymorphism

	Both polymorphisms (n = 14)	No polymorphisms (n = 9)	P value
Age (y, mean $\pm$ SD)	65.8 $\pm$ 8.9	69 $\pm$ 3.7	NS
Gender (female/male)	2:7	0:14	NS
Ejection fraction (%)	63 $\pm$ 10	63 $\pm$ 10	NS
Bypass time (min, mean $\pm$ SD)	103 $\pm$ 14	89 $\pm$ 17	NS
Crossclamp time (min, mean $\pm$ SD)	60 $\pm$ 8	53 $\pm$ 11	NS
Intensive care unit stay (d, mean $\pm$ SD)	2.1 $\pm$ 1.4	1.5 $\pm$ 0.8	NS
Intubation time (min, mean $\pm$ SD)	1515 $\pm$ 691	810 $\pm$ 257	.022
Transfusions (U, mean $\pm$ SD)	3 $\pm$ 2.7	0.79 $\pm$ 0.9	.033
Infections (No.)	0	1	NS
Atrial fibrillation (No.)	0	1	NS
Hospital stay (d, mean $\pm$ SD)	9.1 $\pm$ 1.9	10.3 $\pm$ 3.4	NS

NS, Not significant.

to a linked process with other genetic variants. Newman and colleagues²³ hypothesized that patients with the *APOE***E4* allele are predisposed toward coronary artery disease and would be seen earlier for surgery.²³ Whether the patients in that study demonstrated the *APOE***E4* polymorphism alone or had additional genetic variances is unfortunately unknown.

In our patients who underwent elective CABG, no significant differences could be demonstrated in the clinical outcome except for the length of intubation and the amount of blood transfusion, both of which were significantly higher in patients with both genetic variants. However, it is hard to prove a clear causal relationship between the elevation of cytokines and the clinical outcome. Whether the increased cytokine release in patients with polymorphisms is due to more transfusions given is debatable, but we believe that it is rather an aspect of inflammation. A patient

population with more severe conditions would probably have demonstrated more clinical relevance than what we saw in our study. Turner and associates²⁴ reported on a heart transplantation cohort in which the combination of a low IL-10 producer genotype *1082AA with the high TNF- α producer allele *308A was correlated with increased levels of early heart transplant rejection.²⁴

In conclusion, preoperative *APOE4* and *TNFB* genotyping may be a useful tool in deciding whether patients with genetically determined increased proinflammatory cytokine production and release could benefit from avoiding CPB now that off-pump CABG has evolved as a true alternative to on-pump procedures. Furthermore, genotyping may be used for selecting high-risk patients for intensive monitoring and additional perioperative measures for the treatment of the increased systemic inflammatory response.

References

1. Cambien F, Poirier O, Mallet C, Tiret L. Coronary heart disease and genetics: an epidemiologist's view. *Mol Med Today*. 1997;3:197-203.
2. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis*. 1988;8:1-21.
3. Lenzen HJ, Assmann G, Buchwalsky R, Schulte H. Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. *Clin Chem*. 1986;32:778-81.
4. Ilveskoski E, Perola M, Lehtimäki T, Laippala P, Savolainen V, Pajarinen J, et al. Age-dependent association of apolipoprotein E genotype with coronary and aortic atherosclerosis in middle-aged men: an autopsy study. *Circulation*. 1999;100:608-13.
5. Nassar BA, Dunn J, Title LM, O'Neill BJ, Kirkland SA, Zayed E, et al. Relation of genetic polymorphisms of apolipoprotein E, angiotensin converting enzyme, apolipoprotein B-100, and glycoprotein IIIa and early-onset coronary heart disease. *Clin Biochem*. 1999;32:275-82.
6. Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J Lipid Res*. 1992;33:447-54.
7. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking. A preliminary report from the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *JAMA*. 1990;264:3018-24.
8. Ross R. Atherosclerosis and inflammatory disease. *N Engl J Med*. 1999;340:115-26.
9. Vaddi K, Nicolini FA, Mehta P, Mehta JL. Increased secretion of tumor necrosis factor-alpha and interferon-gamma by mononuclear leukocytes in patients with ischemic heart disease. Relevance in superoxide anion generation. *Circulation*. 1994;90:694-9.
10. Jacob CO, Fronek Z, Lewis GD, Koo M, Hansen JA, McDevitt HO. Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: relevance to genetic predisposition to systemic lupus erythematosus. *Proc Natl Acad Sci U S A*. 1990;87:1223-37.
11. Weinshenker BG, Hebrink D, Wingerchuk DM, Klein CJ, Atkinson E, O'Brien PC, et al. Genetic variants in the tumor necrosis factor receptor 1 gene in patients with MS. *Neurology*. 1999;52:1500-3.
12. Komata T, Tsuchiya N, Matsushita M, Hagiwara K, Tokunaga K. Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus. *Tissue Antigens*. 1999;53:527-30.
13. Messer G, Spengler U, Jung MC, Honold G, Blomer K, Pape GR, et al. Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF-beta production. *J Exp Med*. 1991;173:209-19.
14. Al Ansari AS, Ollier WE, Villarreal J, Ordi J, Teh LS, Hajeer AH. Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus. *Tissue Antigens*. 2000;55:97-9.
15. Aslanidis C, Schmitz G. High-speed apolipoprotein E genotyping and apolipoprotein B3500 mutation detection using real-time fluorescence PCR and melting curves. *Clin Chem*. 1999;45:1094-7.
16. Cleveland JC, Shroyer AL, Chen AY, Peterson E, Grover FL. Off-pump coronary artery bypass grafting decreases risk-adjusted mortality and morbidity. *Ann Thorac Surg*. 2001;72:1282-9.
17. Matata BM, Sosnowski AW, Galinanes M. Off-pump bypass graft operation significantly reduces oxidative stress and inflammation. *Ann Thorac Surg*. 2000;69:785-91.
18. Inbal A, Freimark D, Modan B, Chetrit A, Matetzky S, Rosenberg N, et al. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. *Blood*. 1999;93:2186-90.
19. Wilson AG, di Giovine FS, Duff GW. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *J Inflamm*. 1995;45:1-12.
20. Meldrum DR, Cleveland JC, Cain BS, Meng X, Harken AH. Increased myocardial tumor necrosis factor-alpha in a crystalloid-perfused model of cardiac ischemia-reperfusion injury. *Ann Thorac Surg*. 1998;65:439-43.
21. Stuber F, Petersen M, Bokelmann F, Schade U. Genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. *Crit Care Med*. 1996;24:381-4.
22. Elliott MJ, Finn AH. Interaction between neutrophils and endothelium. *Ann Thorac Surg*. 1993;56:1503-8.
23. Newman MF, Laskowitz DT, White WD, Kirchner JL, Grocott HP, Stafford-Smith M, et al. Apolipoprotein E polymorphisms and age at first coronary artery bypass graft. *Anesth Analg*. 2001;92:824-9.
24. Turner D, Grant SC, Yonan N, Sheldon S, Dyer PA, Sinnott PJ, et al. Cytokine gene polymorphism and heart transplant rejection. *Transplantation*. 1997;64:776-9.