Chronic infections and genetic factors in the development of ischemic stroke

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SUMMARY

The aim of this study was to examine whether chronic infections and genetic factors of the host play roles in the pathophysiology of acute noncardioembolic ischemic stroke. Blood samples from 59 subjects with ischemic stroke and 52 control patients were investigated by nested PCR for the presence of *C. pneumoniae* DNA, HCMV DNA and enterovirus RNA, by ELISA for the levels of antibodies to *C. pneumoniae*, HCMV, HSV, HHV-6, EBV and the inflammatory chemokine IL-8, and by PCR for promoter polymorphism of the IL-8 and CD14 host genes. Associations of stroke with the HCMV IgG and HSV-1 IgA antibody levels were observed. No association of stroke was detected with the presence of *C. pneumoniae*, HCMV or enterovirus nucleic acids in the peripheral blood, *C. pneumoniae* IgM, IgG and IgA, the HSV IgG, the EBV IgG, or HHV-6 IgG antibody levels, the pathogen burden, the IL-8 or CD14 promoter polymorphisms, or with the serum levels of IL-8 in the overall study population. These results are consistent with the hypothesis that certain pathogens are involved in the development of ischemic stroke.

KEY WORDS: Ischemic stroke, Risk factors, HCMV IgG, HSV-1 IgA, IL-8, CD14

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INTRODUCTION

Conventional risk factors may not be fully responsible for the development of ischemic stroke, especially in young individuals. Chronic infections with certain pathogens, such as *Chlamydia pneumoniae*, human cytomegalovirus (HCMV), herpes simplex virus type 1 (HSV-1), varicella-zoster virus, *Helicobacter pylori*, bacteria of periodontitis and genetic parameters that influence inflammatory reactions are suggested to contribute to the disease (Freidank *et al.,* 2002; Tarnacka *et al.,* 2002; Lindsberg and Grau, 2003; Anzini *et al.*, 2004; Ngeh *et al.,* 2005; Elkind *et al.,* 2006; Park *et al.,* 2006). However, other studies have not confirmed these data (Heuschmann *et al.*, 2001; Apfalter *et al.,* 2004). It is possible that further pathogens with the capacity to establish persistence in humans and to impair endothelial functions, such as the Epstein-Barr virus, human herpesvirus-6 (HHV-6) and enteroviruses (Conaldi *et al.,* 1997; Galbraicht *et al.,* 1997; Murono *et al.,* 2001; Caruso *et al.*, 2003; Xiong *et al.,* 2004; Vallbracht *et al.,* 2005), increase the risk of ischemic events.

Genetic variations of certain host genes, such as IL-8, CD14, IL-6, P-selectin and cathepsin G, may also influence the immune response to the pathogens or to other inflammatory stimuli.

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Chemokines, including IL-8, are potent mediators of immune cell migration through the endothelium. Thus, an increased expression of IL-8 may induce plaque rupture and accelerate atherothrombosis (Kostulas *et al.,* 1998; Grau *et al.,* 2001a). IL-8 promoter polymorphism has previously not been directly associated with acute ischemic stroke. CD14 is a monocytic lipopolysaccharide receptor, and a polymorphism of nt -260 C → T in the promoter of the gene has been suggested to be associated with vascular diseases (Risley *et al.,* 2003; Krüger *et al.,* 2005). Other investigators, however, found that CD14 polymorphism is not a risk factor for ischemic stroke. Because of the conflicting results, and since most studies have been restricted to the investigation of 1-3 pathogens, or the polymorphism of one or only a few of the inflammatory factors in separate studies, and in samples obtained from populations heterogeneous for the time before or after the development of stroke, we investigated these associations further. We were interested in whether these associations exist when several of these parameters are investigated concurrently in a study population that is relatively homogeneous for the time relating to the development of acute ischemic stroke, and when pathogens or proinflammatory genes that have not been examined so far from the aspect of the development of stroke are included.

Accordingly, we evaluated the presence of *C. pneumoniae* DNA, HCMV DNA and enterovirus RNA in the peripheral blood of ischemic stroke patients and control subjects, the levels of IgG, IgM and IgA antibodies to *C pneumoniae*, of IgG and IgA to HSV-1, of IgG and IgM to HCMV, and of IgG to EBV and HHV-6. Further, we determined the IL-8 and CD14 promoter polymorphisms, and also the serum level of IL-8 in the study populations.

MATERIALS AND METHODS

Subjects

Fifty-nine consecutive stroke patients (mean age: 52.8 years, range: 32-65 years) hospitalized in 2003 were selected for the study at the Department of Neurology at the University of Szeged, Hungary, if they met the following criteria:

1) first noncardiogenic ischemic stroke;
2) stroke treatment in the Department;
3) admission to the Department within the first 72 hours after stroke onset;
4) age <65 years.

This relatively young age at stroke onset was chosen to decrease the potential influence of the traditional vascular risk factors on the evaluation of the role of chronic infections and host gene polymorphism in the development of acute ischemic stroke. Ischemic stroke was diagnosed on the basis of newly formed focal neurological signs, if other causes of the symptoms could be excluded by the clinical examinations and the standardized diagnostic tests, including the computed tomography scan or magnetic resonance imaging, vascular imaging, electrocardiography and echocardiography. Patients with a known myocardial infarction in the past, or with atrial fibrillation, valvular or myocardial heart disease were excluded from the study. These criteria were determined by reviewing the previous medical reports and the anamnesis of the patients. The controls comprised 52 patients (mean age: 50.4 years, range: 22-76 years) admitted to the Department of Neurology during the same time period, but with no indications for ischemic stroke; the same exclusion criteria were applied for the controls as for the stroke patients, with the exception of broader range of age for controls. The control patients were hospitalized for low back pain (spondylarthrosis, discus degeneration, ischias syndrome) or mono- or polyneurophathy. The study was approved by an institutional review committee. All patients gave their written informed consent. Neither the stroke nor the control patients reported any signs of an acute infection within several weeks preceding admission. The clinical characteristics of the subjects were determined by standard clinical and laboratory methods. Blood samples were taken within one week after symptom onset.

DNA/RNA of pathogens and IL-8 and CD14 promoter polymorphisms

DNA was extracted from blood samples with the Wizard Genomic DNA Purification Kit (Promega). The primers for the HCMV-IE-exon4-based and for the *C. pneumoniae ompA*-based nested PCR (nPCR) were reported earlier (Frank *et al.,* 1992; Tong and Sillis, 1993). The sensitivities of the semiquantitative nPCR

assays were evaluated by testing blood samples from seronegative individuals which were spiked with 10-fold serial dilutions of purified elementary bodies of *C. pneumoniae* strain TW183 (ATCC) and HCMV strain Towne (ATCC). Each DNA sample from the stroke and control patients was tested in 5 replicates with the nPCR. The sensitivity threshold of the *C. pneumoniae* or HCMV nPCR when at least 1 of the 5 replicates was positive was 0.005 inclusion-forming units or 0.05 plaque forming-units, respectively. The β-actin housekeeping gene was amplified from each sample. Contamination in the PCR reactions is not likely, since none of the negative PCR controls (every fifth reaction) was positive. For the extraction of RNA for enterovirus amplification, the phenol-chloroform extraction method was used, the reverse transcription was performed with MuLV reverse-transcriptase (Applied Biosystems) and 5'NTR region-specific primers were applied for the amplification (Diedrich *et al.,* 1995). The IL-8 promoter nt -251 T→A polymorphism was detected with the amplification-refractory mutation system. Previously described allospecific primers were used (Hull *et al.,* 2001), and the PCR for the IL-8 promoter polymorphism was carried out with the Adventage-CG cDNA Polymerase Kit (Clontech). The CD14 promoter nt -260 C→T polymorphism was identified with primers via melting point analysis (Gyulai *et al.,* 2004).

Serum antibody and IL-8 levels

Serum antibody levels were determined in ELISA: HSV types 1 and 2 IgG (ETI-HSVK-G 1/2; Diasorin); HSV-1 IgA (EIAgene; Adaltis); CMV IgM and IgG (Enzygnost, Dade Behring); EBV IgG (Captia EBV VCA (P18); Trinity); HHV-6 IgG (EIA; Biotrin); and *C pneumoniae* IgM, IgG and IgA (NovaTec Immundiagnostica). The serum IL-8 level was measured with an ELISA kit (Biosource). The dilutions of sera in ELISA and threshold values for positivity were set according to the respective manufacturer's instructions. The results are quantitative for IgG isotypes of HCMV, HSV-1 and 2, EBV, HHV-6 and *C. pneumoniae* antibodies, with OD values divided into tertiles; antibody levels were considered high at an OD in the highest tertile. For the IgM and IgA isotypes of the HCMV, HSV-1 and *C. pneumoniae* antibodies, the results are qualitative (positive or negative). Samples falling within the equivocal range were retested, and those that were again equivocal were regarded as negative.

Statistics

All statistical analyses were performed with the Statistical Package for the Social Sciences, Windows program version 13.0. Differences between groups in continuous variables were calculated with the nonparametric Mann-Whitney U-test. To evaluate the association of high levels of IgG antibodies with ischemic stroke, dichotomous variables were created, i.e. high (highest tertile) versus low (lower two tertiles). This approach was selected because of the high seroprevalence of these pathogens, and the skewed distribution of the antibodies, and because the risk associated with these antibodies did not differ in the lower tertiles of distribution. For dichotomous variables, Fisher's exact test and/or the χ^2 probe and logistic regression were applied. In the joint effect analyses, subjects with low levels of HCMV-IgG antibodies or negative results for HSV-1 IgA were used as references to estimate the relative risks of the other 3 combinations. All tests were 2-tailed; a $p<0.05$ was taken as significant.

RESULTS

Risk factors

Analysis of the clinical characteristics and the traditional risk factors (Table 1) revealed significant differences between the stroke and control patients for hypertension ($p<0.001$), alcohol consumption ($p=0.005$) and hyperlipidemia ($p=0.007$). A close to significant difference was found for cigarette smoking ($p=0.084$). No differences were seen for age, gender or sedimentation rate.

Chronic infections as risk factors

The role of certain chronic infections in the development of acute ischemic stroke was investigated by determining the presence of the nucleic acids of *C. pneumoniae*, HCMV and enteroviruses in the peripheral blood and the serum antibody levels to *C. pneumoniae*, HCMV, HSV, EBV and HHV-6 in the stroke and control patients. Table

TABLE 1 - *Clinical characterization of stroke patients.*

	Patients (n = 59)	*Controls (n = 52)*	*p OR (95% CI)*
Age, mean (years)	52.8	50.4	0.111
Age, range (years)	32-65	22-76	
Male gender*	64.4	50	0.178
Regular alcohol consumption*†	18.6	1.9	0.005 11.62 (1.45-9.09)
Smoking*‡	52.5	34.6	0.084
Hyperlipidemia*§	55.9	29.4	0.007 3.05 (1.38-6.72)
Hypertension*¶	62.7	21.2	<0.001 6.25 (2.68-14.71)
Sedimentation rate*#	15.3	5.9	0.134

*Percentage of positive samples in total number of samples; †>24 g alcohol daily vs. occasionally or never; ‡ vs never, or stopped more than 1 year ago; §high serum cholesterol (>6.2 mmol/l) or tryglyceride (>2.3 mmol/l) levels after an 8-hour starvation, or if lipid-lowering drugs were taken; ¶blood pressure above 140/90 mmHg (measured several times in the subacute phase of the stroke, or if antihypertensive medication was taken; #>18 mm/h.

TABLE 2 - *Chronic infections as risk factors in stroke patients.*

	Patients	*Controls*	*p*	*OR (95% CI)*
DNA/RNA*				
C. pneumoniae	16.9	9.8	0.404	
HCMV	1.7	0	1.000	
Enterovirus	0	0		
Antibodies*				
HCMV IgM	0	0		
HCMV IgG†	44.1	21.2	0.015	2.94 (1.27-6.81)
HSV-1 IgA‡	40.7	15.7	0.006	3.68 (1.47-9.20)
HSV-1-2 IgG†	39.0	27.4	0.305	
EBV IgG†	37. 3	28.8	0.421	
HHV-6 IgG†	32.2	34.6	0.842	
C. pneumoniae				
IgM	0	0		
IgG†	28.8	35.2	0.313	
IgA‡	33.9	23.5	0.294	
Pathogen burden§	27.1	22.0	0.657	

*Percentage of positive samples in total number of samples; †high OD values; ‡positive OD values; §high IgG antibody levels to HSV, EBV, HHV-6 and *C. pneumoniae*, and detectability of *C. pneumoniae* IgA; percentage of subjects with positivity for 3-5 of these antibodies.

TABLE 3 - *Logistic regression analysis of HCMV IgG and HSV-1 IgA antibody levels in stroke vs control patients, adjusted for confounders.*

Adjusted for	p	OR (95% CI)
HCMV IgG† (no adjustment)	0.012	2.94 (1.27-6.81)
age, gender, smoking, alcohol, lipid, hypertension, sedimentation rate	0.014	4.95 (1.38-17.80)
HSV-1 IgA‡ (no adjustment)	0.005	3.69 (1.47-9.21)
age, gender, smoking, alcohol, lipid, hypertension, sedimentation rate	0.015	4.65 (1.34-16.16)

†high OD values; ‡positive OD values; definitions for confounders are given in the legend to Table 1.

2 shows that the differences between the detectability of *C. pneumoniae* and HCMV DNA in the stroke and control patients were not significant. None of the RNA samples of the stroke or control patients was positive for enterovirus RNA. Significant differences were observed between the stroke and control patients for the levels of antibodies to HCMV IgG (p=0.015) and HSV-1 IgA (p=0.006). However, no differences were detected for HSV IgG, EBV IgG, HHV-6 IgG and *C. pneumoniae* IgM, IgG and IgA, or for the combined presence of high antibody levels to these pathogens (pathogen burden). After adjustment of the HCMV IgG and HSV-1 IgA OD values for confounders by logistic regression analysis, the differences remained significant (Table 3).

TABLE 4 - Association of IL-8 and CD14 promoter polymorphisms with stroke.

	Patients %	*Controls %*	*p*
IL-8*			
TT	36.2	32.0	0.192
AT	36.2	52	
AA	27.6	16	
TT + AT	72.4	84	0.149
AA	27.6	16	
AT + AA	65.5	68	
TT	34.5	32	0.785
TT	56.8	66.7	0.439
AA	43.2	33.2	
CD14*			
CC	33.9	28.8	0.682
CT	40.7	38.5	
TT	25.4	32.7	
CT+CC	74.6	67.3	0.399
TT	25.4	32.7	
TT+CT	66.1	71.2	0.568
CC	33.9	28.8	
CC	57.1	46.9	0.401
TT	42.9	53.1	

*Percentage of samples in the various genotypes is given

IL-8 and CD14 promoter polymorphisms and serum IL-8 levels

The contribution of genetic determinants to the risk for acute ischemic stroke was analyzed by determining the polymorphisms in the promoter of IL-8 and CD14 genes, and the serum IL-8 levels in the stroke and control populations. No differences were observed in the distribution of the IL-8 promoter genotypes in the stroke and control patients or for the prevalence of the T or A alleles or homozygotes for the normal TT vs. AA variant genotype (p>0.05 for all combinations). The genotype distributions of the CD14 promoter polymorphism were also similar in the stroke and control groups (p>0.05 for all combinations) (Table 4).

To evaluate the IL-8 serum level as a risk factor for stroke, and to assess the possible effect of IL-8 -251 T/A alleles on the synthesis of IL-8 chemokine, the serum concentration of IL-8 was determined. The mean serum IL-8 level (continuous variable) was higher in the stroke patients (116.15 pg/ml) than in the controls (89.78 pg/ml), but the difference was not significant in the study population (p=0.287).

Associations and joint effects for risk factors

No association was observed between the HCMV IgG level and the HSV-1 IgA positivity of the total study population or of the stroke and control patients, nor between *C. pneumoniae* PCR positivity and high levels of *C. pneumoniae* IgG antibodies or IgA positivity in the overall study population (p=>0.05). There was no association between the IL-8 or CD14 promoter polymorphisms and the HCMV IgG level, or HSV-1 IgA

positivity, or C. pneumoniae antibody levels, or the presence of pathogen DNA in the blood, or hypertension, or lipid status, or alcohol consumption, in the combined study population (p=>0.05 for all combinations).

We did not detect any joint effect for stroke of a high level of HCMV IgG and HSV-1 IgA positivity (OR=6.41, CI 1.60-25.76) in the overall study population. There was no association and no joint effect of a high level of HCMV IgG and HSV-1 IgA positivity with hypertension, lipid status and alcohol consumption (p=>0.05 for all combinations).

DISCUSSION

Our results disclosed *C. pneumoniae* DNA in the peripheral blood of 10-17% of the population, with no significant difference between acute ischemic stroke patients and control individuals, and a very rare detectability of HCMV DNA in the study population. Some earlier investigations demonstrated a higher prevalence of *C. pneumoniae* DNA and HCMV DNA in the peripheral blood, and significant differences between patients with vascular diseases and control subjects (Larsson *et al.*, 1998; Lindberg and Grau, 2003), while others indicated a prevalence similar to or lower than our own result and no difference between patients and controls (Roback *et al.,* 2003, Apfalter *et al.,* 2004). Since we used a sensitive nPCR, and controls for contamination and PCR inhibitors, we are confident that our results are correct. We could not establish a quantitative relationship between the *C. pneumoniae* DNA in the peripheral blood of the stroke and the control patients, since the number of positive PCR replicates of the individual blood samples was very low (1 or 2) by nPCR, regardless of the origin of the samples (obtained from stroke or control patients).

Our results are in agreement with the finding of no association of *C. pneumoniae* antibodies with ischemic stroke (Heuschmann *et al.,* 2001). However, we did detect increased levels of HCMV IgG, as suggested earlier (Tarnacka *et al.,* 2002), and HSV-1 IgA antibodies in patients with acute ischemic stroke, suggesting an association of these infections with the disease. HCMV and HSV-1 antigens have been detected in carotid atheromas, but the HCMV IgG antibody titers did not correlate with the tissue findings and HSV-1 antibodies were not investigated (Chiu *et al.,* 1997).

No association of stroke with the IL-8 and CD14 promoter polymorphisms was observed in our overall study population. Earlier, a higher number of IL-8 mRNA-expressing blood mononuclear cells correlated with the serum level of IL-8 in patients with ischemic stroke, suggesting that IL-8 could be involved in recruiting blood polymorphonuclear leukocytes to the sites of cerebral ischemia, and higher IL-8 levels were found in patients aged <50 years with acute ischemic stroke, as compared with control subjects (Kostulas *et al.,* 1998; Grau *et al.,* 2001a, 2001b). Our results tend to support the higher serum levels of IL-8 in acute ischemic stroke. Other investigators, however, did find an association between the extent of hypoperfused brain area with a cytokine release of TNF-alpha and IL-6, but not with IL-8 in hyperacute stroke, indicating a complex interaction of neuroinflammatory markers with tissue destruction in cerebral ischemia (Montaner J, 2003). Interestingly, when our subjects were divided into groups < or ≥50 years old, for the subjects aged <50 years the distribution of the IL-8 genotypes differed in the stroke (n=13) and the control (n=26) patients (p=0.016). Whereas a strongly significant difference was observed for the combined group of TT and AT vs. AA genotypes between the stroke and the control subjects (p=0.005), no difference was detected between the AT+AA vs. TT genotypes (p=0.795), indicating a role of the T allele in the protection against stroke. A higher proportion of stroke patients had AA genotypes and a lower proportion had TT genotypes than among the control patients, and the difference was close to being significant (p=0.058). No differences were seen in the stroke (n=46) and control (n=26) patients aged ≥50 years for the IL-8 genotypes. For the CD14 promoter nt -260 C → T polymorphisms, no difference between our stroke and control patients aged <50 or ≥50 years was observed (p=>0.05 for all homozygote and heterozygote combinations). A polymorphism in the CD14 promoter has been suggested to be associated with various inflammatory diseases. The T allele has been shown to display a higher preva-

lence in vascular diseases in a Japanese population or in certain subgroups of Caucasian patients (Hohda *et al.,* 2003; Rishley *et al.,* 2003). However, other studies have not demonstrated differences in the distribution of CD14 genotypes in patients or control subjects (Ito *et al.,* 2000, Park *et al.,* 2006), suggesting the role of CD14 polymorphisms only in certain subgroups of patients with vascular diseases.

There were significant differences between our stroke and control patients for hypertension, the lipid status and alcohol consumption. Moreover, our results suggest an association of acute ischemic stroke with chronic HCMV and HSV-1 infection. The lack of a higher prevalence of HCMV DNA in the peripheral blood of stroke victims indicates that HCMV did not enter the circulation in a detectable level before or during the acute ischemic stroke. The lack of an association of stroke with additional pathogens of persistent infection may possibly indicate specific roles of HCMV and HSV-1 in the development of acute ischemic stroke, or may be related to the local population in our study, or could be due to chance. The lack of an association with HSV IgG, but the strong association with HSV-1 IgA, may indicate a specific role of chronic HSV-1 infection in the development of the disease. However, the causability of these factors is not established in our study.

Chronic infections with a number of pathogens, chronic inflammatory vascular conditions, specific genetic predispositions and traditional risk factors are included in the list of the factors that are suggested to modify stroke risk. These fields are interrelated and the factors probably cooperatively enhance the risk of stroke. In addition, the various interacting conditions must be considered by stratifying the analyses for different subtypes of stroke, and the age of the stroke victims. Further difficulties include the selection of proper controls from the general population, especially within older age groups, who are more likely to be disabled or suffering from severe comorbidities. The published results are conflicting partly because of the heterogenous nature of stroke disease and the many interacting factors involved in the development of stroke. We examined some of these factors in a population of ischemic stroke with the aim to establish an association between these factors and the disease. However, multicentric, more accurate clinical and epidemiological studies are needed to define an etiological link between one or more pathogens, or genetic predispositions with stroke events, caused by atherosclerotic mechanism, possible in subgroups of patients stratified by stroke subtypes and age.

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REFERENCES

Anzini, A., Cassone, A., Rasura, M., Cervo, A., Beccia, M., Di Lisi, F., Fieschi, C. (2004). *Chlamydia pneumoniae* infection in young stroke patients: a case-control study. *Eur. J. Neurol.* **11**, 321-327.

Apfalter, P., Baroush, W., Nehr, M., Willinger, B. Rotter, M., Hirschl, AM. (2004). No evidence of involvement of *Chlamydia pneumoniae* in severe cerebrovascular atherosclerosis by means of quantitative real-time polymerase chain reaction. *Stroke* **35**, 2024-2028.

Caruso, A., Favilli, F., Rotola, A., Comar, M., Horejsh, D., Alessandri, G., Di Luca, D., Fiorentini S. (2003). Human herpesvirus-6 modulates RANTES production in primary human endothelial cell cultures. *J. Med. Virol.* **70**, 451-458.

Chiu, B., Viira, E., Tucker, W., Fong, IW. (1997). *Chlamydia pneumoniae*, cytomegalovirus, and herpes simplex virus in atherosclerosis of the carotid artery. *Circulation* **96**, 2144-2148.

Conaldi, PG., Serra, C., Mossa, A., Falcone, V., Basolo, F., Camussi, G., Dolei, A., Toniolo A. (1997). Persistent infection of human vascular endothelial cells by group B coxsackieviruses. *J. Infect. Dis.* **175**, 693-696.

Diedrich, S., Drisesel, G., Shreier, E. (1995). Sequence comparison of echovirus type 30 isolates to other enteroviruses in the 5' noncoding region. *J. Med. Virol.* **46**, 148-152.

Elkind, MS., Tondella, ML., Feikin, DR., Fields, BS., Homma, S., Di Tullio, MR. (2006). Seropositivity to *Chlamydia pneumoniae* is associated with risk of first ischemic stroke. *Stroke* **37**, 790-795.

Frank, TS., Cook, SM., Del Bueno, EA., Wilson, MD. (1992). A simplified method for detecting cytomegalovirus by polymerase chain reaction from histologic sections of small biopses. *Mod. Pathol.* **5**, 449-454.

Freidank, HM., Lux, A., Dern, P., Meyer-Konig, U., Els, T. (2002). *Chlamydia pneumoniae* DNA in

peripheral venous blood samples from patients with carotid artery stenosis. *Eur. J. Clin. Microbiol. Infect. Dis.* **21**, 60-62.

GALBRAITH, DN., NAIRN, C., CLEMENTS, GB. (1997). Evidence for enteroviral persistence in humans. *J. Gen. Virol.* **78**, 307-312.

GRAU, AJ., AULMANN, M., LICHY, C., MEISER, H., BUGGLE, F., BRANDT, T., GROND-GINSBACH, C. (2001a). Increased cytokine release by leukocytes in survivers of stroke at young age. *Eur. J. Clin. Invest.* **31**, 999-1006.

GRAU, AJ., REIS, A., BUGGLE, F., AL-KHALAF, A., WERLE, E., VALOIS, N., BERTRAM, M., BECHER, H., GROND-GINSBACH, C. (2001b). Monocyte function and plasma levels of interleukin-8 in acute ischemic stroke. *J. Neurol. Sci.* **192**, 41-47.

GYULAI, Z., KLAUSZ, G., TISZAI, A., LENART, Z., KASA, IT., LONOVICS, J., MANDI, Y. (2004). Genetic polymorphism of interleukin-8 (IL-8) is associated with Helicobacter pylori-induced duodenal ulcer. *Eur. Cytokine Netw* **15**, 353-358.

HEUSCHMANN, PU., NEUREITER, D., GESSLEIN, M., CRAIVAN, B., MAASS, M., FALLER, G., BECK, G., NEUNDOERFER, B., KOLOMINSKY-RABAS, PL. (2001). Association between infection with *Helicobacter pylori* and *Chlamydia pneumoniae* and risk of ischemic stroke subtypes. Results from a population-based case-control study. *Stroke* **32**, 2253-2258.

HOHDA, S., KIMURA, A., SASAOKA, T., HAYASHI, T., UEDA, K., YASUNAMI, M., OKABE, M., FUKUTA, N., KUROSAWA, T., IZUMI, T. (2003). Association study of CD14 polymorphism with myocardial infarction in a Japanese population. *Jpn Heart J* **44**, 612-622.

HULL, J. THOMSON, A., KWIATKOWSKI, D. (2000). Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* **55**, 1023-1027.

ITO, D., MURATA, M., TANAHASHI, N., SATO, H., SONODA, A., SAITO, I., WATANABE, K., FUKUUCHI, Y. (2000). Polymorphism in the promoter of lipopolysaccharide receptor CD14 and ischemic cerebrovascular disease. *Stroke* **31**, 2661-2664.

KOSTULAS, N., KIVISAKK, P., HUANG, Y., MATUSEVICIUS, D., KOSTULAS, V., LINK, H. (1998). Ischemic stroke is associated with a systemic increase of blood mononuclear cells expressing interleukin-8 mRNA. *Stroke* 29, 462-466.

KRÜGER, S., GRAF, J., MERX, MW., STICKEL, T., KOCH, KC., JANSSENS, U., HANRATH, P., KUNZ, D. (2005). CD14 gene -260 C/T polymorphism is associated with chronic heart failure. *Eur. J. Intern. Med.* 16, 345-347.

LARSSON, S., SÖDERBERG-NAUCLER, C., WANG, FZ., MÖLLER, E. (1998). Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time. *Transfusion* **38**, 271-278.

LINDSBERG, PJ., GRAU, AJ. (2003). Inflammation and infections as risk factors for ischemic stroke. *Progress Review Stroke* **34**, 2518-2532.

MONTANER, J., ROVIRA, A., MOLINA, CA., ARENILLAS, JF., RIBO, M., CHACON, P., MONASTERIO, J., ALVAREZ-SABIN, J. (2003). Plasmatic levels of neuroinflammatory markers predict the extent of diffusion-weighted image lesions in hyperacute stroke. *J. Cereb. Blood. Flow. Metab.* **23**, 1403-1147.

MURONO, S., INOUE, H., TANABE, T., JOAB, I., YOSHIZAKI, T., FURUKAWA, M., PAGANO, JS. (2001). Induction of cyclooxygenase-2 by Epstein-Barr virus latent membrane protein 1 is involved in vascular endothelial growth factor production in nasopharingeal carcinoma cells. *Proc. Natl. Acad. Sci. USA* **98**, 6905-6910.

NGEH, J., GOODBOURN, C. (2005). Chlamydia pneumoniae, Mycoplasma pneumonia, and Legionella pneumoniae in elderly patients with stroke (C-PEPS, M-PEPS, L-PEPS). A case-control study on the infectious burden of atypical respiratory pathogens in elderly patients with acute cerebrovascular disease. *Stroke* 259-263.

PARK, MH., MIN, JY., KOH, SB., KIM, BJ., PARK, MK., PARK, KW., LEE, DH. (2006). Helicobacter pylori infection and the CD14 C(-260)T gene polymorphism in ischemic stroke. *Thromb. Res.* **118**, 671-667.

RISLEY, P., JERRARD-DUNNE, P., SITZER, M., BUEHLER, A., VON KEGLER, S., MARKUS, HS. (2003). Promoter polymorphism in the endotoxin receptor (CD14) is associated with increased carotid atherosclerosis only in smokers: Carotid Atherosclerosis Progression Study (CAPS). *Stroke* **34**, 600-604.

ROBACK, JD., DREW, WL., LAYCOCK, ME., TODD, D., HILLYER, CD., BUSCH, MP. (2003). CMV DNA is rarely detected in healthy blood donors using validated PCR assays. *Transfusion* **43**, 314-321.

TARNACKA, B., GROMADZKA, G., CZLONKOWSKA, A. (2002). Increased circulating immune complexes in acute stroke. The triggering role of *Chlamydia pneumoniae* and cytomegalovirus. *Stroke* **33**, 936-940.

TONG, CY., SILLIS, M. (1993). Detection of *Chlamydia pneumoniae* and *Chlamydia psittaci* in sputum samples by PCR. *J. Clin. Pathol.* **46**, 313-317.

VALLBRACHT, KB., SCHWIMMBECK, PL., KUHL, U., RAUCH, U., SEEBERG, B. SCHULTHEISS, HP. (2005). Differential aspects of endothelial function of the coronary microcirculation considering myocardial virus persistence, endothelial activation, and myocardial leukocyte infiltrates. *Circulation* **111**, 1784-1791.

XIONG. A., CLARKE-KATZENBERG, RH., VALENZUELA, G., IZUMI, KM., MILLAN, MT. (2004). Epstein-Barr virus latent membrane protein 1 activates nuclear factor-kappa B in human endothelial cells and inhibits apoptosis. *Transplantation* **78**, 41-49.