TNF- α , TNF- β , IL-6, and IL-10 polymorphisms in patients with lung cancer

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Abstract. Apart from cigarette smoking, genetic factors seem to be of importance in the development of lung cancer. The present case-control study investigated frequencies of five inflammatory response gene polymorphisms (TNF- α -308, TNF- β -Intron1-252, IL-6-174, IL-10-819 and IL-10-1082) in patients with lung cancer and controls. The study population consisted of 117 patients with lung cancer (77 patients with NSCLC, including 40 Squamous Cell Carcinoma and 26 Adenocarcinoma, and 40 patients with SCLC), 117 matched controls without pulmonary disease and 243 healthy individuals (population control).

Genotype analyses revealed no difference in genotype frequencies using matched-pair analysis. However, in comparison to the population control, the IL-10-1082 genotypes carrying the G allele appeared with higher frequency in the SCLC group ($p = 0.006$) [SCLC: 84.6%, population controls: 64.6%]. This yields an odds ratio of 3.01 for SCLC (95% CI = [1.21, 7.48]). No associations were seen for all other polymorphisms analysed.

The study raises the possibility of a correlation between the IL-10-1082 G allele and the presence of SCLC in a German population. The functional IL-10-1082 polymorphism correlates with altered IL-10 levels and might influence lung cancer susceptibility by altered inflammatory responses in the airways.

1. Introduction

Lung cancer is a growing health problem that causes high morbidity and mortality in all industrial countries. Cigarette smoking and exposure to other exogenous carcinogens is clearly related to the development of lung cancer. However, only 11% of tobacco smokers ultimately develop lung cancer, and family studies suggest genetic factors to modulate the risk among those who are exposed to carcinogens [2]. Individual cancer risk may be mediated by local factors in the airways and the individual response to environmental mutagens.

Independent of smoking habits, age, and family history, incidence and mortality of lung cancer is increased in patients with impaired lung function [39]. Furthermore, patients suffering from chronic obstructive lung disease (COPD) – a disease characterized by impaired lung function and chronic inflammation of the airways [16,33] – are at risk of developing lung cancer. Chronic inflammation by itself is known to contribute to lung cancer [4,5,28] and abnormalities in cytokine serum and bronchoalveolar lavage fluid (BAL) levels were shown, both in smokers and patients with lung cancer [3,8,9].

Tumor Necrosis Factor-alpha (TNF- α), Tumor Necrosis Factor-beta (TNF- β), Interleukin-6 (IL-6), Interleukin-8 (IL-8) and Interleukin-10 (IL-10) are significant modulators of airway inflammation. Cytokine promoter polymorphisms, including TNF- α -308, TNF- β -Intron1-252, IL-6-174 and IL-10-1082, are associated with altered protein levels and/or transcription rates [14,29,43,47]. In the present case-control study

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frequencies of five cytokine polymorphisms (TNF- α -308, TNF- β -Intron1-252, IL-6-174, IL-10-819 and IL-10-1082) were analysed in patients with Non Small Cell Lung Cancer (NSCLC), Small Cell Lung Cancer (SCLC) and controls.

2. Methods

2.1. Patients

The present case-control study investigated the frequencies of five interleukin (IL) promoter polymorphisms in 365 Caucasian individuals: 117 patients with lung cancer (77 patients with Non Small Cell Lung Cancer and 40 with Small Cell Lung Cancer), 117 matched controls, and 243 healthy individuals (population control). The study was conducted at the University of Marburg, Germany. Samples from lung cancer patients with primary disease were collected between January 1998 and March 1999 in the Department of Oncology. Patients with prior diagnosed serious pulmonary disease, such as COPD II°–III°, pneumonia, lung fibrosis or other interstitial lung diseases were not included. From a total of 600 patients undergoing lung function testing in the Department of Internal Medicine those without pulmonary disease and with matching age, gender and smoking habits to above mentioned patients were included to set up matched pairs. Smoking habits were classified using a cut-off of 10 pack years (py; 1 py corresponds to smoking one packet of cigarettes every day over a period of 1 year) according to a European multicenter study demonstrating a significant excess risk of 70% with every 10 pack years smoked [1]: A) Never smokers and ever smokers who had smoked less than 10 pack years were classified as never/light smokers, B) former smokers and current smokers who smoked more than 10 pack years were classified as former/current smokers.

There was a history of smoking in 40.5% of the population control group, 88.3% of the NSCLC group, and 97.5% of the SCLC group. Highest average number of pack years (43.8) was reported by patients with SCLC, lowest (23.5) by patients with Adenocarcinoma. Highest number of individuals who smoked more than 60 pack years were presented in the SCLC and lowest in the Adenocarcinoma group (Table 1b). Group characteristics of different subgroups are shown in Table 1a and b and described below.

Blood specimens were drawn for genotype analysis after obtaining the patients' informed consent.

2.2. Population group

To provide a comparable baseline of polymorphism frequencies in an ethnically matched group of German individuals, we recruited a population control group. This group consisted of 243 healthy, German, Caucasian individuals. Of these 131 individuals underwent the common physical examination by the staff medical service at the University of Marburg upon entering a new position or changing positions between October 1998 and July 1999. 112 were included between March and April 2000 from the Department of Surgery having had accidental injuries, without reporting a history of pulmonary diseases or symptoms. The group consisted of 134 males and 109 females (gender ratio: 1.22) with a mean age of 37.9 years. A history of at least 10 p/y cigarette smoking was reported by 79 (39.9%) individuals.

2.3. NSCLC group

Of 77 patients included in the NSCLC group, 40 suffered from Squamous Cell Carcinoma (SCC), 26 from Adenocarcinoma (AC), 5 from large cell carcinoma, and 6 from other tumor histology. The group consisted of 69 males and 8 females, with mean age 65.4 (42–82 years). Out of 77 patients, 68 (88.3%) smoked more than 10 pack years. Ten patients smoked less than 20 p/y (12.9%), 25 patients smoked between 21–40 p/y (32.4%), 17 patients between 41–60 p/y by (14.5%) and 10 patients smoked more than 60 p/y (12.9%). Average number of pack years was 36.5 for all NSCLC patients, 43.9 for the SCC-, and 23.5 for AC- subgroup (Table 1a and b).

2.4. SCLC group

The SCLC group consisted of 40 patients, including 35 males and 5 females (gender-ratio: 7.0). Patients' age ranged from 44–84 years, mean age was 63.3 years. Thirty-nine patients (97.5%) were classified as current or former smoker. Two patients (5.0%) smoked less than 20 p/y, 15 (37.5%) between 21–40 p/y, 13 (32.5%) between 41–60 p/y and 6 patients (15.0%) smoked more than 60 p/y (Table 1a and b). Mean number of p/y was 43.8.

Table 1
Characteristics of the groups under study and smoking habits (pack years) of the lung Cancer groups

1a Group Characteristics

	Control group			Lung Cancer group			
	Population	Matched NSCLC	Matched SCLC	NSCLC	SCLC	SCC	AC
<i>n</i>	243	77	40	77	40	40	26
Male/Female	1.22	8.7	14.5	8.7	14.5	10.7	10.5
Mean Age	37.9	65.4	63.3	65.4	63.3	65.4	65.6
(range)	(25–62)	(42–82)	(44–84)	(42–82)	(44–84)	(34–82)	(42–81)
Former/current Smokers	79	68	39	68	39	38	20
(> 10 py) n(%)	(39.9%)	(88.0%)	(97.5%)	(88.0%)	(97.5%)	(95.0%)	(76.9%)

1a Pack years (p/y) smoked by former/current smokers

n (%)	NSCLC	SCLC	SCC	AC
11–20 p/y	10 (12.9)	2 (5.0)	2 (5.0)	6 (23.0)
21–40 p/y	25 (32.4)	15 (37.5)	14 (35.0)	6 (23.0)
41–60 p/y	17 (14.5)	13 (32.5)	13 (32.5)	5 (19.2)
> 60 p/y	10 (12.9)	6 (15.0)	5 (12.5)	1 (3.8)
Unavailable	6 (7.8)	3 (7.5)	4 (10.0)	2 (7.7)
Mean p/y	36.5	43.8	43.9	23.5

NSCLC = Non small cell lung cancer, SCLC = Small cell lung cancer, SCC = Squamous Cell Carcinoma, AC = Adenocarcinoma. % (of the whole group) are given in parenthesis.

2.5. Matched controls

A matched control (regarding age [$\pm$ 1 year], gender and smoking habit) for each lung cancer patient was included. Inclusion criteria were absence of any pulmonary disease, normal lung function and no history or symptoms of respiratory disease. Smoking habits were matched using a cut-off of 10 pack years as described above. From 117 control patients, 100 (85.4%) had coronary heart disease, 4 (3.4%) had sleep apnea, 3 (2.5%) patients underwent surgery for cancer (excluding lung cancer) and 10 (8.5%) patients suffered from other diseases.

2.6. Analysis of polymorphisms by RFLP based (converted) PCR method

With the exception of TNF polymorphisms, which contain a natural restriction enzyme recognition site, for analyses of polymorphisms RFLP based (converted) PCR was used: If a polymorphism does not contain a natural restriction enzyme recognition site, amplification using specifically designed primers (Table 2) containing a mismatched base can create PCR products including a restriction enzyme recognition site in one allele. Consequently, in case of a single nucleotide polymorphism (SNP), none, one or both alleles will be digested by the appropriate enzyme.

Genomic DNA was isolated from blood specimens using a commercial kit (Qiagen, Hilden, Germany). PCR products were generated in a reaction volume of 50 μ l with 10 ng DNA as template, 0.2 units Taq polymerase provided by Boehringer Mannheim (Mannheim, Germany), 1.5 μ l buffer 1 (17.5 mM MgCl₂) and buffer 2 (22.5 mM MgCl₂) 10 $\times$ concentrated, provided by Boehringer Mannheim (Mannheim, Germany), 1 μ l of each specific primer (100 ng/ μ l) and 1 μ l dNTPs (1.25 mM each dNTP). PCR cycling conditions were as follows: Each PCR reaction started at 95°C for 2 minutes, followed by 30–35 cycles at the respective annealing temperature [IL-10 1082, IL-10 819: 59°C. IL-6-174, TNF- α : 60°C. TNF- β : 65°C] and finished at 72°C for 5 minutes. For all PCR reactions a Gene Amp PCR system 9600 Perkin Elmer Thermocycler was used. Each PCR reaction was run including a positive and negative control.

Specific PCR products were digested by the corresponding restriction enzyme (Table 2), separated by 8% polyacrylamide gel electrophoresis, and stained with ethidium bromide to analyse the given genotype. Several spot checks from PCR products were sequenced for each genotype.

2.7. Statistical analysis

Hardy-Weinberg-Equilibrium was checked in both control groups by chi-square tests for each polymor-

Table 2
Primers and restriction enzymes for genotyping

	Specific Primer Pairs	Restriction Enzyme
TNF- α	TAC ACC ATC TCC AGC ACA TAG AA CAA GAC AAC ACT AAG GCT TCT TGA GGA [46]	NCO I
TNF- β	CTC CTG CAC CTG CTG CCT GGA TC GAA GAG ACG TTC AGG TGG TGT CAT [37]	NCO I
IL-6-174	TTG TCA AGA CAT GCC AAG TGC T GCC TCA GAG ACA TCT CCA GTC C [32]	Nla III
IL-10-819	GAT TCT CAG GCA CAT GTT TCC AC GTT AGC ACT GGT GTA CCC TTG TAC AGG TGA ATT AA	BseR I
IL-10-1082	TAC ACC ATC TCC AGC ACA TAG AA CAA GAC AAC ACT ACT AAG GCT TCT TGA GGA	ASE I

Table 3
Frequencies of different genotypes in the groups under study (SCLC)

	SCLC (<i>n</i> = 40)	Population control (<i>n</i> = 243)	p-value (SCLC vs. population controls)	Matched controls (<i>n</i> = 40)	p-value (SCLC vs. matched controls)
IL-6-174-CC	4 (10.3)	46 (18.9)	0.10	7 (17.5)	0.27
-GC	16 (41.0)	107 (44.0)		19 (47.5)	
-GG	19 (48.7)	90 (37.1)		14 (35.0)	
IL-10-819-CC	24 (60.0)	140 (57.8)	0.78	24 (60.0)	0.72
-TC	14 (35.0)	88 (36.4)		12 (30.0)	
-TT	2 (5.0)	14 (5.8)		4 (10.0)	
IL-10-1082-AA	6 (15.4)	86 (35.4)	0.006	7 (17.5)	0.48
-AG	21 (53.8)	115 (47.3)		24 (60.0)	
-GG	12 (30.8)	42 (17.3)		9 (22.5)	
TNF- α -308-AA	0 (0)	4 (1.6)	0.68	1 (2.5)	1.00
-AG	11 (27.5)	67 (27.7)		9 (22.5)	
-GG	29 (72.5)	171 (70.7)		30 (75.0)	
TNF- β -Intron 1-AA	18 (45.0)	115 (47.9)	0.75	25 (62.5)	0.26
-AG	17 (12.5)	97 (40.4)		10 (25.0)	
-GG	5 (12.5)	28 (11.7)		5 (12.5)	

Absolute numbers with % in parenthesis are shown: n(%), p-values are for comparison between SCLC and respective control group, for population controls using Cochran-Armitage test for trend based on genotypes, for matched controls using conditional logistic regression.

phism. In the population control group, all markers were in HWE ($p > 0.1$). In the matched control group, the polymorphism IL-10-1082 ($p = 0.05$) showed slight deviations from HWE. Since genotypes were checked independently by two raters, we do not consider this as evidence of genotyping errors. Instead it could be an effect of the composition of this control group which contains mostly coronary heart disease patients and this marker might be relevant in coronary heart disease as well.

For each polymorphism and each of the four lung cancer subgroups (SCLC, all NSCLC, Squamous Cell Carcinoma, Adenocarcinoma), two univariate group comparisons were performed. First, genotype frequencies between LC cases and the whole population control sample were compared using the Cochran-Armitage test for trend (an exact test was used whenever one of the expected cell counts was below 5). This test does not require specification of a genetic model, but just assumes that risk of disease increases with the num-

ber of risk alleles at a putative disease locus. Second, genotypes between LC cases and one-to-one matched controls were compared using conditional logistic regression.

For the IL-10-1082 polymorphism, which was nominally significant ($p < 0.05$) and seemed to follow a dominant model with genotypes AG and GG more common in cases, we performed a post-hoc test and compared the frequency of carriers of the G allele in cases and population controls using a chi-square test. Odds ratio estimates for being a case when carrying at least one G allele and corresponding 95% confidence intervals (CI) are also given for IL-10-1082. The frequency of the IL-10-1082 G allele in our study groups was compared by chi-square test with that in other published population samples (control groups in 12, 15, 18, 24).

Analyses were performed with SAS V8 (SAS Institute, Cary, USA) and StatXact 5 (Cytel Software) for

Table 4
Frequencies of different genotypes in the groups under study (NSCLC)

	NSCLC (<i>n</i> = 77)	Population control (<i>n</i> = 243)	p-value (NSCLC vs. population controls)	Matched-controls (<i>n</i> = 77)	p-value (NSCLC vs. matched controls)
IL-6-174-CC	13 (16.9)	46 (18.9)	0.88	5 (6.7)	0.28
-GC	36 (46.8)	107 (44.0)		41 (54.7)	
-GG	28 (36.4)	90 (37.1)		29 (38.7)	
IL-10-819-CC	42 (54.5)	140 (57.8)	0.73	47 (61.8)	0.54
-TC	31 (40.3)	88 (36.4)		24 (31.8)	
-TT	4 (5.2)	14 (5.8)		5 (6.6)	
IL-10-1082-AA	24 (31.6)	86 (35.4)	0.22	16 (21.3)	0.81
-AG	33 (43.4)	115 (47.3)		44 (58.7)	
-GG	19 (25.0)	42 (17.3)		15 (20.0)	
TNF- α -308-AA	1 (1.3)	4 (1.6)	0.53	1 (1.3)	0.13
-AG	25 (32.5)	67 (27.7)		15 (19.5)	
-GG	51 (66.2)	171 (70.7)		61 (79.3)	
TNF- β -Intron 1-AA	33 (42.9)	115 (47.9)	0.99	36 (47.4)	0.58
-AG	39 (50.6)	97 (40.4)		35 (46.0)	
-GG	5 (6.5)	28 (11.7)		5 (6.6)	

Absolute numbers with % in parenthesis are shown: n(%), p-values are for comparison between NSCLC and respective control group, for population controls using Cochran-Armitage test for trend based on genotypes, for matched controls using conditional logistic regression.

Table 5
Frequencies of different genotypes in the groups under study (SCC)

	SCC (<i>n</i> = 40)	Population control (<i>n</i> = 243)	p-value (SCC vs. population controls)	Matched controls (<i>n</i> = 40)	p-value (SCC vs. matched controls)
IL-6-174-CC	4 (10.0)	46 (18.9)	0.24	4 (10.3)	1.0
-GC	19 (47.5)	107 (44.0)		18 (46.1)	
-GG	17 (42.5)	90 (37.1)		17 (43.6)	
IL-10-819-CC	16 (40.0)	140 (57.8)	0.04	25 (62.5)	0.12
-TC	20 (50.0)	88 (36.4)		12 (30.0)	
-TT	4 (10.0)	14 (5.8)		3 (7.5)	
IL-10-1082-AA	13 (32.5)	86 (35.4)	0.38	7 (18.4)	0.72
-AG	17 (42.5)	115 (47.3)		24 (63.2)	
-GG	10 (25.0)	42 (17.3)		7 (18.4)	
TNF- α -308-AA	1 (2.5)	4 (1.6)	0.18	1 (2.5)	0.20
-AG	15 (37.5)	67 (27.7)		8 (20.0)	
-GG	24 (60.0)	171 (70.7)		31 (77.5)	
TNF- β -Intron 1-AA	14 (35.0)	115 (47.9)	0.58	15 (38.5)	0.67
-AG	24 (60.0)	97 (40.4)		22 (56.4)	
-GG	2 (5.0)	28 (11.7)		2 (5.1)	

Absolute numbers with % in parenthesis are shown: n(%), p-values are for comparison between Squamous Cell Carcinoma (SCC) and respective control group, for population controls using Cochran-Armitage test for trend based on genotypes, for matched controls using conditional logistic regression.

exact tests. Power calculations were done with S-Plus 6.0 (Lucent Technologies).

Nominal p-values for each test are reported and should be evaluated against an adequate level of significance [7,31]. This has to be adjusted for the multiple testing in this study and possibly even for the potential multiple testing of a much larger number of candidate polymorphisms. Using a conservative Bonferroni correction, this would mean $\alpha = 0.00125$ (5 polymorphisms with 8 tests each) or as suggested in the literature, a general significance level for genetic association studies of as little as $\alpha = 10^{-6}$ [36].

In the analysis of the different lung cancer groups (SCLC: *n* = 40, NSCLC: 77, SCC: *n* = 40 and AC: *n* = 26) vs. population controls (*n* = 243) we have 80% power (at $\alpha = 0.00125$) for carrier frequency differences larger than about 0.30 (SCLC), 0.25 (NSCLC), 0.30 (SCC) and 0.38 (AC), respectively. In the matched analysis, we have 80% power (at $\alpha = 0.00125$) to detect carrier frequency differences of about 0.24 (SCLC), 0.17 (NSCLC), 0.24 (SCC) and 0.30 (AC), respectively. Since also smaller effects could certainly be clinically and biologically relevant, we cannot exclude a role in the aetiology of lung cancer for those genes for which no association is found.

Table 6
Frequencies of different genotypes in the groups under study (AC)

	Adeno carcinoma (<i>n</i> = 26)	Population control (<i>n</i> = 243)	p-value (AC vs. population controls)	Matched controls (<i>n</i> = 26)	p-value (AC vs. matched controls)
IL-6-174-CC	5 (19.2)	46 (18.9)	0.94	0 (0)	0.62
-GC	11 (42.3)	107 (44.0)		17 (68.0)	
-GG	10 (38.5)	90 (37.1)		8 (32.0)	
IL-10-819-CC	18 (69.2)	140 (57.8)	0.16	15 (60.0)	0.42
-TC	8 (30.8)	88 (36.4)		8 (32.0)	
-TT	0 (0)	14 (5.8)		2 (8.0)	
IL-10-1082-AA	5 (19.2)	86 (35.4)	0.08	7 (26.9)	0.44
-AG	14 (53.9)	115 (47.3)		14 (53.9)	
-GG	7 (26.9)	42 (17.3)		5 (19.2)	
TNF- α -308-AA	0 (0)	4 (1.6)	0.98	0 (0)	0.18
-AG	8 (30.8)	67 (27.7)		4 (15.4)	
-GG	18 (69.2)	171 (70.7)		22 (84.6)	
TNF- β -Intron 1-AA	11 (42.3)	115 (47.9)	0.91	16 (61.5)	0.11
-AG	13 (50.0)	97 (40.4)		10 (38.5)	
-GG	2 (7.7)	28 (11.7)		0 (0)	

Absolute numbers with % in parenthesis are shown: n(%), p-values are for comparison between Adenocarcinoma (AC) and respective control group, for population controls using Cochran-Armitage test for trend based on genotypes, for matched controls using conditional logistic regression.

3. Results

Genotype frequencies and results of both the matched and unmatched analysis are given in Tables 3–6: For none of the markers and none of the different lung cancer groups comparison between cases and one-to-one matched controls showed a significant difference in genotype frequencies. Comparison between lung cancer groups and population controls showed relevant differences only for the IL-10-1082 marker. These were most pronounced in the SCLC group ($p = 0.006$), and showed the same trend in the subgroup of adenocarcinoma ($p = 0.08$), while there was no clear association in the subgroup of Squamous Cell Carcinoma ($p = 0.38$) or the whole NSCLC patient group ($p = 0.22$). The association is marked by a higher rate of genotypes carrying the G allele in the cases (SCLC: 84.6%, NSCLC: 68.4%, AC: 80.8%, SCC: 67.5%, population controls: 64.6%). The estimated odds ratio of being a SCLC case when carrying at least one G allele is 3.01 (95% CI = [1.21, 7.48]), for NSCLC we have OR = 1.19 (95% CI = [0.68, 2.06]), for Adenocarcinoma OR = 2.30 (95% CI = [0.84, 6.32]), and for Squamous Cell Carcinoma OR = 1.14 (95% CI = [0.56, 2.32]). Even though the observed associations for SCLC and AC are quite strong, they fail to reach the strict Bonferroni-corrected level of significance of 0.00125 due to the limited sample size.

The allele frequency of IL-10-1082 G in other published control groups [12,15,18,25] is homogenous and varies between 47% and 52% (50% over all individuals), while it is 41% in our population sample. This

difference is highly significant ($p = 0.0002$, chi-square test).

4. Discussion

To our knowledge this is the first analysis investigating IL-6, IL-10, and TNF- α polymorphisms in patients with lung cancer. No significant differences were observed between the lung cancer and control groups, except for IL-10-1082. There, the analysis showed relevant differences in the frequency of genotypes with the IL-10-1082 G allele between population controls and the SCLC group.

Lung cancer risk may be influenced by local factors of the airways, such as inflammatory reactions, e.g. in response to environmental mutagens. A chronic inflammatory disease of the airways related to cigarette smoke, COPD, is known to contribute to lung cancer [16,33], as well as chronic inflammation by itself [4,28]. Moreover, abnormalities in serum cytokine and bronchoalveolar lavage fluid (BAL) levels were demonstrated in smokers and patients with lung cancer [3,8,9]. New experimental evidence emphasising the role of inflammation in lung tumour development came from different inbred mouse strains [5], suggesting inflammation to be important in both, initial and further promoted epithelial cell alteration by inflammation related release of reactive oxygen species (ROS), chemokines and pro-angiogenetic factors. Several susceptibility loci for lung neoplasia found in mice, being homologous with human asthma loci, contain suscepti-

bility genes for lung injury and inflammation, including TNF- α , that had been implicated in the development of lung cancer before [41]. The TNF- β polymorphism was implicated in lung cancer before [37]. The present study did not show any association of TNF- α , TNF- β , or IL-6-polymorphisms with lung cancer, but we cannot exclude a role in the aetiology of the disease for these polymorphisms since our study only had power to detect large effects.

However, the analyses suggest genotypes carrying the IL-10-1082 G to be associated with the presence of SCLC.

IL-10, a cytokine that is postulated to inhibit inflammatory responses by reducing proinflammatory cytokine production, is produced by T-cells and macrophages [10,21,30,45]. The promoter polymorphism IL-10-1082 (G/A) was shown to be functional as the alleles show different effects on IL-10 transcription *in vitro* [43], on binding activity to transcription factors [35] and regulation of constitutive mRNA levels [40]. *In vitro* and *in vivo* studies had reported conflicting results concerning the modulation of IL-10 secretion: Concanavalin A stimulation results in higher IL-10 production by human lymphocytes associated with the IL-10-1082 G allele [43], while other studies demonstrated reduced IL-10 production after LPS stimulation [13] and *in vivo* after cardiopulmonary bypass [15]. Patients with postulated "low producer allele" IL-10-1082 A seem to develop autoimmune inflammatory diseases, such as inflammatory bowel disease [42], severe forms of rheumatoid arthritis [18] systemic lupus erythematosus [24] and in patients with severe asthma [25]. In the present study a shift towards the IL-10-1082 G allele in patients with SCLC was observed.

Expression of IL-10 has been reported to correlate with tumour vascularisation [20] and clinical prognosis in NSCLC [9,19]. IL-10 is increased in serum and effusions of lung cancer patients [8], is known to be produced [22] and induced in lymphocytes [23] by lung tumour cells, and seems to provide several properties that may be inhibitory to the generation of anticancer immunity [17]. Interestingly, IL-10 serum levels had been previously correlated with prognosis in advanced NSCLC [9]. To date, no specific information about IL-10 in BAL, serum or local cancer environment in patients with SCLC are available. However, an effect of IL-10 on lung tumour susceptibility in general is shown in animal models, as haploinsufficiency in knock out mice increase tumourgenesis of lung tumours [6]. In humans, IL-10 seems to be involved in immunescape

in lung cancer [44] and was demonstrated to influence the release of proteases and antiproteases by airway macrophages in cigarette smokers, a balanced system that seems to be important in lung cancer development, as well [26].

Currently, neither the impact of different IL-10 expression on lung cancer development in general nor its potential importance for different lung cancer subgroups is exactly known. Lung cancer is a multifactorial disease with a complex interplay of environmental and genetic background. Associations might be specific for different populations and may correlate with multiple events anywhere in cancer genesis or may be linked to mechanisms that enhance lung cancer susceptibility indirectly. Thus, genetically based altered IL-10 levels may play a role in chronic inflammation, in the development of airway obstruction, or other unknown mechanisms. Previously, the IL-10-1082 G allele was demonstrated to be more common in patients with COPD [38], a disease with a prominent chronic inflammation component and airway obstruction.

The observed difference in IL-10-1082 genotype frequencies between SCLC patients and our population control sample could possibly be caused by population stratification [34]. However, all groups in the present study are of the same geographical location and ethnic background and migration/immigration in the study region was small in the relevant time frame. Moreover, although the age distribution among cases and controls differs, we do not expect this to contribute to the observed genotype difference since there are no known or suspected effects of IL-10 on survival. Lastly, neither age nor gender (which also differs in distribution between cases and controls) should be confounders, since the polymorphism is almost surely independent of either [11,27]. Thus, we are not aware of any systematic population differences between our groups that could explain the different genotype frequencies. Nevertheless, it has to be pointed out, that the allele frequency of IL-10-1082 G in other control groups varies between 47% and 52% [12,15,18,24] (50% over all 1442 control individuals in these four studies), while it is 41% in our population sample, raising the possibility that for some unknown reason the allele frequency in our population control sample is smaller than in the general German population (which might be more similar to the other control samples). However, the cited control groups came from different populations, thus we would not necessarily expect the allele frequencies to be the same in German individuals. Interestingly, the IL-10-1082 G allele appeared with similar high frequency in

the matched control group that was adjusted for age, gender, and smoking with the majority of the individuals suffering from coronary heart disease. The high frequency of the IL-10-1082 G might be associated with mechanisms that enhance susceptibility to chronic inflammation in general. Therefore, failure to observe significant differences between these two groups may be explained by shared risk factors (i.e. chronic inflammation) that may work as confounders.

In summary, following investigation of differences in the frequency of five cytokine polymorphisms, no association between lung cancer and IL-6, TNF- α and TNF- β polymorphisms was found. However, a functional IL-10 promoter polymorphism, correlated with altered IL-10 expression *in vitro*, appears to be associated with the presence of SCLC. The underlying mechanisms are currently uncertain, but genetically influenced altered responses to inflammatory events may lead to local airway conditions influencing lung cancer susceptibility. A large study is warranted to confirm these findings and to determine their impact on disease pathogenesis.

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