Abstract  We investigated, in a random sample of a German population, the association of polymorphisms in the genes encoding the cytokines interleukin 2 (IL-2), interleukin 4 receptor (IL-4R), interleukin 6 (IL-6), interleukin 10, interferon gamma (IFNG), tumor necrosis factor (TNF) and intercellular adhesion molecule 1 (ICAM-1) with (1) secreted levels of the respective proteins after T-cell stimulation and (2) data on selected diseases obtained from a questionnaire. The scope of this investigation was to further the understanding of the genetic background of allergies and common colds and the observed heterogeneity of many immune responses in the human population. In contrast to previous reports that showed associations of promoter polymorphisms of cytokine genes with the production of the corresponding protein, we did not find associations with protein release after T-cell stimulation in vitro. Among the correlations with the clinical parameters, we observed an increased risk of allergies (odds ratio, OR=4.1; confidence interval, CI: 1.6–10.4), particularly hay fever (OR=5.6, CI: 1.8–17.1) in individuals homozygous for IFNG 13 CA-repeats. In combination with the TNF wildtype, the risk for hay fever increased to OR=8.4 (CI: 2.7–25.6). Furthermore, individuals with a combination of IL2, IL6 and ICAM-1 polymorphisms tended to have higher frequencies of reported common colds than individuals with the alternate genotypes. As these are results of an explorative investigation, these findings require confirmation in material from a different source. If confirmed, these relationships could contribute to a better characterisation of the genetic component of allergies.

Introduction

In the light of increasing evidence of the importance of cytokine gene polymorphisms in inter-individual differences in the immune response, we present the re-analysis of a previous sero-epidemiological study on an extended database and correlate polymorphisms in genes of interest with secreted levels of the respective proteins after T-cell stimulation and selected diseases obtained from a questionnaire. In 1992 and 1993, we collected, from a random sample of a German population, in two waves (1) information on the frequency of common colds and other clinical parameters (e.g. allergies, serious chronic illnesses, etc.) during personal interviews, and (2) blood specimens for serological analysis and T-cell proliferation experiments. Briefly, an inverse correlation was found between the frequency of common colds and the level of soluble CD58 in serum (Becker et al. 1992) and a pattern of increased in vitro T-cell proliferation and cytokine release among individuals with a higher frequency of common colds (Becker et al. 1996).

In this study, we have tested whether the inter-individual variability in secreted cytokines interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 10 (IL-10), interferon gamma (IFNG) and intercellular adhesion molecule 1 (ICAM-1) levels after T-cell stimulation is associated with population heterogeneity attributable to polymorphisms in the respective genes. Therefore, we have determined the polymorphism status of all our previous study participants for whom frozen blood specimens are still available. Since some of these and other cytokine genes and ICAM-1 have been implicated as candidate genes for allergies (interleukin 4 receptor (IL4R), IL10, tumor necrosis factor (TNF) and IFNG; see, for example, Anderson and Cooksen 1999) or are involved in the pathogenesis of common colds (IL2, IL6, TNF, IFNG; see, for example, Ramshaw et al. 1997), we have additionally investigated the associations of these clinical conditions and polymorphisms in the respective genes.
Materials and methods

Subjects

The original project comprised a random sample of 156 men and women of a German population, aged 45–65 years, who were personally interviewed in 1992 and asked for a blood sample from which serum was stored. The entire group was re-contacted in 1993. Altogether 144 individuals participated in the second round and again interview data and blood samples were collected (for details, see Becker et al. 1992, 1996).

This present study is based on data material of 111 subjects for whom frozen peripheral blood mononuclear cells (PBMC) were still available. Accordingly, the data material included the following information: (1) interview data of 1992; (2) interview data of 1993; (3) findings of the serological analysis of 1992 (Becker et al. 1992); (4) findings of the proliferation experiments and serological analysis of 1993 (Becker et al. 1996). Methodologies for proliferation assay, cytokine assay and enzyme-linked immunosorbent assay have been described in detail elsewhere (Becker et al. 1992, 1996).

Genotyping

Genomic DNA from PBMC was extracted by using the QiaAmp Blood kit, in accordance with the provider’s instructions (Qiagen). Polymerase chain reaction (PCR) amplifications were carried out in a volume of 10 µl in a Mastercycler gradient (Eppendorf). Final concentrations of reagents, if not specified otherwise, were: 1×Thermoprime polymerase, 200 µM each dNTPs, 1.5 mM MgCl₂, 0.25 U Thermoprime Polymerase DNA polymerase (all from ABgene), 2.5 µM specific forward primer, 2.5 µM specific reverse primer (for allele-specific PCRs: 1 µM internal control primer mix), and 20–30 ng genomic DNA. Table 1 summarises the investigated polymorphisms, the primers, the references of publications from which the genotyping methods were adapted, the PCR conditions

![Table 1](https://example.com/table1.png)

**Table 1** Primer pairs, conditions, and detection methods used in gene assays based on PCR (f forward, r reverse)

<table>
<thead>
<tr>
<th>Gene and Polymorphism</th>
<th>Primers</th>
<th>PCR conditions</th>
<th>PCR-type</th>
<th>Product Size</th>
<th>Analysis</th>
</tr>
</thead>
</table>
| **IL2** –330 T/G      | 5'-TATTCACATGGTCATGTAGTTC (f)  
5'-TGGATTCACCCGGATTACA (r) (John et al. 1998) | 95°C 60 s,  
30×(95°C 15 s,  
59°C 50 s,  
72°C 40 s) | PCR-RFLP, Mael | 150 bp | 3% Metaphor;  
T: 150 bp;  
G: 124 bp;  
26 bp |
| **IL6** –174 G/C      | 5'-CAGAAGAATCAGATGACTG (f)  
5'-GCTGGGCTCTGAGGAG (r) (Bagli et al. 2000) | 95°C 60 s,  
30×(95°C 15 s,  
63°C 50 s,  
72°C 50 s) | PCR-RFLP, SfAI | 611 bp | 2% Agarose  
C: 367 bp;  
244 bp;  
G: 611 bp |
| **IL10** –1082 G/A    | 5'-CTACTAAGGGTCTTGGGAG (f)  
5'-ACTACTAAGGGTCTTGGGA (f) (Perrey et al. 1999) | 95°C 60 s,  
10×(95°C 15 s,  
65°C 50 s,  
72°C 40 s) | Allele-specific | 258 bp | 2% Agarose |
| **IL10** –819 G/T     | 5'-CCCTTGTACAGGTAGATGAA (f)  
5'-ACCCCTGTACAGGTAGTAAT (f) (Perrey et al. 1999) | 20×(95°C 20 s,  
59°C 50 s,  
72°C 50 s) | Allele-specific | 233 bp |
| **IL4** Q576R A/G     | 5'-TCGGCCCCCAACCTGCTTC (f)  
5'-CTCTGGGACACGACCGCTG (r) (Olavesen et al. 2000) | 95°C 60 s,  
30×(95°C 15 s,  
65°C 50 s,  
72°C 40 s) | PCR-RFLP, DdeI | 150 bp | 3.5% Metaphor;  
G: 150 bp;  
A: 130 bp;  
20 bp |
| **IFNG** intron 1, CA-repeat | 5'-CGTCTCATAATAATATCCAG (f)  
5'-CGAGCTTTAAAAGATAGTT (r) (Pravica et al. 1999) | 95°C 60 s,  
30×(95°C 15 s,  
55°C 50 s,  
72°C 40 s) | VNTR | 121 bp - 124 bp | 3% Metaphor |
| **TNF** –307 G/A      | 5'-ATAGGGTTTTAGGGCCATG (f)  
5'-CTCTGGGTCTTGGCAGT (f)  
5'-TCTCGTTTCTCACCGTCA (r) (Perrey et al. 1999) | 95°C 60 s,  
10×(95°C 15 s,  
65°C 50 s,  
72°C 40 s) | Allele-specific | 184 bp | 2% Agarose |
| **ICAM1** G241R A/G   | 5'-GTGGTCTGTTCCTCGGAC (f)  
5'-GGTGCTGGTCTTCCGGACA (f)  
5'-ACGACATCGCCGCTCCCT (r) (Verity et al. 2000) | 95°C 60 s,  
10×(95°C 25 s,  
70°C 45 s,  
72°C 60 s) | Allele-specific | 929 bp | 1% Agarose |