SHORT PAPER

Association of IL-10 polymorphism with severity of illness in community acquired pneumonia

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Thorax 2003;**58**:154–156

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Revised version received 11 September 2002
Accepted for publication 2 October 2002

Background: The influence of genetic polymorphisms of interleukin (IL)-10, tumour necrosis factor (TNF)-α, and IL-6 gene promoters on severity of systemic inflammatory response syndrome (SIRS) associated with community acquired pneumonia (CAP) was studied.
Methods: Using PCR-RFLP analysis we analysed a –1082G/A single nucleotide polymorphism (SNP) of the anti-inflammatory IL-10 gene, a –308G/A SNP of the pro-inflammatory TNF-α gene and a –174G/C SNP of the IL-6 gene. Illness severity was stratified according to SIRS score, calculated by presence of up to four physiological indices: temperature, white blood cell count, heart rate and respiratory rate (non-SIRS, SIRS 2, SIRS 3, and SIRS 4).
Results: A statistically significant stepwise increase in frequency of the IL-10 G allele, associated with higher expression of the gene, was observed in patients with increasing severity of illness from non-SIRS (n=19) to SIRS 2 (n=17), SIRS 3 (n=33) and SIRS 4 (n=24). This was primarily due to a higher frequency of the GG genotype with increasing severity from non-SIRS through to SIRS 4. IL-10 G allele frequency was also increased in patients who died as a result of CAP (n=11) compared with CAP survivors (n=82) (p=0.01). No association was seen between the TNF-α –308G/A and IL-6 –174G/C SNPs and disease. Additionally, no interaction between all three SNP genotypes and disease severity was observed.
Conclusions: A polymorphism affecting IL-10 expression may influence the severity of illness in patients with CAP.

Community acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide. A significant number of patients with CAP develop a systemic inflammatory response syndrome (SIRS) and sepsis, in which there is an ongoing balance between pro-inflammatory and anti-inflammatory cytokines.

Major pro-inflammatory cytokines include tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6). Inflammatory stimuli in sepsis also induce production of counter-inflammatory cytokines such as IL-10, which inhibits synthesis of pro-inflammatory cytokines including IL-1α and β, IL-6, IL-8, and TNF. We recently reported that circulating IL-10 levels are increased in CAP, are correlated with disease severity, and may be of prognostic significance.[1]

The response of various cytokines to stressful stimuli was recently shown to be partly due to interindividual variation at a genetic level.[2–4] This study investigates single nucleotide polymorphisms (SNPs) in the promoter regions of the IL-10, TNF-α, and IL-6 genes which have shown variability in induction of gene expression: (A) IL-10 -1082G/A,[4] (B) TNF-α –308G/A,[2] and (C) IL-6 –174G/C.[3] We compared the distribution of these SNPs in patients with CAP and healthy age matched controls and correlated them with illness severity in CAP patients measured using the SIRS score.[5]

METHODS

Subjects

We studied 93 CAP patients of mean (SE) age 72 (1) years and 90 controls of mean (SE) age 80 (1) years. Controls were recruited from medical outpatient clinics at Beaumont Hospital and had no history of pneumonia or current infection. Ethical approval was obtained and all subjects consented to participate in the study. CAP was diagnosed using established criteria.

SIRS is manifested by two or more of the following conditions: (1) temperature >38°C or <36°C; (2) heart rate >90 beats/min; (3) respiratory rate >20 breaths/min or $Paco_2$ <4.3 kPa; (4) white blood cell count >12 000/mm^3, <4000 cells/mm^3, or >10% immature forms.[5] Patients were categorised by SIRS score: non-SIRS was defined as having one or none of the criteria, SIRS 2 as having any two, SIRS 3 as having any three, and SIRS 4 as having all four criteria.

SNP detection

DNA was isolated from white blood cells by a standard method. Using polymerase chain reaction (PCR) followed by restriction enzyme digestion (PCR-RFLP), DNAs from patients with CAP and controls were genotyped for the IL-10, TNF-α, and IL-6 SNPs. PCR-RFLP products were visualised using ethidium bromide and agarose gel electrophoresis.

Statistical analysis

All statistical analyses were performed using the Stata Version 7.0 software (StatCorp, Texas, USA). CAP and control populations were tested for conformity to Hardy-Weinberg equilibrium using the χ^2 test between observed and expected numbers. Associations between the SIRS score and SNP were examined using the χ^2 test or Fisher's exact test as appropriate. A non-parametric trend test was used to test for trend across SIRS. Odds ratios were calculated using logistic regression allowing adjustment for additional risk factors. Ordered logistic regression was used to determine interactions between SNPs of all three genetic loci and disease severity. All values are for two tailed tests.

RESULTS

Analysis of 93 patients with CAP and 90 control subjects for the IL-10 –1082G/A, TNF-α –308G/A and IL-6 –174G/C SNPs was performed. Neither group deviated significantly from Hardy-Weinberg equilibrium (p>0.05). No significant differences in allele frequencies for the three SNPs were seen between the two groups.

Table 1 IL-10, TNF-α and IL-6 SNP distribution in patients with CAP grouped according to systemic inflammatory response syndrome (SIRS) score

	Illness severity group			
SNP	Non-SIRS	SIRS 2	SIRS 3	SIRS 4
IL-10 GG*	5 (26%)	3 (18%)	12 (36%)	12 (50%)
IL-10 GA	7 (37%)	10 (59%)	12 (36%)	9 (38%)
IL-10 AA	7 (37%)	4 (24%)	9 (27%)	3 (13%)
IL-10 G allele**	17 (45%)	16 (47%)	36 (55%)	33 (69%)
TNF-α GG	10 (53%)	7 (41%)	20 (61%)	14 (58%)
TNF-α GA	9 (47%)	9 (53%)	11 (33%)	10 (42%)
TNF-α AA	0 (0)	1 (6%)	2 (6%)	0 (0)
TNF-α A allele	9 (24%)	11 (32%)	15 (23%)	10 (21%)
IL-6 GG	3 (16%)	6 (35%)	9 (27%)	7 (29%)
IL-6 GC	12 (63%)	8 (47%)	19 (58%)	11 (46%)
IL-6 CC	4 (21%)	3 (18%)	5 (15%)	6 (25%)
IL-6 G allele	18 (47%)	20 (59%)	37 (56%)	25 (52%)

SNP=single nucleotide polymorphism.
*p<0.05, non-parametric test for trend; **p=0.02, non-parametric test for trend.

Table 1 shows the genotype and allele distributions of the three SNPs in patients with CAP grouped according to SIRS score. The IL-10 –1082 G allele, which predicts a higher level of expression of the gene, was observed with significantly greater frequency in patients with increasing illness severity, from non-SIRS (45%, chromosome n=38) to SIRS 2 (47%, chromosome n=34), SIRS 3 (55%, chromosome n=66) and SIRS 4 (69%, chromosome n=48) (p=0.02). GG homozygote frequency increased significantly with increasing SIRS score (p<0.05), suggesting a recessive mode of action of the IL-10 variant. These findings remained unchanged after adjustment for age, sex, smoking status, and presence of chronic obstructive pulmonary disease (COPD).

Eleven (12%) of the 93 patients with CAP died. A significantly higher G allele frequency (82%) was observed in the patients who died compared with the remaining CAP patients (51%, OR=4.3, 95% CI 1.39 to 13.22, p=0.01) and the control population (55%, OR=3.7, 95% CI 1.20 to 11.32, p=0.02). These findings remained significant when adjusted for illness severity, COPD, smoking, age, and sex. However, this observation must be interpreted with caution as the small sample size (n=93) results in wide confidence intervals.

Forty (43%) of the 93 patients with CAP were current smokers and 25 (27%) had COPD. Illness severity was influenced by presence of COPD, with significantly more COPD patients manifesting SIRS scores of 3–4 (p<0.05). In addition, there was a trend towards more severe illness in current smokers, although this finding was not significant. Twelve patients (13%) had bacteraemia as assessed by positive blood culture. No association was observed between patients with bacteraemia and illness severity or IL-10 genotype.

In contrast with the IL10 –1082G/A SNP, the TNF-α –308G/A and IL6 –174G/C SNPs showed no association with severity of illness in patients with CAP (table 1). There was also no association between these two SNPs and bacteraemia or death subsequent to CAP.

DISCUSSION

To our knowledge this is the first study to show an association between an IL-10 promoter polymorphism and severity of SIRS in patients with CAP. Use of the SIRS criteria as an index of illness severity is validated by our observance of a significant trend towards greater numbers of COPD patients with increasing SIRS scores from non-SIRS up to SIRS 4 (p<0.05). The SIRS score was also evaluated by a large prospective study conducted by the University of Iowa.[6] Of 3708 patients admitted to the ICU, 2527 met the SIRS criteria. A progressive increase in mortality rate was observed with an increasing number of SIRS criteria, from 3% (non-SIRS) up to 17% (SIRS 4).

Several studies have shown a genetic basis for variation in IL-10 production. This mutation falls within a region of the IL-10 promoter which has been implicated in modulation of IL-10 gene activity; the SNP is located within an ETS-like transcription factor recognition site.[4] However, it is possible that IL-10 –1082G/A may not be functional in itself, but may be in linkage disequilibrium with other functional mutations. Several mutations occurring in this region have been associated with various diseases, including systemic lupus erythematosus.[7]

Our finding is in accordance with the observation that levels of IL-10 are higher in patients with sepsis and septic shock. Furthermore, non-survivors of sepsis have persistently raised levels of IL-10, while survivors show decreasing IL-10 levels over time.[8] We previously reported that circulating IL-10 levels are raised in CAP and directly correlate with disease severity.[1] Other studies have shown that in vitro stimulation of monocytes from septic patients resulted in decreased production of TNF-α in response to endotoxin, while IL-10 levels were persistently high. Incubation of normal monocytes with septic serum elicited a similar dampened TNF-α response which was abrogated by co-incubation with anti-IL-10 antibodies.[9]

In addition to the IL-10 –1082G/A SNP, we analysed the TNF-α–308G/A and IL-6 –174G/C SNPs. No association was seen between these SNPs and disease severity (table 1). No interaction between the genotypes of these three SNPs in relation to their combined influence on illness severity was observed; however, a larger population size is required to elucidate fully any possible interactions.

In conclusion, we have shown that a polymorphism of the IL-10 gene (–1082G/A) affecting the level of expression of the cytokine influences illness severity in patients with CAP. This polymorphism should therefore be added to the spectrum of immunogenetic factors involved in the systemic response to infection in CAP.

ACKNOWLEDGEMENT

This work was supported by the Health Research Board of Ireland and the Royal College of Surgeons in Ireland.

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