Short Report

Differential association of tumour necrosis factor-α single nucleotide polymorphism (–308) with tuberculosis and bronchial asthma

VIJAY KUMAR, RAJIV KHOSLA, VIKAS GUPTA, B. C. SARIN, P. K. SEHAJPAL

ABSTRACT

Background. Tumour necrosis factor (TNF)-α is a pleiotropic, pro-inflammatory cytokine of 17 kDa, whose gene is localized on the short arm of chromosome 6. It has a G-308A polymorphism in the promoter region, which is known to be associated with its differential production; the A allele being the high producer. The circulating level of TNF-α is under genetic control and implicated in the pathophysiology of asthma and tuberculosis. Since raised levels of TNF-α have been found in asthma and tuberculosis, we looked for the association of TNF-α G-308A polymorphism in patients with these diseases.

Methods. A total of 300 blood samples from patients (155 with asthma, 145 tuberculosis) and 211 normal healthy controls were collected. The G-308A polymorphism was studied using amplification refractory mutation system analysis.

Results. The distribution of G/A alleles in the two patient groups when compared with normal controls revealed a statistically significant association with asthma (p=0.016) but not with tuberculosis (p=0.178).

Conclusion. The data support the common variant common disease hypothesis, which emphasizes that common genetic variations may participate as critical players in inciting common diseases.


INTRODUCTION

Tumour necrosis factor-alpha (TNF-α) is a non-glycosylated protein of 17 kDa with a polypeptide chain that is 157 amino acid long. This pleiotropic and pro-inflammatory cytokine is produced only in response to discrete stimuli by lymphocytes, monocytes, macrophages and other cells. The gene for this cytokine has a G–A single nucleotide polymorphism (SNP) at the –308 position in the promoter region and the presence of the A allele has been associated with high TNF-α production.2

The TNF-α cytokine is involved in the pathophysiology of bronchial asthma and tuberculosis (TB). Raised levels of TNF-α have been observed in the airways and bronchoalveolar lavage (BAL) fluid of symptomatic patients with asthma,1 and in patients with advanced TB.3 Previous studies have reported an association between asthma and the A allele of the G-308A polymorphism,4 while other investigations either failed to find any association5 or observed an association with the G allele.6 Likewise, the association of this allele has also been evaluated in patients with TB; some studies reported a positive association of TB with the A allele,7 while others did not show any association.8 We, therefore, investigated the association between allelic variations responsible for high or low production of this cytokine with susceptibility or resistance to asthma and TB in a Punjabi population.

METHODS

Study population
A total of 511 subjects were studied—145 patients with TB, 155 patients with asthma and 211 normal healthy controls. The diagnosis of asthma and TB was made by a physician. Informed consent was taken from all the subjects participating in the study. Information related to name, age, sex, endogamous group and family history was also collected. Venous blood samples from the subjects were collected in sterile storage vials containing EDTA as an anticoagulant. Samples were collected from patients by visiting the Department of TB and Chest, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar; Rai Bahadur Sir Gujarmal and Kesradevi Tuberculosis Sanatorium (TB and Chest Hospital), Amritsar; TB and Chest Wing, Civil Hospital, Gurdaspur and Civil Hospital, Batala. Normal healthy controls were from Amritsar or nearby areas with no history of TB, asthma or other allergic diseases. Their age, sex and endogamous group was matched with that of the patients with TB and asthma.

DNA extraction and genotyping of G-308A TNF-α polymorphism
The modified inorganic method was used for extraction of DNA from the blood specimens.9 Extracted DNA was then quantified using UV-spectrophotometry. The G-308A TNF-α polymorphism was genotyped using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method developed in our laboratory.10 The amplified PCR products were electrophoresed on a 2% gel and visualized after ethidium bromide staining. DNA samples with known –308 genotypes were amplified as controls with each batch of amplification. Presence or absence of the G or A allele was indicated by the occurrence of a specific PCR product of 273 bp. The validity of the PCR products was also confirmed by automated DNA sequencing (data not presented).

Statistical analysis
Genotypic percentage and allele frequencies were measured by direct count. Allelic frequencies in patients and normal healthy populations were compared using 2×2 contingency tables and chi-square statistics. Statistical significance was defined at the standard 5% level. All the study groups were checked for their fit to the
RESULTS

The demographic features of the control and patient groups are shown in Table I. Figure 1 shows a representative agarose gel of genotypes of the G-308A TNF-α polymorphism among the study population. A sample was designated to have the GG or AA genotype if it gave a 273 bp product with AP1 and CP, or AP2 and CP primers, respectively. For the GA genotype, PCR products were amplified with both sets of primers (Fig. 1, panel A and B, lanes 3 and 5). There is a 3’ end mismatch of primer AP1 with the DNA strand of the A allele, and of AP2 with the G allele. Owing to this property of ARMS primers, control DNA with the GG genotype behaved as a positive control for the G allele, and DNA from an AA genotype as a negative control and vice versa. The genotypic distribution of G-308A TNF-α SNP was found to be in the Hardy–Weinberg equilibrium with a non-significant chi-square value of 1.25 (after applying Yates correction), 0.01 and 0.12 for patients with TB, asthma and normal healthy controls, respectively. All the three groups had a high percentage of GG homozygotes—normal controls had the highest (84.4%) and patients with asthma the lowest (74.8%) percentage. None of the patients with TB had the AA genotype, while normal healthy controls and patients with asthma had a minimum share of 0.47 and 1.94 for this genotype (Table II). When the frequency of the G and A alleles of normal healthy controls was compared with that of patients with TB and asthma, only the latter group showed a statistically significant difference ($\chi^2=5.79; p=0.016$).

DISCUSSION

TNF-α has been strongly implicated in the aetiology of two commonly occurring respiratory diseases with very different pathophysiology—asthma and TB. Asthma is a complex genetic disorder of the lungs accompanied by breathlessness, chest congestion and wheezing, while TB is a granulomatous pulmonary or extrapulmonary infectious disease caused by Mycobacterium tuberculosis. In TB, TNF-α has both protective and pathological effects; it plays a key role in granuloma formation and mycobacterial elimination, while its excessive production may cause tissue necrosis, fever and cachexia.11 It has been reported that granuloma formation in mice infected with Mycobacterium bovis Bacille Calmette–Guerin (BCG) coincides with local TNF-α synthesis, and injection of anti-TNF-α antibody interferes with granuloma formation and elimination of mycobacteria.12 The pathological effects of TNF-α are supported by a study which documented that peripheral blood mononuclear cells (PBMCs) from TB patients with cachexia and high grade fever produce more TNF-α than from patients without these findings.13,14 Thus, the production of TNF-α is important for antimycobacterial immune defence and the genetic make-up of the host may play a role in regulating the production of this cytokine. Various studies report conflicting results of an association of G-308A TNF-α-SNP with TB. In the Bashkorst population, the TNF-α A allele has been shown to be a risk factor for TB,1 and a similar association has been reported in the Sicilian population.15 We failed to find any significant association of the –308 A allele of TNF-α with TB, an observation supported by an earlier study.3

TNF-α has also been implicated as a major candidate cytokine in the pathogenesis of asthma.1 It is involved in both airway inflammation and increased airway responsiveness.4 In the early phase of bronchoconstriction, its presence leads to smooth muscle contraction,16 while in the late phase TNF-α upregulates adhesion molecules17 and allows influx and activation of inflammatory cells.18 Given the potential role of TNF-α in the pathophysiology of asthma, it has been suggested that neutralizing the deleterious effect of TNF-α by drugs may be useful in the management of chronic severe asthma.1 Studies in different populations to unravel the association of G-308A polymorphism with asthma revealed varying results.4,6,8 Our study shows an association of asthma with the A allele of G-308A TNF-α polymorphism, which is in agreement with a majority of studies that show an increase in frequency of the A allele in patients with asthma.4

Available reports suggest that the genetic element plays a significant but differential role in the susceptibility to asthma (heritability quotient [$\alpha$] >0.6)19 and TB ($\alpha$=0.2).3,20 The observation of a positive association of G-308A TNF-α polymorphism with asthma and not with TB thus supports the variable involvement of

<table>
<thead>
<tr>
<th>Item</th>
<th>Healthy controls</th>
<th>Patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender</td>
<td>Tuberculosis</td>
<td>Asthma</td>
</tr>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Mean age (in years)</td>
</tr>
<tr>
<td></td>
<td>95</td>
<td>116</td>
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<td></td>
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<td>33.8</td>
</tr>
<tr>
<td></td>
<td>260</td>
<td>251</td>
<td>33.5</td>
</tr>
</tbody>
</table>

Table I. Distribution of normal healthy controls and patient populations

![Agarose gel photograph showing amplified PCR products of G-308A TNF-α polymorphism. Panels A and B depict the DNA samples amplified with G and A allele-specific primer pairs, respectively. A 100 bp DNA ladder (Lane M) was included as a control marker for sizing the PCR products. Lanes 1 and 2 correspond to control samples of the GG and AA genotypes, respectively. Lanes 3 to 8 show random amplified samples. The genotype obtained for these samples is given at the bottom of the respective lane.](image)

Fig 1.

Table II. Distribution of genotype and allele frequencies of TNF-α G-308A polymorphism

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>TNF-α genotypes (%)</th>
<th>Allele frequency (G/A)</th>
<th>Chi-square, p value (df=1)</th>
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</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>211</td>
<td>GG 178 (84.4) GA 32 (15.2) AA 1 (0.5)</td>
<td>0.92/0.08</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>145</td>
<td>GG 111 (77.9) GA 32 (22.1) AA 2 (1.4)</td>
<td>0.89/0.11</td>
<td>1.81, 0.178</td>
</tr>
<tr>
<td>Asthma</td>
<td>155</td>
<td>GG 116 (74.8) GA 36 (23.2) AA 3 (1.9)</td>
<td>0.86/0.14</td>
<td>5.79, 0.016</td>
</tr>
</tbody>
</table>
genetic elements in the aetiology of the two diseases. The disease with a high heritability quotient showed an association with genetic variants of the −308 site, while the incidence of the susceptibility allele in TB was not much different from that of controls. This observation also lends credence to the fact that association of the A allele at the −308 site with asthma is not a chance occurrence as the observation also lends credence to the fact that association of the A allele in TB was not much different from that of controls. This suggests that common genetic variations could play a major role in the pathophysiology of common diseases.

It can thus be concluded that the A allele at the −308 site of TNF-α promoter is a risk factor for asthma but not for TB in a Punjabi Indian population.

ACKNOWLEDGEMENT
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REFERENCES
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