Association of tumor necrosis factor polymorphisms with asthma and serum total IgE

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Tumor necrosis factors (TNF; TNFA and TNFB) are major pro-inflammatory cytokines that are thought to be important in the pathogenesis of asthma. However, the functions of genetic polymorphisms in these cytokines have not been thoroughly examined in the context of asthma pathology. In an effort to discover polymorphism(s) in genes whose variant(s) have been implicated in asthma phenotypes, we examined the genetic effects of TNF (TNFA and TNFB) polymorphisms on asthma and total serum IgE level. Seven common single-nucleotide polymorphisms (SNP) in TNF genes were genotyped in a Korean asthma cohort (asthmatics n=550, normal controls n=171). Six common haplotypes could be constructed in the TNF gene cluster due to very strong LD between TNFA and TNFB, located 13 kb apart on chromosome 6p21. One SNP (TNFA-308G > A) showed a significant association with the risk of asthma (P=0.0004). The frequency of TNFA-308A allele-containing genotype in asthmatics (9.8%) was much lower than that in normal controls (22.9%). The protective effects of this polymorphism on asthma were also evident in separated subgroups by atopic status (P=0.05 in non-atopic subjects and P=0.003 in atopic subjects). The most common haplotype of the TNF gene (TNF-ht1[GGTCCGG]) was associated with total serum IgE (immunoglobulin E) levels in asthma patients, especially in non-atopic patients (P=0.004). Genetic variants of TNF might be involved in development of asthma and total serum IgE level in bronchial asthma patients. The results of this study could be helpful to understand the function of important TNF genes in asthma and IgE production.

INTRODUCTION

Tumor necrosis factor alpha (TNFA; MIM 191160) and tumor necrosis factor beta (TNFB, alternative names lymphotoxin alpha, LTA; MIM 153440), on chromosome 6p21, share a common receptor on tumor cells (1). Tumor necrosis factors are cytokines usually associated with cell-mediated immunological responses. TNFA production was initially described in macrophages and monocytes (2). Since then, other cells of the hematopoietic lineage have been shown to have an ability to generate this cytokine de novo. The sensitized mast cell is known to store a number of cytokines, including TNFA, within its granales and to release them upon antigen presentation, making it a pivotal cell in the allergic asthmatic response to allergens (3,4). In addition, other cells in the airway have the ability to generate TNFA: eosinophils (5), epithelial cells (6) and airway macrophages (7).

Asthma is recognized as a T-helper type 2 (Th2) disease with a particular profile of cytokine release, including interleukin 4 (IL4) and interleukin 5 (IL5). However, increasing evidence...
indicates that other cytokines, which were classically considered to belong to Th1-type profiles, are also associated with inflammatory response that characterizes human asthma. One such mediator is TNFA, which has been implicated in asthmatic inflammation by a broad series of in vitro, ex vivo, in vivo and genetic studies. Mast cells have been shown to generate a range of mediators including cytokines, and recent work has documented that the mast cell granule itself contains preformed TNFA (4,8). This indicates that the cytokine is co-released with the more extensively characterized preformed mast cell granule mediators, such as histamine, chymase and tryptase. Mast cell mediators are classically associated with immediate bronchospasm, and now TNFA has also been shown to induce airway hyper-reactivity (9,10).

A number of independent studies have indicated an association of the TNFA-308G>A promoter polymorphism with the risk of asthma (11,12). Although some studies have shown no significant association (13), the TNFB+252A>G intronic polymorphism was reported to be associated with asthma (14). In the studies reported so far, there are still controversies over the effects of TNF polymorphisms on asthma. In the present work, we performed a genetic association study in a Korean asthma cohort with all published polymorphisms (nine single-nucleotide polymorphisms, SNPs) and haplotypes in TNF genes (TNFA and TNFB).

RESULTS

In initial genotyping of TNF polymorphisms, two SNPs (TNFA-376 G>A and TNFA-163 G>A), which had been reported in other ethnic groups (15), were not polymorphic in the Korean population (at least in the first 340 individuals genotyped). The physical distance between TNFA and TNFB is about 13 kb on chromosome 6p21 (Fig. 1A), and all SNPs in both TNF genes were in very strong linkage disequilibrium (LD; Fig. 1C); graphical LDs among TNF SNPs are available at www.snp-genetics.com/user/news_list.asp. Six common haplotypes (frequency > 0.04) could be constructed along with TNFA and TNFB, which account for more than 96% of the distribution (Fig. 1B). The frequencies of those seven SNPs are shown in Table 1. Among six haplotypes, TNF-ht3[GACACGG], TNF-ht4[CATCTGG], TNF-ht5[GGTCCAG] and TNF-ht6[GACCCGA] were not analyzed because they are almost (>98%) equivalent to TNFA-863C>A, TNFA-857C>T, TNFA-308G>A and TNFA-238G>A, respectively.

By logistic regression analyses controlling age, sex and smoking status, one SNP (TNFA-308G>A) showed a very significant association with the risk of asthma (P = 0.0007; Table 1). The frequency of TNFA-308A containing genotype in asthmatics (9.8%) was much lower than that in normal controls (22.9%; P = 0.0004; Fig. 2). The protective effects of this polymorphism on asthma were also evident in separated subgroups by atopic status (P = 0.05, OR = 0.47 in non-atopic subjects and P = 0.003, OR = 0.33 in atopic subjects; Fig. 2). The frequencies of this TNFA-308A-containing genotype in the general Korean population [our Korean osteoporosis (n = 560) and hepatitis (n = 1750) cohorts; data not shown] were about intermediate (15%) between asthmatic and normal controls, as expected.

We also tested the genetic effects of TNF polymorphisms on peripheral blood eosinophil percentage (%), PC20 methacholine initial and total serum IgE levels. No significant associations were detected with peripheral blood eosinophil percentage (%), PC20 methacholine initial (data not shown). In analyses of the association of TNF polymorphisms with total serum IgE levels, no significant associations were detected with single SNPs. But further analyses by haplotypes, the most common TNF haplotype, TNF-ht1[GGTCCGG], showed an association with serum total IgE levels by a co-dominant model (P = 0.05). Patients with TNF-ht1-bearing genotype had higher levels of serum log [total IgE (IU/ml)] (2.30) than the other group (2.19; P = 0.02). The association of this haplotype was stronger in non-atopic patients (P = 0.004) than in atopic patients (P = 0.40; Table 3).

DISCUSSION

TNFA has been demonstrated to cause an increase in airway hyper-reactivity (10). This increased airway smooth muscle responsiveness may be by recruitment of inflammatory cells (16), by direct effects upon the airway smooth muscle (17), or by generating a cascade of inflammatory responses with the release of mediators, including increased sensitization with elevated histamine release (18). It is of particular interest that TNFA can also cause an increase in smooth muscle eosinophil generation and secretion (along with IL1B) from the human airway smooth muscle, with eosinophils being clearly demonstrated within the asthmatic airway muscle (19). TNFA may have additional indirect remodeling activity because it is able to induce eosinophils to release the matrix metalloproteinase (MMP) (20), and to stimulate glycosaminoglycan synthesis in human lung fibroblasts (21). These data indicate the potential variety of roles that TNFA can play in asthma by increasing smooth muscle responsiveness, activating myofibroblasts and fibroblasts, and by regulating the activity of eosinophils via IL-4 and IL-5. While the presence of the eosinophil is recognized as the hallmark of asthmatic inflammation, evidence for TNFA-mediated neutrophil involvement in the pathogenesis of asthma is increasing and is supported by the fact that neutrophils from asthmatic subjects show increased migratory responses (22,23), increased superoxide generation, and that their secretory products increase bronchial ring contractility (24–26).

A number of independent studies of TNF polymorphisms have indicated an association with asthma, especially focused on TNFA-308G>A, which is particularly interesting because of its known involvement in differential transcriptional activator (27), elevated plasma TNFA levels and higher amounts of TNF on stimulation in vivo and ex vivo (28). It has been reported to be associated with increased risk of asthma (12,14,29–31), atopy (13) and bronchial hyperreactivity (32). On the other hand, several negative associations of this TNFA-308G>A with asthma and/or asthma-related phenotypes have also been published (33–36). In spite of several studies performed, the effects of TNFA-308G>A on the risk of asthma (and related phenotypes) are still controversial. The frequencies of minor alleles (TNFA-308A) were higher in asthmatics than in those in controls from several studies (12,30,31). However, opposite results (lower frequencies in asthmatics) were also reported in one study (14).
Our results presented here, which are concordant with the results of Australian Caucasians studied (14), strongly suggest that TNFA-308A has protective effects on asthma based on the high significance ($P = 0.05–0.0004$) and consistent protective effects through analyses in sub-separated groups by status of atopy (Fig. 2).

Although it is hard to decipher the discrepancies among studies on the effect of this important TNF variant on asthma, the low sample sizes (cases or controls) and/or marginal significances in those studies could be plausible explanations, i.e. 293 subjects (43 non-asthmatics versus 251 asthmatics, $P = 0.02$) (30), 400 subjects (44 non-asthmatics versus 312 asthmatics, $P = 0.001$), 436 subjects (416 non-asthmatics versus 20 asthmatics, $P = 0.03$), 481 subjects (275 non-asthmatics versus six asthmatics, $P = 0.015$) (31) and 511 subjects (236 asthma cases versus 275 non-asthmatic controls, $P = 0.04$) (29).

Two previous papers studied the effects of TNF haplotypes on asthma. However, one study (37) had not included TNF A-308G $\rightarrow$ A and TNFB intronic polymorphisms. They reported that, by transmission disequilibrium test, TNF A-857C alleles were associated with asthma in the analysis of 52 families (36 transmitted versus 16 not transmitted, $P = 0.006$), and TNFA-1031T-863C-857C haplotype was also transmitted more often to asthmatic offspring in 74 families (53 transmitted versus 21 not transmitted; $P = 0.0002$). TNFA-1031T-863C-857C haplotype in their study corresponds to the combined haplotype (ht 1, 2 and 5) in our study. Although positive associations were reproduced ($P = 0.03$), the direction of effect was opposite (OR $= 0.47$, 95% confidential interval; 0.24–0.93) in the dominant model (ht1, ht2 and ht5 containing individuals versus others) in this study (data not shown). The other study (12) genotyped only two SNPs: TNF A-308G $\rightarrow$ A and TNFB $+252A \rightarrow G$. The haplotype (TNF-308A$-252G$, constructed by two SNPs), which was significantly associated with asthma, was an exactly equivalent model with TNF A-308G $\rightarrow$ A.

The relationship of TNF polymorphisms and total serum IgE has not been studied thoroughly. The homozygous minor allele for TNFB $+252A \rightarrow G$ has been associated with increased IgE levels in females (38,39). In this study, similar increases of IgE levels were observed in TNF-ht1[GGTCCGG]-bearing individuals (Table 3), which could be compatible with previous results, because TNF-ht1[GGTCCGG] has tagging more than 90% of TNF-ht1[GGTCCGG] (Fig. 1B). The significance of the
association of TNF-ht1[GGTCCGG] with increased levels of total serum IgE was more apparent in non-atopic asthmatics ($P = 0.004$). Although significant increases were not detected, the same direction of increased IgE level in atopic asthmatics was also observed.

Correction of $P$-values obtained from multiple tests of SNPs in single gene (or single LD block) would be a complicated issue when considering the following facts: (1) generally, most of markers in single genes are in strong LD and not independent (and not completely dependent either); (2) haplotypes constructed by combinations of SNPs are not independent (and not completely dependent either); generally, most of markers in single genes are in strong LD. Nevertheless, it could be speculated, by the results of our study, that individuals with higher TNF-A secretion. TNF-A has been demonstrated to cause an increase in airway hyper-reactivity (10) by increased airway smooth muscle responsiveness (16), and/or by generating a cascade of inflammatory responses with the release of mediators (18). Nevertheless, it could be speculated, by the results of our study, that individuals with higher TNF-A production ability by TNFA-308A variant might be protective to development of asthma. Therefore it could be suggested that TNF might have the opposite effect on onset and progress of asthma, i.e. a protective effect on the risk of asthma development and detrimental effect on progress of asthma.

It has been demonstrated that bronchial epithelial cells can synthesize and release a wide range of mediators both spontaneously and following stimulation. These include GM-CSF, TNFA, IL6, IL8 and RANTES. TNFA-308A variant has been shown to be associated with elevated TNFA transcriptional activity (27,40), which may lead to increased TNFA secretion. TNFA has been demonstrated to cause an increase in airway hyper-reactivity (10) by increased airway smooth muscle responsiveness (16), and/or by generating a cascade of inflammatory responses with the release of mediators (18). Nevertheless, it could be speculated, by the results of our study, that individuals with higher TNFA producing ability by TNFA-308A variant might be protective to development of asthma. Therefore it could be suggested that TNF might have the opposite effect on onset and progress of asthma, i.e. a protective effect on the risk of asthma development and detrimental effect on progress of asthma. Further functional and mechanistic studies should be performed to confirm the pathway speculated by this study.

In summary, seven TNF polymorphisms were genotyped in a Korean asthma cohort, and six common haplotypes (frequency $>0.04$) were constructed, along with TNFA and TNFB. Statistical analyses revealed that one single nucleotide polymorphism (TNFA-308G>A) showed a strong association with the risk of asthma, and that the most common TNF haplotype,
was weakly associated with increased serum total IgE levels.

**Materials and Methods**

**Subjects**
Subjects were recruited from the Asthma Genome Research Center that consists of four tertiary hospitals in Korea (Soonchunhyang University Hospitals). Ethical approval was obtained from the institutional review board of each hospital. All patients had the clinical symptoms and the physical examination compatible with asthma. Each patient showed airway reversibility as documented by an inhalant bronchodilator-induced improvement of more than 15% of forced expiratory volume in one second (FEV1) and/or an airway hyperreactivity of less than 10 mg/ml of methacholine. Normal subjects were recruited from spouses of the patients and the general population who answered negatively to a screening questionnaire for respiratory symptoms and had FEV1 greater than 75% predicted, the provocation concentration to cause a fall in the FEV1 of 20% (PC20) by methacholine greater than 10 mg/ml, and normal finding on a simple chest radiogram. Total IgE and specific IgE to *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dp) were measured using the CAP system (Pharmacia Diagnostics, Sweden). Atopy was defined by the presence of immediate skin reaction (histamine of greater than 3 mm in diameter) to one or more of 24 common aeroallergens [dust mites (*D. farinae* and *D. pteronyssinus*), cat fur, dog fur, cockroaches, grass, tree pollens, and ragweed], and/or by the positive response (score $\geq 1$) of specific IgE to Dp or Df. The clinical parameters are summarized in Table 4.

**Genotyping**
Nine single nucleotide polymorphisms (SNPs) in TNF genes (*TNFA-1031T>C*, *TNFA-863C>A*, *TNFA-857T>C*, *TNFA-
Table 3. Regression analyses of Log [total IgE] as functions of TNF-ht1[GGTCCGG] among bronchial asthma patients.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Genotype</th>
<th>N</th>
<th>Means</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>-/-</td>
<td>195</td>
<td>2.19</td>
<td>0.63</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>-/ht1 or ht1/h1</td>
<td>326</td>
<td>2.30</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Atopic BA</td>
<td>-/-</td>
<td>137</td>
<td>2.45</td>
<td>0.50</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>-/ht1 or ht1/h1</td>
<td>231</td>
<td>2.51</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Non-atopic BA</td>
<td>-/-</td>
<td>58</td>
<td>1.56</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-/ht1 or ht1/h1</td>
<td>95</td>
<td>1.79</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

TNF-ht1 containing genotype distribution, means, standard deviations (SD) of log [total IgE] and P-values for regression analyses of TNF-ht1 dominant models are shown.

Table 4. Clinical profiles of the study subjects

<table>
<thead>
<tr>
<th>Clinical profiles</th>
<th>Normal controls</th>
<th>Asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>171</td>
<td>550</td>
</tr>
<tr>
<td>Age [mean (range)]</td>
<td>28.7 (7–75)</td>
<td>35.2 (7–80)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>85/86</td>
<td>248/302</td>
</tr>
<tr>
<td>Current smoker</td>
<td>30.8%</td>
<td>19.6%</td>
</tr>
<tr>
<td>FVC1%, predicted (±SD)</td>
<td>89.0 (±1.7)</td>
<td>87.9 ± 0.7</td>
</tr>
<tr>
<td>FEV1%, predicted (±SD)</td>
<td>93.7 (±1.2)</td>
<td>83.0 ± 0.9*</td>
</tr>
<tr>
<td>PC_{20}, methacholine (mg/mL (±SD))</td>
<td>24.0 (±1.4)</td>
<td>28.0 ± 0.7*</td>
</tr>
<tr>
<td>Total IgE (IU/mL (±SD))</td>
<td>212.0 (±41.7)</td>
<td>537.0 ± 52.4*</td>
</tr>
<tr>
<td>Skin test positive (% (no.))</td>
<td>32 (18.7%)</td>
<td>318 (57.8%)*</td>
</tr>
<tr>
<td>Positive rate of specific IgE (Df)</td>
<td>32.2%</td>
<td>45.5%*</td>
</tr>
<tr>
<td>Positive rate of specific IgE (Dp)</td>
<td>36.3%</td>
<td>49.8%*</td>
</tr>
<tr>
<td>Positive rate of atopy</td>
<td>46.7%</td>
<td>66.6%*</td>
</tr>
</tbody>
</table>

*P-value < 0.001 for difference between asthmatics and normal controls.

376G>A, TNFA-308G>A, TNFA-238G>A, TNFB-252A>G and TNFB+318G>C were genotyped by single base extension method as described previously (41). Information about the primers is available at www.snp-genetics.com/user/news_list.asp.

Statistics

We examined a widely used measure of linkage disequilibrium between all pairs of biallelic loci, Lewontin’s D’ ([D’]) (42). Haplotypes of each individual were inferred using the algorithm developed by Stephens et al. (43), which (PHASE) uses a Bayesian approach incorporating a priori expectations of haplotypic structure from population genetic and coalescent theory. Genetic effects of inferred haplotypes were analyzed in same way as SNPs. Phase probabilities of each site were calculated for each individual by this software. Individuals with phase probabilities less than 97% were excluded in analysis. Genotype distribution of TNF SNPs and haplotypes among bronchial asthmatics and normal subjects were analyzed with logistic regression models controlling age (continuous value), sex (male = 0, female = 1) and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as co-variables. The log-transformed total serum IgE levels in asthmatics were analyzed by multiple regression, controlling age and sex as co-variables.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGMENTS

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