Asthma is a multifactorial disorder and both genetic and environmental factors contribute to its development. The present study explored cytokines interleukin (IL)-17A and IL17F levels as usable parameters for the diagnosis of asthmatics Saudi patients. Blood samples were collected from 100 asthma patients and 100 matched controls. The transcript mRNA levels in whole blood were determined by real-time reverse-transcription polymerase chain reaction. Expression studies showed that levels of IL17A and IL17F were significantly higher in asthma patients compared to controls [IL17A: 1.112 (2.088) vs 0.938 (1.363)]; IL17F: 0.707 (1.33) vs 0.667 (0.590). The mRNA transcripts of IL17A and IL17F were positively and significantly correlated in all subjects examined in this study; controls as well as asthma patients (r = 0.455, P < 0.01 for controls and r = 0.644, P < 0.01 for patients). These findings suggest that asthma is characterized by an elevation of cytokines IL17A and IL17F and the measurement of their expression can be a valuable parameter for the diagnosis of asthma.

Key words: Asthma, interleukin-17F (IL17F), interleukin-17A (IL17A), gene expression, real time-polymerase chain reaction (RT-PCR), Saudi Arabia.

INTRODUCTION

Asthma is a frequently encountered chronic airway inflammatory disorder, characterized by mucus production and airway hyperresponsiveness (AHR) with airway remodeling. The disease affects children and young adults at a higher frequency and is a cause of increased morbidity and mortality (Barrett, 2008; Eijkemans et al., 2008). Asthma is T-cell driven disorder and both T helper (Th1 and Th2) lymphocytes play an important role in its pathophysiology. A third subset of helper T cells, named Th17 cells, which exhibits functions distinct from Th1 and Th2 cells and preferentially produces interleukin IL17, have been shown to plays a critical role in the pathophysiology of asthma (Benchetrit et al., 2002; Ivanov et al., 2007; Evans and Koo, 2009). Th17 cells have been considered as one of the important pathogenic cells associated with pathogenesis and development

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Abbreviations: AHR, Airway hyperresponsiveness; SNPs, single nucleotide polymorphism; BAL, bronchoalveolar lavage fluid.
of many autoimmune and inflammatory diseases (Annunziato et al., 2009; Louten et al., 2009; Pernis, 2009). There is emerging evidence that an increase in IL17 level is closely related to a variety of inflammatory conditions including rheumatoid arthritis, inflammatory bowel diseases and psoriasis (Starnes et al., 2002; Chen and O’Shea, 2008).

In asthmatic patients, IL17 expression has been shown to increase in sputum, lung, bronchoalveolar lavage fluids and peripheral blood (Rouvier et al., 1993; Yao et al., 1995; Molet et al., 2001; Barczyk et al., 2003; Hellings et al., 2003). IL17 was first identified by Rouvier et al. (1993), and was shown to be mainly expressed in activated CD4+ T cells, but not in the resting state. T cells, neutrophils, eosinophils, and CD8+ T cells are all good sources of IL17A (Kawaguchi et al., 2001; Borish and Steinke, 2003). The functional roles of the different members of IL17 family differ toward asthma or asthma-related phenotypes (Wang and Liu, 2008). In asthmatic fibroblasts, IL17A enhances the production of IL-6 and IL11, whereas IL17F induces the expression of TGF-β and IL11 (Molet et al., 2001). Chakir et al. (2003) reported that IL17 is upregulated in patients with moderate to severe asthma compared with mild asthmatic and control subjects (Chakir et al., 2003).

In this study, we detected the transcript mRNA levels of IL17A and IL17F in whole blood of asthma patients and normal donors. The change of their levels was evaluated by real-time reverse transcription polymerase chain reaction (qRT-PCR), for exploring whether these cytokines are potential parameters for the diagnosis of asthma.

MATERIALS AND METHODS

Asthmatic patients and control subjects

One hundred Saudi asthmatic patients attending clinic at the King Khalid University Hospital in Riyadh were used for the study and 100 healthy volunteers were recruited as control. This study was approved by the hospital institutional review board (IRB). Informed consent was obtained from the patients or their guardians and the controls. The diagnosis of asthma was based on clinical assessment by the physicians. The demographic and clinical data were recorded on specially designed data-capture sheets.

Blood samples

Blood samples were drawn by venepuncture from the patients and control attending the King Khalid hospital, King Saud University. Immediately after withdrawal, at least 2.5 ml whole blood was added into PAXgene Blood RNA tubes, and the samples were transferred to Genome Research Chair facility for analysis.

RNA isolation and cDNA synthesis

RNA was isolated from whole blood using the PAXgene Blood RNA kits (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. RNA integrity was assessed by formaldehyde agarose gel electrophoresis and by 260/280 nm absorption ratio. Reverse transcription was performed, in 20 μl reaction volume using the High Capacity RNA to cDNA Reverse Transcription Kits, according to the manufacturer’s instructions. The quality of constructed cDNA was examined by electrophoresis on 1% agarose. The quantity of IL17 transcripts was estimated by the standard curve using GAPDH as a reference.

Real-time real time-polymerase chain reaction (RT-PCR)

For real-time RT-PCR assays, 2 μl of the synthesized cDNAs was amplified with gene-specific primers and a gene-specific TaqMan probe. All assays were run in duplicates. The threshold cycle (ct) of samples was automatically calculated by the 7500 fast Real-Time PCR software. The data were analyzed with the comparative CT method for gene expression relative to GAPDH.

Statistical analysis

The results were analyzed using the Statistical Package for the Social Sciences (SPSS) program version 17. Because of the asymmetric and non-normal distribution of the IL17A and IL17F mRNA, the Mann–Whitney test was used to assess the differences in the expression of IL17A and IL17F in asthmatic patients and control subjects. The Spearman’s correlation test was used to evaluate the correlation between mRNA levels of IL17A and IL17F.

RESULTS

Subjects recruited

A total of 100 asthma patients (age range: 42:58; 38.4 ± 17.2 years) and 100 controls (97:3; 26.9 ± 17.7 y) were recruited for this study. Table 1 shows demographics data of subjects.

RNA preparation

On average, RNA preparations of patients had 206.9 (±105.8) ng RNA/μl and exhibited A260/A280 of 2.1 (±0.07), while those of the control subjects had 201.1 (±105.8) ng RNA/μl with A260/A280 of 2.2 (± 0.07). Since the A260/A280 for all RNA samples was 1.9 or above, all RNA preparations were considered pure by this criterion. All examined RNA preparations exhibited two strong bands corresponding to 28S rRNA and 18S RNA, with relative intensity of approximately 2:1 for most samples. Figure 1 shows representative results obtained with selected samples. The relative intensities of rRNA bands and the fact that the gel showed minimal smearing suggested all RNA samples had good quality.

Integrity of cDNA

RNA preparations were converted into cDNA preparations via reverse transcription. All cDNA preparations exhibited
Table 1. Subject demographics.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients (n=100)</th>
<th>Controls (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>42:58</td>
<td>97:3</td>
</tr>
<tr>
<td>Age (year)</td>
<td>38</td>
<td>27</td>
</tr>
</tbody>
</table>

**Case (Yes: No)**

- Smoking                                  | 4:96             | 34:66            |
- Exercise induced asthma-like symptoms    | 90:10            | 6:94             |
- Aspirin Allergy                          | 13:87            | 0:100            |
- Allergic Rhinitis                        | 81:19            | 28:72            |
- Eczema                                    | 41:49            | 1:99             |

Figure 1. Integrity of total RNA purified from whole blood as assessed by denaturing gel electrophoresis and ethidium bromide staining. Sharp, distinct bands corresponding to 28 and 18 S ribosomal RNA indicate intact RNA.

A faint smear across the entire lane, more pronounced in the low-molecular weight range (Figure 2). These results suggest that cDNA preparations were not contaminated with genomic DNA, and were not grossly degraded. The constructed cDNA was used to quantitate GAPDH, to establish the linear range of quantitation and to establish the input volume of constructed cDNA transcript for IL17A and IL17F quantitation.

Detection of the mRNA transcripts of IL17A and IL17F in asthma patients and controls

The mRNA levels of the IL17A and IL17F cytokines in blood were determined using RT-PCR. The results show that mRNA levels of IL17A and IL17F were significantly up-regulated in asthmatic patient compared to control subjects ($P = 0.004$ and $P < 0.001$, respectively) (Table 2). These data suggest that the levels of mRNA transcripts of IL17A and IL17F might be useful parameters for the diagnosis of asthma. However, the standard deviations of measurements were high, indicating that other factors contributed to the values of IL17.

The level of IL17A mRNAs correlated with the level of IL17F mRNAs

A positive correlation was obtained between the mRNA transcripts of IL17A and IL17F in both subjects of this study: controls as well as asthma patients ($r = 0.455$, $P < 0.01$ for controls and $r = 0.644$, $P < 0.01$ for patients).
**Figure 2.** 1% agarose gel electrophoresis of cDNA. A uniform smearing pattern indicates a high cDNA quality constructed from total RNA.

**Table 2.** Relative levels of IL17A and IL17F transcripts in asthmatic patients and control subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Case</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL17A/GAPDH</td>
<td>Control</td>
<td>0.938</td>
<td>1.363</td>
<td>0.150</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>Asthmatic</td>
<td>1.112</td>
<td>2.088</td>
<td>0.269</td>
<td></td>
</tr>
<tr>
<td>IL17F/GAPDH</td>
<td>Control</td>
<td>0.667</td>
<td>0.590</td>
<td>0.064</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Asthmatic</td>
<td>0.707</td>
<td>1.33</td>
<td>0.150</td>
<td></td>
</tr>
</tbody>
</table>

*Significance for the best-fit model of association of the transcripts with protection against asthma.

**Figure 3.** Linearity of GAPDH transcript level was used to establish the input volume of constructed cDNA transcript for IL17A and IL17F quantitation.
Table 3. Levels of IL17A and IL17F mRNA in patients and controls according to genotypes.

<table>
<thead>
<tr>
<th>Genes / SNPs</th>
<th>Case</th>
<th>Genotyping</th>
<th>No</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL17A</td>
<td>Controls</td>
<td>G/G</td>
<td>91</td>
<td>0.801</td>
<td>1.319</td>
<td>0.160</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>11</td>
<td></td>
<td>1.178</td>
<td>2.039</td>
<td>0.645</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patients</td>
<td>G/G</td>
<td>99</td>
<td>0.728</td>
<td>1.78</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>1</td>
<td></td>
<td>1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL17A / rs17880588</td>
<td>Controls</td>
<td>T/T</td>
<td>74</td>
<td>0.725</td>
<td>1.311</td>
<td>0.159</td>
<td>0.445</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>27</td>
<td></td>
<td>1.141</td>
<td>1.879</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td>IL17F / rs2397084</td>
<td>Controls</td>
<td>T/T</td>
<td>78</td>
<td>0.724</td>
<td>1.421</td>
<td>0.182</td>
<td>0.842</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>21</td>
<td></td>
<td>0.646</td>
<td>0.972</td>
<td>0.235</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>92</td>
<td></td>
<td>0.615</td>
<td>0.619</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>IL17F / rs763780</td>
<td>Controls</td>
<td>C/T</td>
<td>8</td>
<td>0.400</td>
<td>0.220</td>
<td>0.077</td>
<td>0.440</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>91</td>
<td></td>
<td>0.691</td>
<td>1.338</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>7</td>
<td></td>
<td>1.305</td>
<td>1.209</td>
<td>0.855</td>
<td>0.121</td>
</tr>
</tbody>
</table>

(Figure 3). We demonstrate that the levels of IL17A and IL17F proteins in plasma have the diagnostic value in asthma (Bazzi et al., 2011).

Level of IL17A and IL17F mRNAs and gene morphology

The possible association between IL17A and IL17F gene morphology and asthma in Saudis has been examined for four single nucleotide polymorphism (SNPs) (Bazzi et al., 2011). In this study, the effect of gene morphology on expression levels of mRNAs of IL17A and IL17F were compared for individuals with different genotypes (Table 3). The results show no significant differences among the various genotypes on the levels of mRNA transcript.

DISCUSSION

The IL17 family of cytokines has been linked to many diseases (Starnes et al., 2002; Chen and O’Shea, 2008). Elevation of IL17 levels has been reported in chronic inflammatory disorders, inflamed tissue from bacterial infections (Luzza et al., 2000; Johnson et al., 2004), synovial fluid from arthritis patients (Raza et al., 2005), and bronchoalveolar lavage fluid from asthmatic patients (Molet et al., 2001; Wong et al., 2001). IL17 levels greater than 20 pg/mL have been considered as risk factor for severe asthma (Agache et al., 2010). IL17A and IL17F can induce expression of several cytokines that have also been linked to asthma or asthma-related phenotypes (Wang and Liu, 2008). IL17A, produced by type 17 helper T cells, has been linked to neutrophil inflammation in asthma (Bullens et al., 2006). There are also numerous studies that examined the IL17 gene expression, but such studies are often carried out via cell culture approach. Most of these studies showed higher expressions in asthma patients versus control (Schwandner et al., 2000; Kawaguchi et al., 2001; Cai et al., 2002; Koshy et al., 2002; Chabaud, 2001; Kawaguchi et al., 2006).

In this study, we have attempted to correlate level of mRNA transcripts of IL17A and IL17F with asthma using blood samples that were neither stimulated nor cultured. The quantity of IL17A or IL17F transcripts in all subjects showed considerable variations, but remained several orders of magnitude smaller than the reference transcript (GAPDH), suggesting rare expressions. Prior studies showed no detectable IL17 transcript in normal tissues from some mammals (Yao et al., 1995), or expression that was restricted to neonatal tissues (Katoh et al., 2004). The levels of IL17F expression has been reported to increase in cells from the bronchoalveolar lavage fluid (BAL) of asthma patients after allergen stimulation, but not in cells challenged with saline control (Kawaguchi et al., 2001). In this study, some samples showed barely detectable levels of either IL17F or IL17A transcripts. This is consistent with a previous report, which showed that IL17F but not IL17A was strongly expressed in many tissues including liver, lung, ovary, and fetal liver (Kawaguchi et al., 2001).

The present study show positive correlation between levels of mRNA transcript of IL17A versus that of IL17F in controls as well as asthma patients; in any given subject, higher levels of IL17F transcript were correlated with higher levels of IL17A, and vice versa (r = 0.455, P < 0.01 for controls and r = 0.644, P < 0.01 for patients). This
result is expected since both genes are located on the same human chromosome (6p 12), and their promoters and conservative non-coding sequence regions undergo coordinated chromatin modifications (Kawaguchi et al., 2006; Pappu et al., 2008). Furthermore, both IL17A-IL17F act as homodimers or heterodimer, and they both share similar biological functions and expression patterns (Pappu et al., 2008). Therefore, the observed correlation between levels of IL17F and IL17A adds more validity to our approach as well as to the results of this study.

No apparent differences in the levels of IL17F between asthma patients and control subjects when subjects categorized by n the T/T and C/T genotypes of rs2397084 and rs763780 indicating that these sites may not affect expression of mRNA transcript of IL17F (Table 3). The level of IL17A appeared to be influenced by genotypes of rs7880588. However, the apparent statistically significance of results for this SNP was probably due to the fact that virtually all subjects, control and patients, belong to the same genotype for this SNP (Table 3). Studies on a larger population with substantially more individuals with A/G genotype are needed to establish whether this SNP morphology effects gene expression.

In conclusion, this study has attempted to examine the contribution of IL17A and IL17F to the development of asthma in Saudis via measuring the levels of mRNA transcripts. The results have show that levels of IL17A and IL17F mRNA transcripts were significantly up-regulated in the patient group and a positive correlation was obtained between the mRNA transcripts of IL17A and IL17F. These findings are consistent with those for other populations.

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REFERENCES


