ORIGINAL ARTICLE

A human monoclonal anti-TNF alpha antibody (adalimumab) reduces airway inflammation and ameliorates lung histology in a murine model of acute asthma

F. Catal\textsuperscript{a,d}, E. Mete\textsuperscript{a}, C. Tayman\textsuperscript{a}, E. Topal\textsuperscript{b,*,} A. Albayrak\textsuperscript{c}, H. Sert\textsuperscript{d}

\textsuperscript{a} Department of Pediatric Allergy and Asthma, Fatih University Faculty of Medicine, Ankara, Turkey
\textsuperscript{b} Department of Pediatric Allergy and Asthma, Gazi University Faculty of Medicine, Ankara, Turkey
\textsuperscript{c} Department of Pathology, Ankara Numune Education and Research Hospital, Ankara, Turkey
\textsuperscript{d} Department of Anesthesia, Fatih University Faculty of Medicine, Ankara, Turkey

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KEYWORDS
Adalimumab; Anti-TNF alpha; Antibody; Asthma; Cytokine; Inflammation; Murine; Ovalbumin

Abstract

Background: A few experimental studies related to asthma have unveiled the beneficial effects of TNF alpha blocking agents on the airway histology, cytokine levels in bronchoalveolar lavage and bronchial hyper-responsiveness. In the current study, we aimed to assess the effect of adalimumab on the inflammation and histology of asthma in a murine model.

Method: Twelve-week-old BALB/c (H-2d/d) female rats (n = 18) were allocated into three groups, including (group I) control (phosphate-buffered saline was implemented), (group II) asthma induced with OVA (n = 6), and (group III) asthma induced with OVA + treated with adalimumab (n = 6). Rats were executed on the 28th day of the study. The lung samples were fixed in 10% neutral buffered formalin. Lung parenchyma, alveoli, peribronchial and perivascular inflammation were assessed. Lung pathological scoring was performed.

Result: Severity of lung damage was found to be reduced significantly in the asthma induced with OVA + treated with adalimumab group. When compared with the untreated group, adalimumab significantly reduced the inflammatory cells around the bronchi and bronchioles, and reduced inflammation of the alveolar wall and alveolar wall thickness as well (median score = 1, \( p = 0.52 \)). Peribronchial smooth muscle hypertrophy and oedema were significantly reduced after adalimumab administration.

Conclusion: Adalimumab (a human monoclonal anti-TNF alpha antibody) therapy significantly reduced the severity of lung damage by decreasing cellular infiltration and improvement on the lung histology in a murine model of acute asthma.

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\* Corresponding author.
\textit{E-mail address: erdemtopal44@gmail.com} (E. Topal).

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Introduction

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. Additionally, eosinophils and mast cells are important players in the aetiopathogenesis of asthma by promoting the development, differentiation, recruitment, activation and survival of inflammatory cells. TNF-alpha, which is a Th1 and pro-inflammatory cytokine with immunoregulatory activities, is also essential in the pathogenesis of asthma. TNF-alpha serves as a chemoattractant for the neutrophils and monocytes, increasing vascular permeability, and activates T cells, eosinophils and mast cells. The administration of Adalimumab to rats intraperitoneally is essential for the treatment of asthma. The reduction in inflammation in the lungs is achieved by the administration of Adalimumab. Additionally, data about the effects of human monoclonal anti-TNF alpha antibody seem to be limited. In the present study, the aim was to ascertain the effects of Adalimumab on lung histology and lung inflammation in a murine model of asthma.

Materials and methods

Animals and experimental design

BALB/c (H-2\textsuperscript{d/e}) female rats (n = 18) were used for the study. Experimental protocols were approved by the Fatih University Animal Subject Committees. All animals were specified pathogen-free and were maintained under standard animal holding with water and food ad libitum at the Ankara Dışkapı Hospital, Research Center, Animals Research Laboratory in accordance with local and Turkish Home Office regulations. Twelve-week-old rats were randomly divided into three groups, including (group I) control (n = 6) (Phosphate-buffered saline was implemented), (group II) asthma induced with OVA (n = 6), and (group III) asthma induced with OVA + treated with Adalimumab (n = 6).

Administration of OVA and adalimumab

250 μl of “Phosphate-buffered saline” (PBS) was given intraperitoneally to all rats one hour before each intranasal administration. Adalimumab with a dose of 5 mg/kg/day was applied intraperitoneally to the rats in the third group for five days, starting from the day before the first challenge. All rats were sacrificed by cervical dislocation on the 28th day of the study. Lung tissues were removed for the histopathological examination.

Lung histology and scoring

The lung samples were fixed in 10% neutral buffered formalin, after the routine tissue monitorisation, the sections were obtained and stained with haematoxylin and eosin (H&E) and evaluated under light microscope. The lung sections from all groups were examined in a blinded fashion and lung inflammation was scored on the sections stained with H&E. All slides were evaluated over 10 consecutive fields at ×200 magnification. In order to be scored, each field had to contain a complete transection of at least one bronchiole less than half a field width in diameter, a blood vessel and an alveolar airway. Inflammatory cell infiltrate, i.e. the number and type of inflammatory cells present, was evaluated for the perivascular area, the bronchiolar epithelium and the peribronchiolar alveolar tissue. Inflammation was also scored on the basis of increased alveolar wall thickness. The scoring system used to assess inflammation is shown in Table 1.

Statistical analysis

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS) 15.0 software (SPSS Inc., Chicago, IL). Quantitative variables (lung score) were expressed in medians. The median score of groups were compared by using Kruskal–Wallis test. Mann–Whitney U test with Benferoni correction was used to evaluate the differences between groups. A two-sided p < 0.05 was considered statistically significant.

Results

Cell infiltration, inflammation and histological examination

When compared with the normal controls, dense inflammation was seen in the perivascular and peribronchiolar areas, including lymphocytes and eosinophils infiltration; and interstitial space (alveolar walls) was severely thickened due to intense inflammation in the lung tissues of asthma induced rats (median score: 4, p = 0.02). Bronchial smooth muscle hypertrophy and oedema were observed in the lung tissues of asthma induced rats. In the group treated with Adalimumab, the number of inflammatory cells surrounding the bronchi and bronchioles and alveolar wall thickness had a similar histomorphological appearance with the control group (median score: 1, p = 0.52). Furthermore, when compared, peribronchial smooth muscle hypertrophy and oedema were similar between the control group and the Adalimumab-treated group (Fig. 1). Statistically significant
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Table 1  Scoring system for assessing inflammation in a murine asthma model

<table>
<thead>
<tr>
<th>Area scored (number of cells)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perivascular compartment*</td>
<td>No infiltration</td>
<td>&lt;20 cells</td>
<td>&lt;100 cells</td>
<td>&gt;100 cells</td>
</tr>
<tr>
<td>Bronchiolar epithelium</td>
<td>No infiltration</td>
<td>&lt;5 cells</td>
<td>&lt;10 cells</td>
<td>&gt;10 cells</td>
</tr>
<tr>
<td>Peri-bronchiolar alveolar tissue*</td>
<td>No infiltration</td>
<td>&lt;20 cells</td>
<td>&lt;100 cells</td>
<td>&gt;100 cells</td>
</tr>
<tr>
<td>Alveolar walls**</td>
<td>No infiltration</td>
<td>2–3 cells</td>
<td>4–5 cells</td>
<td>&gt;5 cells</td>
</tr>
</tbody>
</table>

* Cells around blood vessel walls.
** Defined as sub-bronchiolar tissue, beneath basement membrane and smooth muscle, not immediately adjacent to a blood vessel.

Scores indicative of focal expansion of alveolar walls by that number of cells.

levels of the lung scores between the groups are shown in Table 2.

Discussion

In the current study, we evaluated the effects of adalimumab (a human monoclonal anti-TNF alpha antibody) on the lung histology and lung inflammation in a murine model of asthma. We found that adalimumab treatment decreased the cell inflammation, and showed similar histological findings to the control group. These findings support the opinion that anti-TNF alpha antibody could be a promising agent in the treatment of asthma.

Table 2  Comparison of the groups according to lung score.

<table>
<thead>
<tr>
<th>Comparison of the groups</th>
<th>Median lung score (min–max)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1, Group 2 &amp; Group 3</td>
<td>1 (1–2), 4 (3–4) &amp; 1 (1–2)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Group 1 &amp; Group 2</td>
<td>1 (1–2) &amp; 4 (3–4)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Group 1 &amp; Group 3</td>
<td>1 (1–2) &amp; 1 (1–2)</td>
<td>0.523*</td>
</tr>
<tr>
<td>Group 2 &amp; Group 3</td>
<td>4 (3–4) &amp; 1 (1–2)</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

* p: Kruskal–Wallis test.
** p: Mann–Whitney U test.

Group 1: Control group, Group 2: Murine asthma model, Group 3: Murine asthma model-adalimumab treated group.

Figure 1  (1a and 1b) Normal alveoli and bronchi (control group), H&E ×40 and ×100. (2a) Peri-bronchiolar and perivascular mix inflammation (asthma group), H&E ×40. (2b) Thickened alveolar walls with inflammation (asthma group), H&E ×100. (3a) The lung parenchyma without inflammation (adalimumab-treated group), H&E ×40. (3b) Mild thickened alveolar septa (adalimumab-treated group), H&E ×100.
Asthma is a disease mediated by Th2 cells, and specific cytokines such as IL4, IL-5, and IL-13 have important roles in the aetipathogenesis of asthma. TNF alpha, which is a Th1 lymphocyte-associated cytokine, has been shown to play an important role in the pathogenesis of asthma in the studies of both animals and humans. TNF-alpha, a strong pro-inflammatory cytokine, is mainly synthesised and stored by mast cells and alveolar macrophages in the lung, and it also has immune-regulatory properties. Furthermore, it contributes to the development of remodelling in asthma by affecting on fibroblasts and triggering the release of matrix metalloproteinase-9. Some studies on rats exposed to house dust and in patients with severe asthma have determined increased TNF-alpha expression in the airways. Therefore, some other studies have been conducted to ascertain the beneficial effects of TNF alpha inhibiting agents on both the animal models and patients with severe asthma. Additionally, some experimental studies have reported that agents inhibiting synthesis and activity of TNF-alpha decrease the infiltration of inflammatory cells in the airways. However, these agents have been reported to be ineffective through some other studies. Furthermore, results of these studies vary according to blocking of TNF alpha activities by different steps. Although Nam et al. have reported that using soluble TNF-alpha receptor in a rat model of asthma reduces eosinophil infiltration; they have declared that no beneficial effects have been found on the airway inflammation, when compared to the control group. In contrast, Kim et al. have determined in an experimental model of asthma that TNF alpha antibodies have provided significant improvement on the pulmonary inflammation, bronchial hyper-responsiveness and pulmonary histology.

Recently, TNF alpha blocking agents have already been successfully used in the treatment of chronic diseases such as Crohn and Rothamoid arthritis. Moreover, asthma is a chronic disease and TNF alpha is believed to have a major role in the pathogenesis of asthma. Therefore, TNF alpha blocking agents would provide favourable effects on the treatment of asthma. Some experimental studies related to asthma have unveiled the beneficial effects of TNF alpha blocking agents on the airway histology, cytokine levels in bronchoalveolar lavage (BAL), and bronchial hyper-responsiveness. However, few studies conducted on patients with severe asthma have indicated increased TNF alpha activity in BAL fluid and on the surface of peripheral blood monocytes. These patients were administered Etanercept (a soluble fusion protein combining two identical chains of the human p75 TNF receptors with an Fc portion of human IgG1) for 12 weeks. At the end of the treatment session, improvement on the asthma symptoms such as bronchial hyper-responsiveness, and pulmonary function tests has been determined.

All in all, asthma is a chronic disease of the airway, and it may sometimes have a severe nature and be treatment-resistant. TNF-alpha, a cytokine associated with Th1, is known to play an important role in the pathogenesis of asthma. It is also known that TNF-alpha blocking agents have beneficial effects on the airway inflammation, lung histology, and asthma symptoms. Adalimumab (a human monoclonal anti-TNF alpha antibody) is a different TNF alpha blocking agent. In the present study, we have evaluated the effects of adalimumab (a human monoclonal anti-TNF alpha antibody) on the lung histology and lung inflammation in a murine model of asthma. The work presented in the current study shows that adalimumab treatment suppresses the airway inflammation, and provides improvement in histological findings in asthma. We may suggest that adalimumab is a promising alternative for the treatment of patients with severe asthma which is unresponsive to treatment.

Conflicts of interest
No competing financial interests exist and there is no conflicts of interest for each author.

Ethical disclosures
Patients’ data protection. Confidentiality of Data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Protection of human subjects and animals in research. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

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The authors thank Tuncay Delibasi, M.D., and the Ankara Dışkapı Hospital, Research Center, Animals Research Laboratory’s workers for providing help in nursing, immunisation with OVA and sacrificing of animals.

References


Altamente Eficaz

• Inmunoterapia con Alérgeno Purificado
• Eficacia Clínica Demostrada
• Inmunoterapia Segura y bien Tolerada
• Dosificación Exacta

Altamente Eficaz
Diater
Alt a 1
Pauta de Administración

VIAL VOLUMEN ADMINISTRAR INTERVALO CONTINUACIÓN
1 0,1 + 0,2 semanal
2 0,4 + 0,4 semanal
3 0,1 + 0,2 semanal
4 0,4 + 0,4 semanal
4 0,8 quincenal

Duración de Tratamiento

6 semanas INICIACIÓN
3 meses CONTINUACIÓN
3 viales
5 meses 3 viales
5 vias

1. COMPOSICIÓN: Diater Alt a 1 es un tratamiento liofilizado estimulador de Alt a 1, alergeno mayoritario de Alternaria alternata, producido por ultrasonidos, vaciado por técnicas inmunológicas, con diversos objetivos de recombinación y que contiene una suspensión estéril de liofilizado de Alt a 1 en un diluyente de solución fisiológica (salina con colorante) y diluyente de recomposición para el tratamiento de iniciación y mantenimiento. La vacuna utilizada ha sido purificada por técnicas de ultrafiltración, valorada por técnicas inmunoquímicas, con diluyente de recomposición y así obtener una suspensión que contiene de 3 a 5 viales.Los viales deberán de mantenerse a una temperatura entre 5 y 30 grados a partir de su recepción, a una temperatura entre 2 y 8 grados desde el momento de la reconstitución hasta la fecha del vencimiento indicado en el envase, ni 6 meses después de la reconstitución. No utilizar después de la fecha de vencimiento indicada en el envase, ni 6 meses después de la reconstitución.

2. FORMA FARMACÉUTICA: VIAL: vial de 1 mL, diluyente de recomposición, con número y color.

3. CONTENIDO DEL ÍN克莱: Diater Alt a 1 consta de dos presentaciones: Diater Alt a 1 - Suspensión de iniciación, presentación que contiene cinco viales con liofilizado de Alt a 1 a la que está sensibilizado el paciente y un sistema de administración por vía subcutánea. Otras dos viales sin liofilizado que contienen un diluyente de recomposición de hidróxido de aluminio en solución salina y su función es diluir el liofilizado de Alt a 1 y así se logre un equilibrio óptimo de concentración de liofilizado de Alt a 1 y hidróxido de aluminio. Para la correcta administración de este tratamiento le resultaría de interés conocer la pauta de administración que se incluye en el presente folleto. En el folleto que se manifiesta a continuación se indica el tratamiento de iniciación, tanto para el tratamiento de iniciación como para el tratamiento de mantenimiento.

4. INDICACIONES: Diater Alt a 1 es un producto indicado en la immunoterapia específica de Alternaria alternata individualizado para el tratamiento de pacientes alérgicos que sean previamente diagnosticados por el especialista de inmunología clínica, con anterioridad, con una respuesta alergina positiva e historia clínica previa. El tratamiento puede contraer una reacción adversa. 6. PRECAUCIONES: Este tratamiento puede entrañar riesgos de reacciones generalizadas, a veces graves (urticaria,sofar,shock,anafilaxis) incluyendo la muerte, por lo que debe seguir durante la duración del mismo las siguientes normas:

- Se debe conservar en un sitio que permita limitar el número de visitas del paciente a la consulta, y se debe realizar una buena orientación del paciente.

- El tratamiento se realizará a cada paciente con antecedentes de reacciones adversas en el pasado.

- Los tratamientos deberán mantenerse a partir de su recepción, a una temperatura entre 2 y 8 grados desde el momento de la reconstitución hasta la fecha del vencimiento indicada en el envase, ni 6 meses después de la reconstitución. No utilizar después de la fecha de vencimiento indicada en el envase, ni 6 meses después de la reconstitución.

- Los viales deberán de mantenerse a una temperatura entre 5 y 30 grados a partir de su recepción, a una temperatura entre 2 y 8 grados desde el momento de la reconstitución hasta la fecha del vencimiento indicada en el envase, ni 6 meses después de la reconstitución. No utilizar después de la fecha de vencimiento indicada en el envase, ni 6 meses después de la reconstitución.

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