Macrophage Colony Stimulating Factor and Monocyte Chemoattractant Protein 2 are elevated in intrinsic asthmatics

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Article history:
Received 28 February 2011
Received in revised form 9 July 2011
Accepted 30 August 2011
Available online 25 September 2011

Keywords:
Chemokines
Cytokines
Intrinsic asthma
Macrophage Colony Stimulating Factor
Monocyte Chemoattractant Protein

ABSTRACT

Background: Intrinsic asthma, etiology unknown, occurs later in life, mostly in females. It is associated with nasal polyps and massive eosinophilic infiltration of the respiratory mucous membrane, aspirin intolerance and steroid dependence. The aim of the study was to determine the cytokine and chemokine profile in sera of intrinsic asthmatics and control subjects.

Methods: Blood was taken from 10 intrinsic asthmatic female and 12 control female subjects. Expression profile of 42 different cytokines and chemokines were measured using a microarray composed of antibodies against the cytokines and chemokines. Complete blood count and C-reactive protein were measured, to assess the state of inflammation in both groups.

Results: We have identified Macrophage Colony Stimulating Factor, a proinflammatory cytokine and Monocyte Chemoattractant Protein 2, a CC chemokine as having significantly higher expression levels (341.71 ± 31.28 SEM Signal intensity) versus (247.97 ± 28.09 SEM Signal intensity), p = 0.036 and (397.07 ± 38.19 SEM Signal intensity) versus (311.33 ± 28.76 SEM Signal intensity), p = 0.036, respectively. There were no significant differences in the other cytokines and chemokines measured nor were there any differences in the inflammatory measurements between the two groups except for eosinophil counts, the hallmark of intrinsic asthma.

Conclusion: Macrophage Colony Stimulating Factor and Monocyte Chemoattractant Protein are elevated in sera of intrinsic asthmatics compared to normal controls. These cytokines may have a critical role in the inflammatory pathology of intrinsic asthma.

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1. Introduction

Asthma is a complex; inflammatory disease of the lungs characterized by reversible airway obstruction, chronic airway inflammation, and airway hyperresponsiveness [1,2]. Asthma is one of the most common chronic diseases in the world, around 300 million people in the world have asthma, and there may be an additional 100 million persons with asthma by 2025 [3]. Asthma is commonly divided into two types: extrinsic asthma which is more common and is IgE mediated and intrinsic asthma [4]. Intrinsic asthma usually occurs in older individuals with preponderance in females. They have onset symptoms in later life and have no history of IgE dependent hypersensitivity [5,6]. Intrinsic asthma is associated with nasal polyps and massive eosinophilic infiltration of the...
respiratory mucous membrane [7]. Patients have negative skin prick test for all aeroallergens, serum levels of immunoglobulin E are within the normal range [8,9], aspirin intolerance and steroid dependency are common [6]. Thus, the intrinsic form of asthma appears to have unique inflammatory mechanisms that differ from those playing a role in the extrinsic form. There is paucity of research dealing with inflammatory mechanisms unique to intrinsic asthma. The reason may be because there is no animal model for intrinsic asthma [10]. The pathogenesis of intrinsic asthma is presently thought to be caused by unknown reasons [7]. The inflammatory mechanism in intrinsic asthma, the role and function of macrophages and chemokines that mobilize and recruit eosinophils have not been thoroughly investigated [5].

The aim of the study was to determine the cytokine and chemokine profile in sera of intrinsic asthmatics and control subjects.

2. Methods and materials

2.1. Human subject description and sample collection

This study was approved by the Hashemite University IRB committee (30/2006). The Hashemite IRB committee follows the Helsinki recommendations. Intrinsic asthmatic women and control women were invited by telephone call to give a blood sample. Informed consent was obtained for participation in the study. All samples of the study group and the controls were taken between 10 and 12 AM in the same lab and by the same technician. The study was carried out from August 2008 to June 2009. The asthmatic patients and controls were referred by the Jordan Allergy Institute in Amman, Jordan. Both groups were identical in being: (1). Non diabetic to avoid complication from other inflammatory diseases (2). Females since it has been shown that intrinsic asthma occurs in women later in life (3). Between 20 and 40 years of age (4). Not pregnant (5). Off of any oral medication for two weeks prior to taking of sample (6). Non smokers. In addition to the above, the study group were asthmatics suffering from airway obstruction, wheezing and cough, with 15% reversibility of pulmonary function tests and having negative skin test to 50 common inhalant allergens (Stallergenes S.A., France) by skin prick test supplementary Table 1. All patients take inhaled corticosteroids and beta agonists to control their disease. It is important to note that medications administered were similar in all patients. Ethical consideration prevented us from stopping the medication prior to taking of the sample. The presence of nasal polyps and history of aspirin intolerance were identified in the study group. The body mass index (BMI) was assessed for both groups.

Sample collection: 12 ml of peripheral blood samples were taken from intrinsic asthmatic and control women in a clinical laboratory. Complete blood count (CBC) and C-reactive protein (CRP) were measured in the clinical lab according to standard procedure. 7 ml of blood were put in a plain tube and centrifuged at 5000 rpm for 5 min after that 5 ml of serum was obtained, transferred to 5 eppendorf tubes each one contained 1 ml of serum and stored at −20 °C.

2.2. Cytokine determination

The cytokines from 22 samples were measured using RayBio® Human Cytokine Antibody Array III (Ray Biotech, Norcross, GA) according to the manufacturer's instructions. The cytokine antibody array is based on the sandwich enzyme-linked immunosorbent assay (ELISA) method for detecting proteins. This array allows for semiquantitative analysis. Actual concentrations are not determined. The array can detect 42 cytokines; the cytokines that will be quantified are listed below:

ENA-78, GCSF, GM-CSF, GRO, GRO-a, I-309, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40p70, IL-13, IL-15, IFN-g, MCP-1, MCP-2, MCP-3, MCSF, MDC, MIF, MIP-1d, RANTES, SCF, SDF-1, TARC, TGFB1, TNFα, TNFB, EGF, IGF-1, Angiogenin, Oncostatin M, Thromboietin, VEGF, PDGF BB, Leptin. The intensities of signals were quantified by densitometer (PTW-Densix, Germany) and positive controls were used to normalize the results from different arrays according to the formula: normalized signal intensity of particular spot = signal intensity of particular spot x (positive1/ positive signal intensity sample in particular spot). positive1: The positive control of first sample. Detection limits for each mediator is in supplementary Table 2.

2.3. Statistical analysis

Clinical data were entered using Excel software running with windows XP2003. Densitometric data were entered and normalized with Array Analysis Tools 3 software provided by manufacturer RayBio® Human Cytokine Antibody Array III (Ray Biotech, Norcross, GA). Data were analyzed using SPSS software version 12. Differences between the means of the intrinsic asthmatic group and control group were tested using Independent t-test. The differences were considered statistically significant at p < 0.05. Data are stated as means ± mean of standard error (SEM).

3. Theory

The etiology of intrinsic asthma is unknown. We theorize that intrinsic asthma involves a unique inflammatory mechanism that may be reflected in a unique cytokine and chemokine profile. We propose to look at the cytokine and chemokine profile in sera of intrinsic asthmatics for changes in levels of expression compared to controls.

4. Results

A total of 10 intrinsic asthmatic women and a total of 12 control women were included in this study. Sixty percent of patients had nasal polyps, and 50% of patients were aspirin intolerant. BMI was not significantly different between control and intrinsic asthmatic groups (p > 0.05). All clinical features are summarized for the two groups in Table 1.

4.1. CBC and CRP measurement

CBC and CRP measurements for the intrinsic asthmatic group and the control group are summarized in Table 2. The data indicate that hemoglobin, Hematocrit, MCV, MCH, MCHC measurements were not significantly different between control and intrinsic asthmatic groups (p > 0.05). In addition the data demonstrate that red blood cell counts and platelet counts displayed no significant differences between the two groups (p > 0.05). Measurement of white blood cell count and differential count specify that the number of eosinophils in the intrinsic asthmatic group is significantly higher than that in the control group (415 ± 104 vs. 170 ± 26, p = 0.02). In contrast there were no significant differences in white blood cells, neutrophils, monocytes, lymphocytes between both groups (p > 0.05). CRP measurements were negative for both groups (<6 mg/L) Table 2.

4.2. Cytokines determination

In an effort to screen and identify cytokines and chemokines that may be associated with intrinsic asthma, we used a microarray composed of antibodies against 42 different cytokines and
chemokines. We measured cytokines and chemokines expressed by each group using serum separated from blood samples of intrinsic asthmatic and control groups as described in the «Methods» section. The signal intensity for each cytokine and chemokine was quantified by densitometry (Fig. 1).

4.3. Determination of lymphokines

Expression levels of lymphokines are presented in (Fig. 2). Data in this figure demonstrates that there are no significant differences in the expression levels of IL-2, IL-3, IL-4, IL-5, IL-7, IL-13 and IL-15 lymphokines between both groups (p > 0.05), but their expression levels were greater in the intrinsic asthmatic group. Exact p values are in Table 3.

4.4. Determination of proinflammatory cytokines

The data in (Fig. 3) confirmed that the expression level of MCSF proinflammatory cytokine was significantly higher in the intrinsic asthmatic group (341.71 ± 31.28 signal intensity) compared to the control group (247.97 ± 28.09 signal intensity). (p = 0.036). While the expression levels of IL-1α, IL-1β, TNF-α, TNF-β, SCF, GCSF, GM-CSF showed no significant differences between intrinsic asthmatic and control groups (p > 0.05). Exact p values are in Table 3.

4.5. Determination of growth factors

The data in (Fig. 4) show that expression levels of growth factors, EGF, IGF-1, VGEF, PDGF-BB and TGF-beta1 were not significantly different between intrinsic asthmatic and control groups (p > 0.05). Exact p values are in Table 3.

4.6. Determination of inhibitory cytokines

The data in (Fig. 5) indicate that expression levels of inhibitory cytokines IL-10, IL-12p40, TNF-gamma were not significantly different between intrinsic asthmatic and control groups (p > 0.05). Exact p values are in Table 3.

4.7. Determination of CXC chemokines

Expression levels of CXC chemokines are presented in (Fig. 6). Expression levels of IL-8, MIG, SDF-1, GRO, GRO-alpha and ENA-78 display no significant differences in expression levels between intrinsic asthmatic and control groups (p > 0.05). Exact p values are in Table 3.

4.8. Determination of CC chemokines

The data in (Fig. 7) revealed that CC chemokine, MCP-2 had a significant higher expression level in intrinsic asthmatic (397.07 ± 38.19 signal intensity) than control (311.33 ± 28.76 signal intensity). (p = 0.036). In contrast MCP-1, MCP-3, I-309, MAD, MIp delta, RANTES and TARC had no significant differences in their expression levels between intrinsic asthmatic and control groups (p > 0.05).

Expression levels of angiogenin, oncostatin M, thrombopoietin, leptin were not significantly different between intrinsic asthmatic and control groups (p > 0.05) (Fig. 8). Exact p values are in Table 3.

5. Discussion

Nasal polyps, aspirin intolerance and eosinophilic infiltration of the respiratory mucous membranes are frequently seen in patients with intrinsic asthma in addition to the hallmarks of the disease; reversible airflow obstruction and airway hyperresponsiveness. The etiology of intrinsic asthma is not known. Progress in the study of intrinsic asthma has been limited and new insights into the etiology of this apparent phenotype of asthma are few. Unfortunately, little pathophysiological data are available that clearly delineate non-asthmatic asthma [11]. Many theories have been suggested. Persistent respiratory infections may play a central role in the development of intrinsic asthma [12]. Other theories proposed are dependent on epidemiological studies which indicate that adult-onset asthma may be initiated by stress (anxiety and depression), obesity and menopause, the ‘Multi-Hit Endocrine Model of Adult-Onset Asthma’ [13]. The BMI measured in our intrinsic asthmatic patients did not support the obesity part of this model Table 1. There are conflicting studies concerning the association between menstrual cycle and asthma [14]. It would be interesting to study the effect of the menstrual cycle on the cytokine profile of intrinsic asthmatic patients. Superantigens may also be important in intrinsic asthma as airway epithelial cells may be colonized by Staphylococci and other superantigen-producing microbes [15]. An alternative view is that an abnormality in the airway smooth muscle cell, which is capable of producing inflammatory, immunological and growth factors as well as molecules, which facilitate interaction with inflammatory cells, is the primary event. Evidence is rapidly accumulating that the smooth muscle is abnormal, in that it proliferates faster, produces more chemokines and cytokines as well as a different profile of extracellular matrix proteins than its non-asthmatic counterpart. Candidates such as eotaxin and IP-10 have been implicated [16].
In our results we found elevated levels of two cytokines, MCP-2 and MCSF in intrinsic asthmatics compared to normal controls. While there were no significant differences in the serum levels of all the other cytokines measured compared to controls. In spite of the small sample size, the size of the sample was statistically considered in assessing the significance of the results.

M-CSF is responsible for the survival, proliferation, differentiation and activation of macrophages at various stages of their development. It is produced by endothelial cells, fibroblasts, and mononuclear phagocytes. Activation of monocytes/macrophages by MCSF is likely to be an important part of the immune and inflammatory response. Prior studies demonstrated that M-CSF produced by human-joint tissue cells (chondrocytes, synovial fibroblasts) in vitro in response to the inflammatory cytokines, IL-1 and TNF-α may play a key role in chronic inflammatory autoimmune diseases, such as rheumatoid arthritis check reference if cell from patients [17]. Thus increased levels of MCSF in the intrinsic asthmatic sera may indicate a similar inflammatory pathway in the pathogenesis of these patients.

MCP-2 is a chemoattractant for eosinophils and basophil. Also MCP-2 induces T cell migration. MCP-2 activates basophils and causes histamine and leukotriene secretion independently of IgE. Prior studies have demonstrated that MCP-1 is detectable in a variety of inflammatory diseases but so far little is known about the

<table>
<thead>
<tr>
<th>CBC Measurement</th>
<th>Mean ± SEM</th>
<th>p value</th>
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<tbody>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.05 ± 1.07</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41 ± 0.03</td>
<td>0.4</td>
</tr>
<tr>
<td>RBC (μL⁻¹)</td>
<td>4819167 ± 386162</td>
<td>0.2</td>
</tr>
<tr>
<td>WBC (μL⁻¹)</td>
<td>7775 ± 359</td>
<td>0.4</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>83.17 ± 6.66</td>
<td>0.5</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>27.75 ± 2.27</td>
<td>0.2</td>
</tr>
<tr>
<td>MCHC (gHb/dl)</td>
<td>32.83 ± 2.71</td>
<td>0.8</td>
</tr>
<tr>
<td>Platelets (μL⁻¹)</td>
<td>289500 ± 23344</td>
<td>0.8</td>
</tr>
<tr>
<td>Neutrophils (μL⁻¹)</td>
<td>4661 ± 233</td>
<td>0.5</td>
</tr>
<tr>
<td>Monocytes (μL⁻¹)</td>
<td>412 ± 40</td>
<td>0.5</td>
</tr>
<tr>
<td>Lymphocytes (μL⁻¹)</td>
<td>2763 ± 297</td>
<td>0.3</td>
</tr>
<tr>
<td>Eosinophils (μL⁻¹)</td>
<td>170 ± 26</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>Negative (&lt;6)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM.

![Fig. 1. A, Panel representing the pattern of cytokine and chemokine expression for intrinsic asthmatic patients, B, Panel representing the pattern of cytokine and chemokine expression for control individuals, C, The map of RayBio® Human Cytokine Antibody Array III.](image-url)
role of MCP-2 in the pathology of inflammatory diseases. In addition MCP-2 levels were significantly high in synovial fluids of rheumatoid arthritic patients [18]. These increased levels of MCP-2 in the intrinsic asthmatic sera suggest that they may play a role in macrophage, eosinophil and basophil recruitment and activation as well as contribute to the inflammatory pathology of
intrinsic asthma. These results may push us to theorize that intrinsic asthma has features common to some autoimmune diseases. We realize that such a statement is an extrapolation yet since there is paucity of research in this area we hope that this will stimulate further research.

The above findings may suggest a common mechanism that links intrinsic asthma with some autoimmune diseases such as rheumatoid arthritis. Recently, in chronic idiopathic urticaria, a significant number of patients have been shown to have IgG auto antibodies [19]. Lacking a definitive animal model for intrinsic asthma, observations in IgE-deficient mice showed that bronchial inflammation is undiminished and it is possible that these mice produce an IgG response. As a result, mast cells sensitized by IgG antibodies might have a role in IgE-deficient mice inflammation, by secreting mediators and cytokines. The fact that eosinophil associated inflammation occurs in this model bears witness [20].

All in all evidence cited above of increased MCSF and MCP2 in intrinsic asthmatics compared to their normal counterparts in this study may open a new venue in the clarification of the etiology and pathophysiology of intrinsic asthma. Both link to an autoimmune disease through MCSF stimulated mechanisms and MCP2 recruitment of eosinophils.

6. Conclusions

In conclusion, MCSF and MCP-2 were elevated in sera of intrinsic asthmatic patients compared to normal controls. These findings provide a promising future direction for research regarding intrinsic asthma including the possible identification of local expression of MCSF and MCP-2 in lungs airways and the role of basophils and monocytes in the inflammatory response as well as correlation with severity of the disease.

Conflict of interest

All authors declare that they do not have any conflict of interest.

Acknowledgements

This research was funded by the Hashemite University. The Hashemite University played no role in study design, in the collection, analysis and interpretation of data, in the writing of the report and in the decision to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cyto.2011.08.040.

References