ORIGINAL CONTRIBUTION

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Increased serum complement component 3 and mannose-binding lectin levels in adult Chinese patients with chronic rhinosinusitis*

Yong-Hua Cui[1], Feng Zhang[1], Zhi-Gang Xiong[2], Xue-Jun You[1], Qi-Xue Gao[1], Zheng Liu[1]

[1] Department of Otolaryngology-Head and Neck Surgery, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China
[2] Department of Laboratory Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China

SUMMARY

Objective: *To study the immune function of adult Chinese patients with chronic rhinosinusitis (CRS) to elucidate its potential role in the pathogenesis of CRS.*

Methods: *A prospective three-arm case-control study. The study population comprised 72 CRS patients without nasal polyps (NPs), 95 CRS patients with NPs, and 110 healthy controls. The concentrations of serum immunoglobulin A (IgA), M (IgM), G (IgG), IgG subclasses (IgG1-4), complement component 3 (C3), and complement component 4 (C4) were measured by nephelometry. Serum mannose-binding lectin (MBL) levels were analyzed by enzyme-linked immunosorbent assay. All CRS patients had a complete blood count with differential, atopic status evaluation, coronal computed tomographic (CT) scan of the sinuses, and nasal endoscopy.*

Results: *Frequency of immunoglobulin, C3, C4, or MBL deficiency showed no difference among groups. The prevalence of coexistence of MBL and immunoglobulin or complement component deficiency did not differ significantly among groups either. However, compared with controls, decreased IgG3 levels were found in CRS patients without NPs, and increased C3 and MBL levels was found in both CRS patients with and without NPs. Moreover, MBL levels were significantly higher in CRS patients with NPs than in CRS patients without NPs, which positively correlated with extent of disease seen on CT scan and endoscopy, and peripheral eosinophil count.*

Conclusions: *Immunoglobulin, C3, C4, and MBL deficiency is not the main cause of CRS in adult Chinese patients. However, on the contrary, increased C3 and MBL levels in serum might play a modulatory role in CRS development.*

Key words: chronic rhinosinusitis, nasal polyp, immunoglobulin, complement, mannose-binding lectin, Chinese

INTRODUCTION

Chronic rhinosinusitis (CRS) is an extremely common and deliberating disease causing a decrease in the quality of life similar to chronic pulmonary disease and rheumatoid arthritis [(1)]. The exact aetiology of CRS has not yet been fully revealed. Bacterial, fungal, and viral infections have been implicated in the development of CRS [(1,2)]. Host defense against microbial pathogen invasion is provided by innate and adaptive immunity. Impairment of adaptive immunity has been linked to CRS. Immunoglobulin A (IgA), M (IgM), G (IgG) and IgG subclasses (IgG1-4) deficiencies have been found in some CRS patients [(3,4)]. However, the significance of these deficiencies is still uncertain [(4)]. Moreover, whether these deficiencies are involved in the development of CRS in Chinese patients is unknown.

Innate immune system serves as the first line of defence against infection. Complement system is an important component of the innate immune response, which mediates humoral and cellular interactions within the immune response, including chemotaxis, cell adhesion, phagocytosis, and B cell differentiation [(5)]. Complement may be activated via three different routes: the classical pathway, the alternative pathway, and the recently discovered lectin pathway. Complement component 3 (C3) is a key protein in all these reaction pathways, whereas complement component 4 (C4) belongs to the classical and lectin pathway. Mannose-binding lectin (MBL), a liver-derived pattern-recognition molecule, can activate the lectin pathway of complement system through binding to arrays of terminal mannose groups on wide variety of pathogens [(6)]. A deficiency

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of complement system can cause increased susceptibility to infections [5-7]. Despite evidence of infection and immune involvement in CRS, few studies have investigated the association between complement system and CRS [8-10], and no study has addressed the serum MBL levels in CRS patients.

The aim of this case-control study was to investigate the association between CRS and serum immunoglobulins, C3, C4, and MBL in a Chinese Han population.

MATERIALS AND METHODS

Subjects

Seventy-two CRS patients without NPs and 95 CRS patients with NPs who underwent endoscopic sinus surgery were recruited. Clinical data of patients are summarized in Table 1. CRS including CRS without and with NPs was diagnosed according to the current European EAACI position paper [2] and American guidelines [1]. Patients were referred for symptoms refractory to conservative therapy, such as antibiotics, nasal steroids, decongestants, antihistamines, mucolytics, and nasal irrigation. Patients refrained from taking oral steroid for one month prior to the surgery. None of the patients had ever received allergen immunotherapy. Subjects who had an antrochoanal polyp, cystic fibrosis, fungal sinusitis, primary ciliary dyskinesia, or gastroesophageal reflux disease were excluded from the study. The atopic status was evaluated by skin prick tests. CT scans were scored using Lund-Mackay system and endoscopy physical findings were scored according to Lanza and Kennedy [11,12]. A complete blood count and differential was performed by automated analysis on blood. As healthy controls, 110 healthy individuals taking routine health examination from the same geographical region were recruited. All control subjects were in generally good health, and had normal results on anterior rhinoscopy. None had a history of sinonasal disease, allergic disease, and recurrent infection. All studied subjects were Han Chinese from central China. Informed consent was obtained from each patient and control and the project was approved by the ethical committee of Tongji Medical College of Huazhong University of Science and Technology.

Laboratory methods

All analyses were performed according to the manufacturers' instructions. Serum IgA, IgM, IgG, IgG1-4, C3, and C4 levels were determined by nephelometry (Dade Behring Marburg GmbH, Marburg, Germany). The normal adult ranges for our laboratory are shown in Table 2. MBL levels were measured in the serum by enzyme-linked immunosorbent assay using mannose-binding enzyme-linked immunosorbent assay kit (Antibody shop, Copenhagen, Denmark). A cut-off of 0.6 μg/ml was adapted to denote MBL insufficiency [6].

Table 1. Case and control demographic and clinical information.

	Control group	CRS without NPs	CRS with NPs
No. of subjects	110	72	95
Sex (male/female)	51/59	39/33	49/46
Age (y)*	31 (25-44)	33 (28-45)	35 (28-49)
No. of patients with asthma	0	7	19
No. of patients with positive skin prick test results	N/A	30	44

* Values are expressed as medians and interquartile ranges.
CRS: chronic rhinosinusitis; NPs: nasal polyps

Statistical analysis

Differences in proportions between groups were tested by the chi-square test or Fisher exact probability test when appropriate. In continuous variables, comparisons between all groups were performed by means of Kruskal-Wallis test because most variables had non-normal distribution in at least one group. If the variance analysis showed significant differences between groups, the Mann-Whitney U test was used to locate them. The spearman test was used to determine correlations. The desired probability of overall type I error was less than 0.05. Bonferroni correction was applied to adjust the significance of multiple comparisons (individual p-values were less than 0.05/k, where k = number of comparisons made). Data analysis was performed through the application of SPSS software for Windows (SPSS, Inc., Chicago, USA).

RESULTS

There was no significant difference in age or sex distribution between controls and CRS patients without and with NPs. The results of serum concentrations of immunoglobulins, C3, C4, and MBL are shown in Table 2.

The frequency of immunoglobulin level above or below the normal range showed no significant difference among healthy controls, CRS patients without and with NPs. Common variable immunodeficiency was observed in 0%, 0%, and 1.05% of healthy controls and CRS patients without and with NPs, respectively; and no significant difference was demonstrated between all groups. No C3 deficiency was found in our present study. On the contrary, the frequency of C3 level above the normal range was higher in CRS patients without NPs than in healthy controls ($p = 0.003$, $p < 0.017$ was considered statistically significant after Bonferroni adjustment). As to C4 and MBL, although the deficiency was found in some subjects, there was no difference in deficiency frequency between different studied groups. In order to investigate the significance of coexistence of different immuno-deficiencies, we analyzed the frequency of combined MBL and immunoglobulin or complement component deficiency. In the control group, we found one subject with MBL plus IgG1 and C4 deficiency; in CRS without NPs group, one patient with MBL plus IgG4 deficiency and one patient with MBL plus IgG1 and C4 deficiency were found; and in CRS with NPs group, we discovered one patient with MBL plus IgG, IgG1, IgG3, and C4 deficiency, one patient with MBL plus IgG3 deficiency, one patient with

Table 2. Serum values in healthy controls, CRS patients without and with NPs.

Value	Control group	CRS without NPs	CRS with NPs
Unit	n=110	n=72	n=95
Reference range		Median (interquartile ranges)	
(M/F)		Values below reference: n (%)	
		Values above reference: n (%)	
IgA	3.14 (1.45-4.46)	2.92 (1.70-4.54)	3.09 (1.88-4.28)
(g/L)	0 (0)	2 (2.78)	2 (2.11)
0.7-5.0	17 (15.45)	13 (18.06)	18 (18.95)
IgM	1.04 (0.76-1.57)	1.04 (0.71-1.61)	1.07 (0.83-1.62)
(g/L)	1 (0.91)	1 (1.39)	3 (3.16)
0.4-2.3/0.4-2.8	3 (2.73)	3 (4.17)	4 (4.21)
IgG	12.13 (9.65-13.88)	11.00 (9.18-14.22)	10.67 (9.12-13.20)
(g/L)	0 (0)	0 (0)	1 (1.05)
7.0-16.0	7 (6.36)	3 (4.17)	5 (5.26)
IgG1	6.71 (5.37-8.28)	6.64 (5.23-8.16)	6.12 (5.11-8.18)
(g/L)	2 (1.82)	2 (2.78)	3 (3.16)
4.9-11.4	9 (8.18)	8 (11.11)	10 (10.53)
IgG2	3.18 (2.41-4.09)	2.44 (1.87-4.56)	2.79 (2.08-3.88)
(g/L)	0 (0)	1 (1.39)	2 (2.11)
1.5-6.4	8 (7.27)	6 (8.33)	10 (10.53)
IgG3	0.47 (0.33-0.61)	0.32 (0.22-0.54) *	0.42 (0.30-0.52)
(g/L)	1 (0.91)	1 (1.39)	2 (2.11)
0.2-1.1	0 (0)	2 (2.78)	2 (2.11)
IgG4	0.40 (0.26-0.51)	0.27 (0.13-0.55)	0.34 (0.21-0.46)
(g/L)	0 (0)	1 (1.39)	1 (1.05)
0.08-1.4	0 (0)	0 (0)	2 (2.11)
C3	0.86 (0.80-0.96)	0.99 (0.88-1.07)*	0.98 (0.86-1.08)*
(g/L)	0 (0)	0 (0)	0 (0)
0.7-1.13	0 (0)	6 (8.33) *	5 (5.26)
C4	0.23 (0.17-0.30)	0.19 (0.14-0.27)	0.21 (0.16-0.29)
(g/L)	5 (4.55)	3 (4.17)	5 (5.26)
0.1-0.4	0 (0)	1 (1.39)	2 (2.11)
MBL	1.16 (0.77-2.80)	2.52 (1.02-3.28) *, #	3.64 (2.13-4.84) *
(µg/mL)	14 (12.73)	11 (15.28)	12 (12.63)
≥0.6			

* Statistically significant difference for CRS without NPs or CRS with NPs compared with controls; # Statistically significant difference for CRS without NPs compared with CRS with NPs.

CRS, chronic rhinosinusitis; NPs, nasal polyps; Ig, immunoglobulin; C, complement component; MBL, mannose-binding lectin.

MBL plus IgG4 deficiency, and one patient with MBL plus IgA deficiency. The prevalence of coexistence of different immuno-deficiencies did not differ significantly between three groups.

Serum concentrations of immunoglobulins, except for IgG3, did not differ significantly between different subject groups. IgG3 concentrations were found to be significantly decreased in CRS patients without NPs compared with healthy controls ($p = 0.0009$, $p < 0.017$ was considered statistically significant after Bonferroni adjustment). Serum C3 levels were shown to be significantly increased in both CRS patients without and with NPs compared with controls ($p = 0.000004$ and 0.000005, respectively; $p < 0.017$ was considered statistically significant after Bonferroni adjustment), whereas serum C4 levels demonstrated no significant difference between CRS patients and controls. In respect of MBL, significantly higher levels were found in CRS patients without and with NPs than in controls ($p = 0.016$ and $p = 0.0000003$, respectively; $p < 0.017$ was considered statistically significant after Bonferroni adjustment). No difference in immunoglobulin, C3, C4, or MBL levels was demonstrated between atopic and nonatopic patients, or asthmatic and non-asthmatic patients in both CRS groups. When comparing CRS patients without and with NPs, significantly higher MBL concentrations were revealed in CRS patients with NPs ($p = 0.001$, $p < 0.017$ was considered statistically significant after Bonferroni adjustment). In addition, a same trend was found when comparing the non-asthmatic patients in CRS without and with NPs groups (Figure 1, $p < 0.013$ was considered statistically significant after Bonferroni adjustment). Furthermore, MBL level was found to positively correlate with CT scan score ($r = 0.58$, $p = 0.000001$), endoscopy score ($r = 0.53$, $p = 0.000001$), and peripheral eosinophil count ($r = 0.40$, $p = 0.000001$).

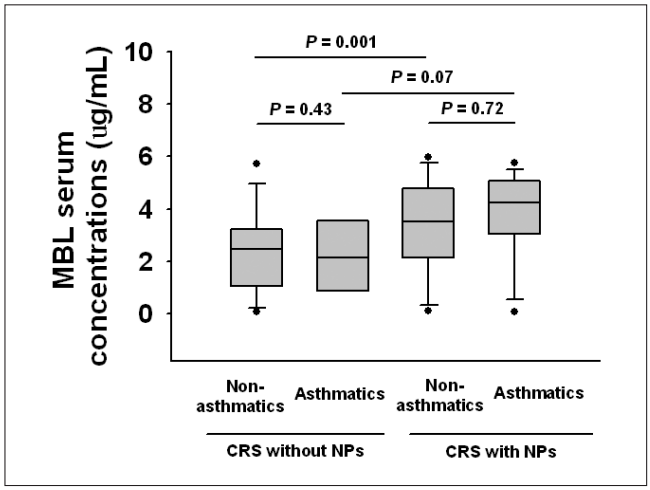

Figure 1. Serum mannose-binding lectin (MBL) levels in asthmatic and non-asthmatic patients in both chronic rhinosinusitis (CRS) with and without nasal polyps (NPs) groups. In CRS without NPs group, n = 72 (7 asthmatics / 65 non-asthmatics); and in CRS with NPs group, n = 95 (19 asthmatics/76 non-asthmatics). Data are expressed in box-and-whisker plot. $p < 0.013$ was considered statistically significant after Bonferroni adjustment.

DISCUSSION

The concept of adaptive immune dysfunction as a risk for chronic or refractory sinus disease has gained a measure of attention in recent years [3,13-15]. However, data on the incidence and exact influence of the adaptive immune deficiency in CRS are rather conflicting. Some reports showed that immunoglobulin deficiencies were found in up to 17% of CRS patients [3,13], whereas other studies did not find any deficiency of total IgG or IgG subclass in patients with CRS [14,15]. The discordance between those reports may be a result of variation in phenotype definitions, ethnicity, and analysis methods used. Moreover, currently, most data have been derived from studies on Caucasians; the immune status in Chinese CRS patients remains unclear. In recent years, academic interest shifted from the adaptive immunity towards the innate immune system, which appears to be more crucial, more complex, and more specific than was previously supposed. The complement system is an important component of the innate immune system. There has been an emerging interest in the complement system due to its important role in innate immunity and correlation with disease [5,6]. However, at present, the role of the complement system in CRS has received little attention.

In order to investigate the role of immune dysfunction in Chinese patients and avoid the possible discrepancy caused by technical differences among the laboratories, we did the three-arm case-control study in a Chinese Han population. Although immunoglobulin deficiencies could be observed in some Chinese patients with CRS, no differences was found when compared with normal individuals; and the clinical pictures of patients with immunoglobulin deficiency were not different from those of patients without deficiency. Moreover, the distribution of the serum levels of all the immunoglobulins, except for IgG3, in CRS patients did not differ significantly from the healthy controls. A previous study demonstrated that most of the low IgG3 levels normalized over time, suggesting that low IgG3 levels in CRS patients may be rather a secondary phenomenon due to consumption of antibodies after infection than a cause of CRS [16]. With respect to the complement system, no higher incidence of C3, C4, or MBL deficiency could be found in Chinese CRS patients. Since some studies indicate that MBL deficiency may not have clinical significance unless it is associated with other immunity defects [17,18], we evaluated the prevalence of coexistence of MBL deficiency and other complement components and immunoglobulin defects in Chinese CRS patients. However, no synergism between MBL and immunoglobulin or complement component deficiency could be found. Taken together, our data suggest that immunoglobulin and complement component deficiencies are not the main causes of CRS in Chinese patients. However, material investigated in this study was relatively small and it should be noted that humoral immunodeficiency is usually sufficiently rare. Moreover, normal concentrations of generalized immunoglobulin classes and subclasses do not exclude the possibility of deficiencies of antigen-specific anti-bacterium or anti-fungus antibodies as the aetiology of CRS, and the cellular response is also an unneglectable part of our defence system. Therefore, further studies are needed to determine the integral immunological responses to specific pathogen antigens in a larger cohort study of CRS patients.

Contrary to expectations, we found C3 and MBL levels were significantly elevated in CRS patients. As a corollary, recently, higher plasma C3a and MBL levels have been found in asthma patients [19,20]. Comparing the asthmatic and non-asthmatic patients in both CRS without and with NPs groups, we did not find a significant difference in serum C3 and MBL levels, which might be due to the limited number of asthmatic patients enrolled in out present study. Several newly published studies propose that the complement system extends beyond its classic role as a first-line host defence molecule to a modulator of inflammation [19,20]. MBL can bind to carbohydrate on microorganisms and initiate the activation of complement components including C3. When activated, C3 is cleaved into two biologically active fragments known as C3a and C3b. C3a can increase small vessel permeability, vasodilatation, and histamine release [5]. Many features of CRS such as mucus hypersecretion and recruitment of inflammatory cells are consistent with actions of C3a. Thus, high MBL and C3 levels may lead to increased and chronic complement activation, which in turn can cause host tissue damage and might therefore contribute to the development of CRS.

Increasing evidence suggests CRS without and with NPs may be two distinct disease entities [1,2], in this regard, we compared

the CRS patients with and without NPs and found serum MBL concentrations were significantly higher in CRS patients with NPs than in CRS patients without NPs. In order to investigate whether the higher MBL concentrations in CRS patients with NPs resulted from higher prevalence of asthma, we compared the difference in MBL concentrations between non-asthmatic patients in CRS without NPs group and CRS with NPs group and found that non-asthmatic patents in CRS with NPs group also showed higher MBL levels than non-asthmatic patients in CRS without NPs group. It indicates that CRS patients with NPs have higher serum MBL concentrations than CRS patients without NPs despite the coexistence of asthma or not. So far the most important difference shown between CRS with NPs and CRS without NPs is the eosinophilic infiltration in CRS with NPs. CRS with NPs also shows more severe disease on CT scans and endoscopy. Therefore, we analyzed the correlation between MBL, peripheral eosinophil, CT scan and endoscopy findings. A significant correlation between MBL concentrations and peripheral eosinophil counts was found, suggesting that MBL may play a role in the eosinophil recruitment in CRS [20]. In addition, we found that MBL levels correlated with extent of disease seen on CT scan and endoscopy, confirming that MBL might play a modulatory role in the progression of CRS. Nevertheless, the causality of these activities in CRS requires further investigation. MBL and C3 are considered to be mainly derived from liver cells; however, recent studies demonstrated that MBL and C3 is also expressed in human sinonasal mucosa [8,10]. Therefore, whether there is altered MBL and C3 expression in sinonasal mucosa during CRS process and whether there is a correlation between local MBL and C3 production and serum levels need further study.

In conclusion, our study indicates that immunoglobulin, C3, C4 and MBL deficiencies alone or in combination are not the main cause of CRS in adult Chinese patients; on the contrary, increased serum C3 and MBL levels may play a modulatory role in CRS development and MBL might play a role in the eosinophil recruitment in CRS.

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Zheng Liu, M.D., Ph.D.
Department of Otolaryngology-Head and Neck Surgery
Tongji Hospital, Tongji Medical College
Huazhong University of Science and Technology
1095 Jiefang Avenue
Wuhan 430030, P.R. China

Tel: +86-27-83663334
E-mail: zhengliuent@hotmail.com