

A characteristic protein in nasal discharge differentiating non-allergic chronic rhinosinusitis from allergic rhinitis*

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SUMMARY

To differentiate non-allergic chronic rhinosinusitis (vasomotor rhinitis) from allergic rhinitis, a characteristic protein in the nasal discharge was studied. The subjects consisted of 10 patients with perennial allergic rhinitis to house dust, 10 patients without perennial rhinitis without antigen (clinically defined as non-allergic chronic rhinosinusitis) and 5 normal volunteers without nasal disease as a control group. The total protein in the nasal discharge was determined by Lowry's method and analysis of the protein components was made by SDS-PAGE. It was found that the nasal discharge obtained from the cases with perennial allergic rhinitis contained a high concentration of albumin (25.9 µg/ml) and a characteristic protein band with an estimated molecular weight of 26 kilo-Daltons (kD) on a SDS-PAGE, in a concentration of 15.8 µg/ml. In contrast, the nasal discharge from non-allergic chronic rhinosinusitis patients contained a lower concentration of albumin (12.9 µg/ml) than that of the allergic rhinitis patients, and the concentration of the characteristic protein 26kD was only 2.3 µg/ml. The 26kD protein was considered to originate from the nasal glands, since its secretion could be provoked by stimulation of the nasal glands of the normal volunteers with a 1% pilocarpine spray. The low concentration of albumin and the characteristic protein 26kD in the nasal discharge thus appeared to differentiate patients with non-allergic chronic rhinosinusitis from those with perennial allergic rhinitis.

Key words: non-allergic chronic rhinosinusitis, allergic rhinitis, nasal discharge, albumin, 26kD protein

INTRODUCTION

Although the pathogenesis of allergic rhinitis (AR) is different from non-allergic chronic rhinosinusitis (NACR), both diseases clinically show very similar symptoms, such as sneezing, watery discharge and nasal obstruction. AR is based on an antigen-antibody reaction, while NACR does not show an immunological reaction. NACR is a non-eosinophilic disease and is characterized by a non-specific reaction. In the past, NACR was called "vasomotor rhinitis", but that term has fallen out of favor recently.

In recent years, the pathological mechanism of AR has been well documented, while the pathophysiology of NACR remains obscure. The classical hypothesis was that NACR was caused by an autonomic imbalance (Malcomson, 1959): 1) hypoactivity of the sympathetic nervous system leading to nasal obstruction, and 2) hyperactivity of the parasympathetic nervous system leading to rhinorrhoea (Wentges, 1979). There is a report claiming that nasal itching and sneezing are less common in NACR than in AR (Togias, 1993).

At present, the differential diagnosis of NACR from AR is

based on the clinical features that its nasal symptoms are exacerbated by odors, alcohol, spicy foods, emotional activity and bright light. In fact, the diagnosis of AR is straight forward in all respects; the allergic history, skin reaction and detection of a specific IgE in the serum (Slavin, 1982; Mikaelian, 1989) while that of NACR is often derived from an exclusion of AR (Knight, 1995; Wilde et al, 1996). However, borderline cases may exist, these clinical methods are not always effective for a clear differentiation of NACR from AR. Moreover, there is no differentiating index for NACR and AR and there have been no studies clearly differentiating NACR from AR on a report basis, except for one in Japanese that AR can be differentiated from NACR (Iguchi, 1995). There has been no report on the difference in the components in the nasal wash between AR and NACR. Accordingly, we have called attention to the components in the nasal discharge, which is one of the common symptoms in AR and NACR. The present paper reports a study of additional cases to find characteristic biochemical parameters in the nasal discharge differentiating NACR from AR.

MATERIALS AND METHODS

Clinical Subject

The subjects of this study were 25 randomly selected patients with similar nasal symptoms of nasal discharge, nasal obstruction and sneezing, and with perennial rhinitis who visited the Nasal Allergy Clinic of Kitasato University from April to September 1997. At each visit, we conducted a routine otorhinolaryngological examination including nasal and paranasal X-ray examination, provocation test and radioallergosorbent test (RAST) in the serum. Patients with severe septum deviation and/or sinusitis were excluded. Twenty of the 25 patients completed the study without dropping out. They were divided into two groups based on the test results: 1) 10 cases of perennial allergic rhinitis (AR group; 6 males, 4 females, mean age 32.4 ± 16.2 years), and 2) 10 cases of non-allergic chronic rhinosinusitis (NACR group; 5 males, 5 females, mean age 37.7 ± 12.5 years). The main symptom of these 20 cases was watery nasal discharge. AR was diagnosed objectively by such findings, the paranasal X-ray was normal, a provocation test with a house dust (HD) disk was positive, and mite was positive in RAST. NACR was diagnosed based on the patient's history including lower sensitivity changes, a lower degree of nasal findings, negative findings on the provocation test and the eosinophil count in the nasal discharge. Five normal subjects (NOR) without nasal disease or nasal symptoms were selected as a control group (1 male, 4 females, mean age 22.7 ± 3.5 years). In addition, 6 other normal subjects were selected only for the pilocarpine spray test.

We obtained a written consent from all patients and volunteers. This study was done under the guidelines of the Ethical Committee at Kitasato University.

Collection of Nasal Discharge

The nasal discharge was vacuum-collected from each subject after introducing 10 ml of warm saline solution to the nasal cavity in one shot according to Proud (1983). The collection time was fixed to be between 10-11:00 a.m. in order to equate the physiological conditions. The collected nasal wash was centrifuged at 1200 $\times g$, and the supernatant fluid was analyzed. For comparison, blood plasma, tear fluid, and saliva were also collected from each subject and analyzed.

Measurement of Total Protein Concentration

A quantitative analysis of the protein content in the obtained samples was made according to Lowry's method (1951). The protein concentration in 1 ml of each sample was obtained with reference to 1 mg/ml bovine albumin used as a standard.

Measurement of Components

A component analysis of the protein contents was made by means of Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) using the method of Laemmli (1970). A slab of 10% polyacrylamide gel was used for separation, with on top a 3% polyacrylamide stacking gel. The specimens were

mixed with 2% sample buffer containing SDS. Fifteen μg of this mixture was applied to the slots of the gels which were connected to Laemmli's electrode buffer, and a current of 20 mA was applied for 2 hours. After the completion of electrophoresis, the proteins on the gels were stained with a silver stain kit, and the protein bands were measured by absorbance at 500 nm using a dual wavelength scanner, and percentage distribution of each band was obtained.

Measurement of the 26kD protein in the nasal wash obtained from normal subjects by pilocarpine spray

To estimate the main origin of the 26kD protein in the nasal discharge, the nasal washes from 6 additional normal subjects were collected before and 5 minutes after a 1% pilocarpine solution was sprayed into the nasal cavity. The 26kD protein percentage was measured before and after the spray test with SDS-PAGE.

Statistical analysis

The results of the concentration of total protein, albumin and the 26kD protein were statistically analyzed (unpaired t-test). The results of pilocarpine spray test were also statistically analyzed (paired t-test). A p-value of less than 0.05 was considered to be statistically significant. The values are shown as mean \pm SD.

RESULTS

After loading 10 ml of saline to the nasal cavity, the average amount recovered in the AR group was 9.0 ± 0.9 ml, and that for the NACR group was 8.0 ± 1.3 ml. The difference was not statistically significant.

As a result of the electrophoretic study on the nasal washes (Figure 1), an albumin band and a protein band with an estimated molecular weight of nearly 26kD were recognized. The molecular weight of the 26kD band was defined based on a plotted graph of a simultaneous electrophoretic analysis of standard samples. The 26kD protein was neither present in tears or saliva that were diluted 500 times, nor in 100 times diluted plasma. On the other hand, its content in the nasal wash increased after 1% pilocarpine was sprayed into the nose, as shown in Figure 2. To be exact, the 26kD protein percentage in the pre-spray sample was $1.2 \pm 0.1\%$ of the total protein amount, whereas in the post-spray sample it was $3.4 \pm 1.8\%$. This difference was statistically significant ($p < 0.01$).

Total Protein

Figure 3 shows the total protein concentration per ml of nasal wash. The AR group showed a high average concentration ($101.7 \pm 30.0 \mu g/ml$). The average protein concentration of the NACR group ($56.3 \pm 19.9 \mu g/ml$) was significantly lower than that of the AR group ($p < 0.01$). Between the normal controls (NOR) ($32.4 \pm 10.9 \mu g/ml$) and the NACR group, the difference was statistically significant ($p < 0.05$).

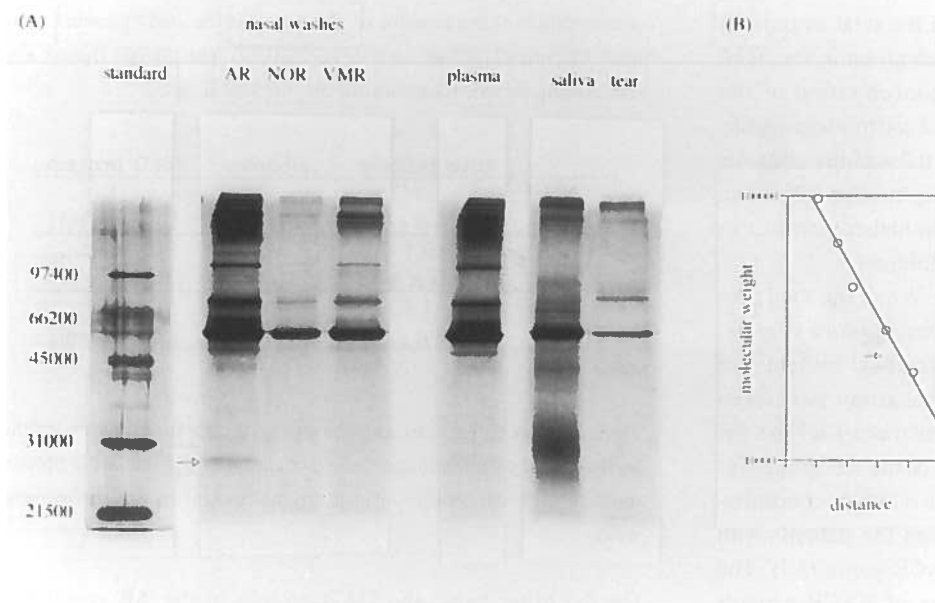


Figure 1. Detection of a 26kD protein by Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE) (A) and the plotted graph of standard protein samples for measurement of the molecular weight of the protein (B). Plasma samples were diluted 500 times with saline. The original fluid from the nasal wash, saliva and tears were applied in the gel slots. Fifteen μ l of each sample was applied. AR: Allergic rhinitis; NOR: normal subjects; NACR; non-allergic chronic rhinosinusitis. Standard: standard proteins with known molecular weight. 26kD protein band. There is a linear relationship between the molecular weight of the standard proteins and the distance of their bands in the gel.

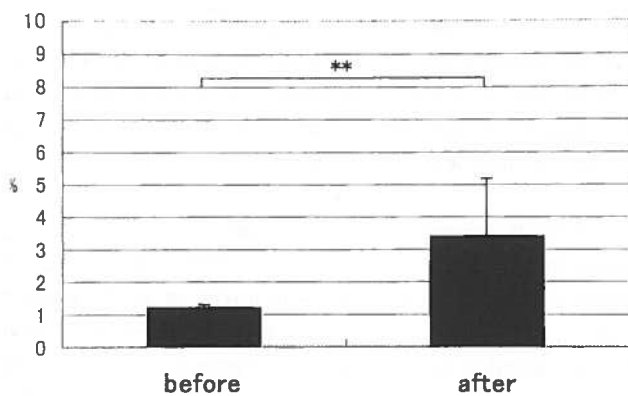


Figure 2. The 26kD protein content in the nasal discharge before and after 1% pilocarpine was sprayed into the nasal cavity of normal subjects. The ordinate indicates the percentage of the 26kD protein in the total protein amounts. The values are shown as the mean \pm SD of the 5 volunteers. **: $p < 0.01$

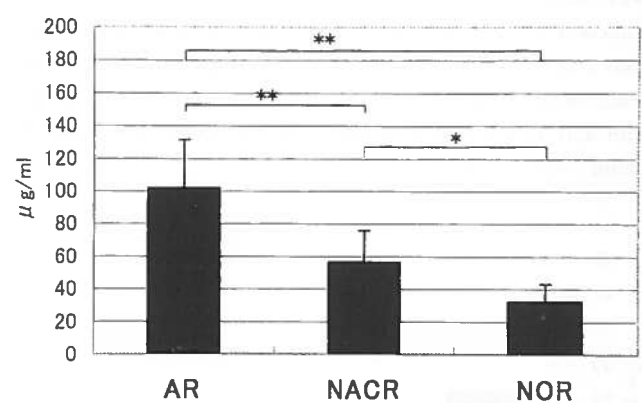


Figure 3. The total protein concentration in the nasal wash. The ordinate indicates the total protein concentration in the nasal wash. AR: allergic rhinitis; NACR: non-allergic chronic rhinosinusitis; NOR: normal control. The values are shown as the mean \pm SD of the 10 patients with AR and NACR and of the 5 normal subjects. *: $p < 0.05$, **: $p < 0.01$

Albumin

Figure 4 shows the concentration of albumin in the entire amount of protein electrophoretically analyzed for each group. In the AR group ($25.9 \pm 10.0 \mu\text{g/ml}$), a significant increase was seen compared to the NOR group ($3.5 \pm 1.7 \mu\text{g/ml}$) ($p < 0.01$). The value in the NACR group ($12.9 \pm 9.2 \mu\text{g/ml}$) was significantly lower than that of the AR group ($p < 0.01$) and larger than that of the NOR controls ($p < 0.05$).

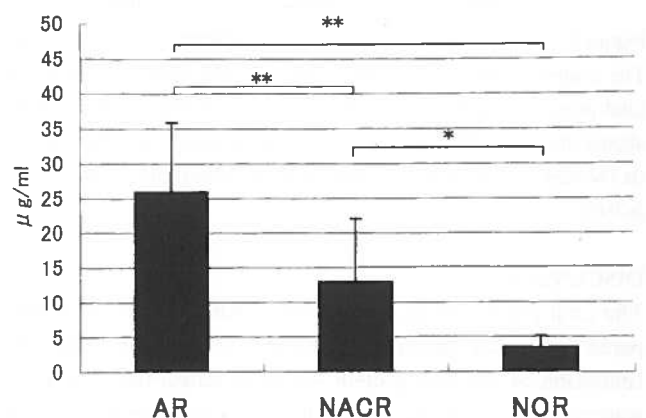


Figure 4. The concentration of albumin in the nasal wash. The ordinate indicates the concentration of albumin in the total protein concentration of the nasal wash of 10 the patients with allergic rhinitis (AR), those with non-allergic chronic rhinosinusitis (NACR) and of the 5 normal subjects. Mean \pm SD **: $p < 0.01$.

The 26kD protein

The concentration of the 26kD protein in the total amount of protein electrophoretically analyzed in each group in the nasal wash is shown Figure 5. The average concentration of the 26kD protein in the AR group ($15.8 \pm 13.2 \mu\text{g/ml}$) was significantly higher than in the NOR ($0.5 \pm 0.7 \mu\text{g/ml}$) controls ($p < 0.01$) or the NACR group ($2.3 \pm 2.6 \mu\text{g/ml}$) ($p < 0.05$). The 26kD protein in the NACR group was also higher than that in the NOR controls, but not significantly different.

These results are summarized in Table 1. When the total protein concentration ($32.4 \mu\text{g/ml}$), the concentration of albumin ($3.5 \mu\text{g/ml}$) and the concentration of the 26kD protein ($0.5 \mu\text{g/ml}$) in the nasal discharge of the normal group were taken as 1, the ratio for the total protein amounts was 3.1 for the AR group and 1.7 for the NACR group. That of the AR group was larger than that of the NACR group. The albumin concentration ratio in the nasal wash obtained from the patients with AR (25.9) was larger than that of the NACR group (3.7). The 26kD protein ratio in the nasal discharge of NACR patients was much less (4.6) than that of AR patients (31.6). This result indicates that the AR patients secreted a large amount of albumin and the 26kD protein during washing of the nasal cavity, whereas the NACR patients secreted less amounts of total protein and albumin. The 26kD protein ratio in their nasal discharge was only 4.6.

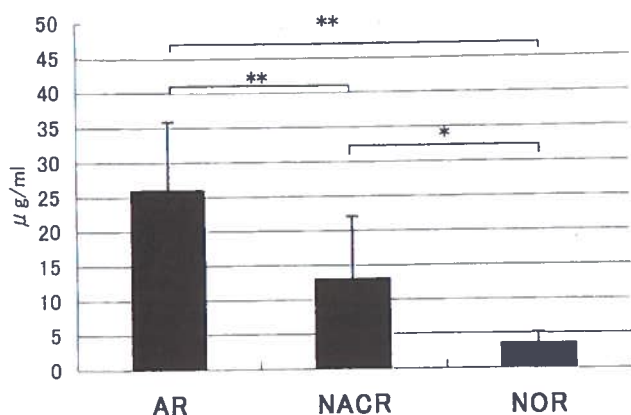


Figure 5. The concentration of the 26kD protein in the nasal wash. The ordinate indicates the concentration of the 26kD protein in the total protein concentration of the nasal wash of the 10 patients with allergic rhinitis (AR), and those with non-allergic chronic rhinosinusitis (NACR) and of the 5 normal subjects. Mean \pm SD *: $p < 0.05$, **: $p < 0.01$.

DISCUSSION

The total protein in the nasal wash in AR was increased compared to normal controls and patients with NACR. The concentration of the total protein seems to reflect the amount of watery nasal discharge. The albumin concentration in AR patients was higher than that in the normal controls or that in the NACR patients. Therefore, we feel that the difference in the amount of albumin can distinguish NACR and/or NOR from AR.

Table 1. Relative ratio of the concentration of the total protein, of the concentration of the amount of albumin and the 26kD protein in the nasal wash from normal controls and patients with allergic rhinitis and non-allergic chronic rhinosinusitis. ns: not significant.

	total protein	albumin	26kD protein
NOR	1 (32.4 $\mu\text{g/ml}$)	1 (3.5 $\mu\text{g/ml}$)	1 (0.5 $\mu\text{g/ml}$)
AR	3.1 (101.7 $\mu\text{g/ml}$)	7.4 (25.9 $\mu\text{g/ml}$)	31.6 (15.8 $\mu\text{g/ml}$)
NACR	1.7 (56.3 $\mu\text{g/ml}$)	3.7 (12.9 $\mu\text{g/ml}$)	4.6 (2.3 $\mu\text{g/ml}$)

The values in parenthesis indicate their concentration relative to the total amount of protein and those for albumin and the 26kD protein indicate their concentration in the total amount of protein in nasal wash.

On the other hand, the 26kD protein in the AR group was markedly increased compared to the NACR group. If the concentration of the 26kD protein was smaller than $2.3 \mu\text{g/ml}$, then the sample could be assumed to be from a NACR patient or NOR control. The differentiation of NACR and NOR could be judged by the albumin concentration and the difference in the nasal findings and symptoms.

Although the existence of the 26kD protein in the nasal discharge has been reported (Itoh et al., 1993), the molecular structure of the 26kD protein is still unknown. In the present study, it was shown that this 26kD protein was secreted from the nasal glands into the nasal fluid of normal volunteers after stimulation of the nasal glands by a 1% pilocarpine spray. This result excludes that the 26kD protein was secreted from the nasal mast cells, since cholinergic stimulation by intradermal injection of acetylcholine into rats does not release histamine from mast cells, unless an extremely large concentration of acetylcholine is used (Kawana and Katori, 1980). The possibility that the 26kD protein is a fragment of the immunoglobulin light chain can also be excluded, since the 26kD protein was secreted in normal subjects as well after pilocarpine stimulation. The chemical structure of the 26kD protein should be analysed in the future.

We consider the possibility that the concentration of albumin is increased in the nasal discharge is mainly caused by the vascular permeability of the nasal mucosa, because albumin can only be synthesized in the liver. On the other hand, the 26kD protein appears to be the result of gland secretion, since its secretion was accelerated by pilocarpine. The fact that the concentrations of albumin and the 26kD protein were higher in the AR group was considered to be due to an acceleration in both vascular permeability and gland secretion. In the case of the NACR group, the factor of vascular permeability might be accelerated over NOR controls, whereas the acceleration of

gland secretion was minimal and the 26kD protein concentration remained below 2.3 $\mu\text{g/ml}$. Thus, it can be concluded that the estimation of the concentration of the 26kD protein in the nasal discharge is useful for an objective differentiation of NACR from AR, and a lower concentration (below 2.3 $\mu\text{g/ml}$) in the nasal discharge may exclude AR and allow the diagnose of NACR.

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