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## Elevated soluble tumor necrosis factor receptor levels in seasonal allergic rhinitis patients

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In this study, we examined the symptom scores and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), p55 soluble tumor necrosis factor receptor (sTNFR1), and p75 soluble tumor necrosis factor receptor (sTNFR2) levels in the sera and nasal epithelial lining fluids (ELF) of 20 patients with Japanese cedar pollinosis from the pre- to the postseason period, and compared the results with those of 10 nonallergic control subjects. The symptom scores of the allergic subjects were significantly ( $P < 0.01$ ) higher than those of the nonallergic subjects during the early stage and mid-stage of the season. There were no statistical differences between the allergic and nonallergic subjects in the TNF- $\alpha$  levels in sera and ELF from the pre- to the post-season. In the allergic subjects, however, the levels of sTNFR1 and sTNFR2 in ELF were significantly elevated during the early stage ( $P < 0.05$ ) and mid-stage ( $P < 0.01$ ) of the season, whereas those in sera did not change from the pre- to the post-season period. The levels of TNF- $\alpha$  in ELF were more than 10 times higher than those in sera, whereas the levels of sTNFR1 and sTNFR2 in ELF were less than half of those in sera in the allergic and nonallergic subjects. These results suggest that sTNFR1 and sTNFR2 may play a role in the pathogenesis of nasal allergic reaction.

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According to the latest research, the nasal allergic response is characterized by the transendothelial migration and activation of various cell populations after allergen provocation (1–5). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) affects both these reactions (1, 6–9). Therefore, TNF- $\alpha$  is assumed to play an important role in the pathogenesis of allergic rhinitis. It has been reported that mRNA expression of TNF- $\alpha$  is

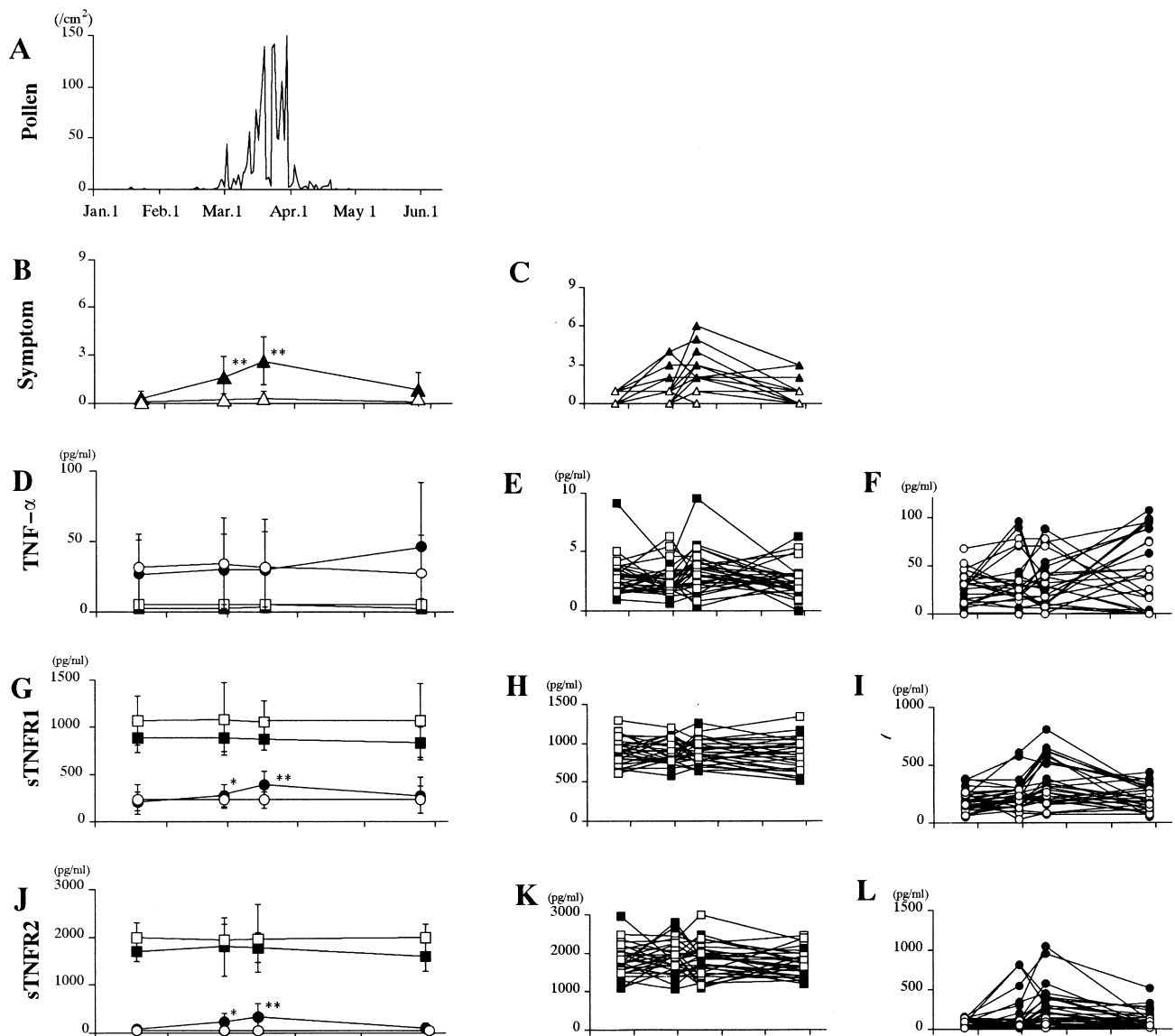


Fig. 1. Dynamics of systemic and local levels of TNF- $\alpha$  and sTNFRs, total subjective symptom scores, and pollen counts. Panel A shows Japanese cedar pollen counts in city of Nagoya. Panels B and C show symptom scores in control ( $\Delta$ ) and allergic ( $\blacktriangle$ ) groups. Panels D–L show levels of TNF- $\alpha$  in sera (D, E;  $\square$  and  $\blacksquare$ ) and ELF (D, F;  $\circ$  and  $\bullet$ ), sTNFR1 in sera (G, H;  $\square$  and  $\blacksquare$ ) and ELF (G, I;  $\circ$  and  $\bullet$ ), and sTNFR2 in sera (J, K;  $\square$  and  $\blacksquare$ ) and ELF (J, L;  $\circ$  and  $\bullet$ ) in control ( $\square$  and  $\circ$ ) and allergic ( $\blacksquare$  and  $\bullet$ ) groups. Each point in panels B, D, G, and J shows mean  $\pm$  standard deviation. Values marked \* ( $P<0.05$ ) and \*\* ( $P<0.01$ ) in allergic group are significantly different from those in control group. Panels C, E, H, K, F, I, and L show subject-by-subject levels.

enhanced by the administration of antigen in nasal mucosal samples of patients with allergic rhinitis (10). However, there are few data on levels of TNF- $\alpha$  in sera and nasal epithelial lining fluids (ELF) from patients with allergic rhinitis under natural allergen provocation.

Recently, researchers have identified naturally occurring inhibitors of TNF- $\alpha$  that block inflammatory changes induced by TNF- $\alpha$  *in vitro* and *in vivo* (11, 12). Current *in vitro* data support a greater inhibitory role for p55 soluble tumor necrosis factor receptor (sTNFR1) than for p75 soluble tumor necrosis factor receptor (sTNFR2) in neutralizing the effects of TNF- $\alpha$  (13, 14). sTNFR protected mice from

lipopolysaccharide-induced lethality (15), and prevented chronic relapsing experimental autoimmune encephalomyelitis (16). These observations suggest that sTNFR may play a key role as a regulator of inflammation. To our knowledge, however, there are no data on systemic and local levels of sTNFR in the patients with nasal allergic inflammation.

In this study, we have measured systemic and local levels of TNF- $\alpha$ , sTNFR1, and sTNFR2, together with subjective symptom scores in patients with seasonal allergic rhinitis, as well as in healthy subjects from the pre- to the postseason period. The data obtained shed new light on the correlation between sTNFR and nasal allergic inflammation.

## Material and methods

### Study design

The allergic group ( $n=20$ ; mean age, 34 years) comprised patients who had a history of Japanese cedar pollen-induced rhinitis and strongly positive radioallergosorbent test (RAST) for only Japanese cedar pollen. They had no other organic manifestations of their allergic disease during the study. The control group ( $n=10$ ; mean age 35 years) comprised volunteers who had no history of allergic or nasal disease. The trial was approved by Nagoya University Branch Hospital (Japan), and each subject gave written informed consent before entering the study. No drugs were permitted for the allergic and control groups during the study, including a period of 3 weeks before the study. Scores of subjective symptoms, and the levels of TNF- $\alpha$ , sTNFR1, sTNFR2, and urea in sera and nasal lavage fluids were examined during the preseason period (22 January), the early stage of the season (28 February), the mid-stage of the season (18 March), and the postseason period (27 May) in both groups, and were compared with each other intragroup or intergroup. According to Kaulbach et al., the plasma, interstitial, glandular, and ELF urea concentrations are invariable, whether or not allergic rhinitis is induced by challenge test (17). Therefore, we used urea as a reliable marker for correction of the parameters measured in nasal lavage fluid. The levels of each parameter in nasal lavage fluids were compared after multiplication by a dilution factor ( $[\text{Urea}]_{\text{serum}}(\text{mg/dl})/[\text{Urea}]_{\text{nasal}}(\text{mg/dl})$ ) and revised to the levels in ELF, as described previously (2–5).

### Nasal lavage procedure

Collections of nasal lavage were performed as described previously (2). A volume of 6 ml of isotonic saline solution (0.9%) was instilled into the right nostril with a pipette while subjects gently flexed their heads backward. Subjects maintained this position without swallowing for a few seconds, and then leaned their heads forward and expelled nasal lavage fluid into a collection vessel. The obtained lavage fluids and sera which were separated from blood were immediately stored at  $-30^{\circ}\text{C}$  until analysis. All the samples were collected from 9 a.m. to 10 a.m.

### Analytic assays

TNF- $\alpha$ , sTNFR1, and sTNFR2 levels in sera and nasal lavage fluids were measured by commercial enzyme-linked im-

munosorbent assay (ELISA) kit (human TNF- $\alpha$  immunoassay kit: R&D Systems, Inc.; USA; human sTNFR1 immunoassay kit: R&D Systems; human sTNFR2 immunoassay kit: R&D Systems), according to the manufacturer's directions for use. Urea in sera and nasal lavage fluids was measured with the Merck auto-UN kit (Kanto Chemical Co, Tokyo, Japan) by the method of Hallett & Cook (18).

### Assessment of symptoms

Subjective symptoms identified by questionnaire consisted of nasal blockage, secretion, and sneezing, as described previously (19, 20). The four-point symptom scale was as follows: 0, no symptoms; 1, mild; 2, moderate; and 3, severe. The sum of each individual's symptom score (full mark: 9 points) was tallied for estimating total individual subjective symptoms.

### Statistical analysis

The changes in all parameters from the pre- to the postseason period in the allergic group compared with that of control group were analyzed with the Mann–Whitney U test. Correlations among the parameters (symptom scores and levels of TNF- $\alpha$ , sTNFR1, and sTNFR2) were carried out with Spearman's rank correlation coefficient.

## Results

### Pollen counts

As shown in Fig. 1A, the airborne Japanese cedar pollen counts in Nagoya, Japan, were reported by the Aichi Prefecture Department of Health.

### Subjective symptoms

Scores of subjective symptoms of the allergic group were significantly higher ( $P<0.01$ ) than those of the control group during the early stage and mid-stage of the season (Fig. 1B).

### Levels of TNF- $\alpha$ , sTNFR1, and sTNFR2

The levels of TNF- $\alpha$  in sera and ELF did not change from the pre- to the postseason period (Fig. 1D, E, F). The levels of sTNFR1 and sTNFR2 in ELF of the allergic group were significantly higher than those of the control group during the early stage ( $P<0.05$ ) and mid-stage of the season ( $P<0.01$ ) (Fig. 1G, I, J, L). There were no statistical differences

between the two groups in the levels of sTNFR<sub>1</sub> and sTNFR<sub>2</sub> in ELF during the pre- and postseason period. The levels of sTNFR<sub>1</sub> and sTNFR<sub>2</sub> in sera from the allergic group were slightly lower than those from the control group, whereas no statistical differences between the two groups could be found from the pre- to the postseason period (Fig. 1G, H, J, K). The levels of TNF- $\alpha$  in ELF were more than 10 times higher than those in sera, whereas the levels of sTNFRs in ELF were less than half of those in sera in the control and allergic groups.

### Correlation among the parameters

In this study, we could find no statistically significant correlation among the parameters (symptom scores and levels of TNF- $\alpha$ , sTNFR<sub>1</sub>, and sTNFR<sub>2</sub>) in the control and allergic groups from the pre- to the postseason period.

## Discussion

In this study, we have demonstrated the dynamics of systemic and local TNF- $\alpha$  and sTNFRs levels from the pre- to the postseason period in patients with seasonal allergic rhinitis under natural allergen provocation.

Bachert et al. reported that there are no differences between grass pollen-allergic subjects and the normal subjects in the levels of TNF- $\alpha$  in nasal lavage, whereas the levels of other cytokines (interleukin [IL]-1 $\beta$ , IL-4, IL-6, and IL-8) from allergic subjects are significantly higher than those from the normal subjects (1). These data partially correspond to our data on the dynamics of the TNF- $\alpha$  levels in allergic subjects.

Increased sTNFR levels have been reported in patients with sepsis, meningitis, and experimental endotoxemia (13, 21). In this study, we displayed elevation of local sTNFRs levels during the season in patients with seasonal allergic rhinitis, whereas the systemic TNF- $\alpha$  and sTNFRs levels in the nasal allergic subjects were slightly lower than those in the healthy subjects. Our results partially correspond to the previous reports of increased expression levels of TNFR in nasal mucosa

from patients with allergic rhinitis (22) and no differences in plasma levels of TNF- $\alpha$  and sTNFRs between atopic and nonatopic children during a stable phase of allergic disease (23). Our results and these reports suggest that sTNFRs may be involved in the pathogenesis of allergic disease.

However, it remains unknown why there was dissociation of the local levels of TNF- $\alpha$  from the local levels of sTNFRs during the season in the patients with seasonal allergic rhinitis. It has been reported that sTNFRs inhibit patient immunity via specific binding with the TNF- $\alpha$  molecule (11–13). Intratracheal administration of sTNFR also inhibits acute pulmonary inflammation induced by endotoxin in the respiratory system (24). Therefore, it is possible that sTNFRs actively bind TNF- $\alpha$  molecules, and locally downregulate them in patients with allergic rhinitis. Downregulation of the local levels of sTNFRs and slight upregulation of the local levels of TNF- $\alpha$  during the postseason period in allergic subjects might indirectly support this hypothesis.

However, in addition to the possibility that we may not have correctly measured the levels of TNF- $\alpha$  and sTNFR due to their complex formation, more study is needed to clarify the role of TNF- $\alpha$  and sTNFRs in the pathogenesis of allergic rhinitis. On the other hand, systemic levels of TNF- $\alpha$  were stable, possibly because of the presence of high levels of regulatory sTNFR in sera from the pre- to the postseason period. Such site-oriented differential regulation of TNF- $\alpha$  by sTNFR should make TNF- $\alpha$  work locally rather than systemically. A naturally occurring antagonistic system in nasal mucosa was also reported for IL-1 and the IL-1 receptor antagonist system (25). This system is believed to regulate nasal allergic inflammation through control of local adhesion receptor expression.

In short, this study discovered a new aspect of the complex pathogenesis of allergic rhinitis under natural allergen provocation by investigating the systemic and local levels of TNF- $\alpha$  and its soluble receptor.

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