

Seasonal changes of proapoptotic soluble Fas ligand level in allergic rhinitis combined with asthma

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The function of apoptosis is to eliminate unnecessary or dangerous cells. The balance between production and death is important in the control of cell numbers within physiological ranges. Cells involved in allergic reactions may have altered apoptosis. The aim of this study was to examine the seasonal changes of programmed cell death in children with pollen allergy. We measured serum levels of soluble Fas (sFas) and soluble Fas ligand (sFasL), and examined whether there was any correlation between soluble apoptosis markers and development of asthma and or rhinitis in children with pollen allergy. We examined two groups of patients with ragweed pollen allergy. The first group consisted of 17 children with 'rhinitis only'. The second group consisted of 16 children with 'asthma + rhinitis'. For seasonal analysis we pooled the two groups and termed this the 'ragweed sensitive' group ($n = 33$, 5–18 yr, 25 boys, eight girls). Measurements (sFas and sFasL) were taken during the ragweed pollen allergy season, while control measurements were performed during the symptom-free period. There was no difference in sFas levels measured during and after [1941 $\pm$ 68, 1963 $\pm$ 83 pg/ml (mean $\pm$ s.e.m, respectively)] the pollen season in the 'ragweed sensitive' group. The sFasL level showed seasonal change, which was significantly higher ($p = 0.0086$) in the symptomatic period compared to the symptom-free state (99 $\pm$ 13 and 53 $\pm$ 16 pg/ml, respectively). There was a difference between the 'rhinitis only' and the 'asthma + rhinitis' groups in the measured parameters of apoptosis. Children having allergic rhinitis combined with asthma had a significantly ($p = 0.03$) higher sFas level in the symptom-free state than the 'rhinitis only' group did (2115 $\pm$ 156 and 1820 $\pm$ 52 pg/ml, respectively). During the allergic symptom state the sFasL level of the 'asthma + rhinitis' group was significantly higher ($p = 0.025$) than that of the 'rhinitis only' group (125 $\pm$ 20 and 75 $\pm$ 14 pg/ml, respectively). In conclusion, the increased level of sFasL during the pollen season may signal its role in the pathogenesis of allergic airway diseases. There was no seasonal change in sFas levels in the examined ragweed allergic group, however in the symptomatic period we observed a diminished level of antiapoptotic factor (sFas) and an elevated level of proapoptotic factor (sFasL) if there was a combined disease with pollen allergic asthma. We suggest that there is a deviation in the apoptotic reaction in children that may increase the seasonal allergic inflammation.

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Eosinophilic inflammation of the airways and T helper (Th)1–Th2 imbalance are key characteristics of allergic diseases. Several studies have suggested that ineffective apoptosis of eosinophil granulocytes and allergen activated T lymphocytes plays a role in the persistence of chronic allergic inflammation (1–7). The Th1–Th2 imbalance can be caused by differences in apoptosis of Th1 and Th2 lymphocytes. Defective apoptosis of the Th2 cells as well as the increased apoptosis of Th1 cells may lead to Th2 dominance (8, 9).

The Fas-mediated signal transduction pathway plays an important role in the apoptotic process. The soluble or cell surface soluble FAS ligand (FasL) induces apoptosis binding to the cell surface Fas receptor.

Fas can occur as a soluble protein as well as a cell-surface receptor (10). Soluble Fas (sFas) is generated by an alternative mRNA splicing event (10, 11), and it was reported to antagonize both anti-Fas and Fas ligand-mediated killing in a dose-dependent manner (10, 12, 13).

The cell surface expression of Fas and FasL in allergy has been widely examined (14–20). The soluble apoptotic markers in atopic diseases have been less extensively studied. Kato et al. has found that allergic rhinitis is a sFas-reduced disease (21, 22) compared to vasomotor rhinitis and to isolated bronchial asthma.

To our knowledge there has been no relevant data in the medical literature concerning the seasonal change of the soluble apoptotic markers (sFas and sFasL) in children suffering from allergic rhinitis and allergic bronchial asthma.

Material and methods

Patients

Patients were enrolled in the study after informed consent from the parents. All patients fulfilled the following inclusion criteria: (i) Children had a history of seasonal allergic rhinitis with or without asthma for at least the past 2 yr. (ii) The diagnosis of ragweed pollen allergy was based on skin prick test or serum ragweed pollen specific IgE levels. (iii) The children developed significant allergic symptoms from August to the end of September, which is the ragweed pollen season in Hungary. (iv) The children were treated with symptomatic medication and follow-up examinations were made regularly in our Department. (v) The first examinations were performed and blood samples were collected in the pollen season, the follow-up measurements were made from November to January (during this period there was no airborne ragweed or other signifi-

cant pollen in Hungary). The symptom severity score of the patients consists of runny nose, nasal obstruction, itchy nose, sneezing, watery eyes, redness of eyes, itching of eyes and cough (scores: 0, symptom absent; 1, mild, present but not annoying for the patient; 2, moderate, present and annoying for the child but does not interfere with sleep or daily activities; 3, severe, interferes with/or unable to sleep or carry out daily activities). The symptom score for asthma consists of night time and/or daytime difficulty in breathing, wheezing, exercise induced bronchospasm. All patients with bronchial asthma had a history of wheezing and met the diagnostic criteria for bronchial asthma of global initiative for asthma (GINA). None of the examined children were in asthmatic attack during the examination. In August, there is usually no febrile epidemic in children.

Criteria for exclusions: (i) Main allergen is other than ragweed. The children with mainly perennial rhinitis were excluded. (ii) Systemic steroid treatment during the last year. (iii) Children with fever or with symptoms of viral infection. We measured qualitative and quantitative blood test and smear for nasal cytology. (iv) Runny nose and stuffiness with cough was considered 'rhinitis only' if the child had no history of wheezing/difficulty in breathing/asthmatic attack. Our patients were screened for lower airways symptoms, however we did not find any ragweed pollen allergic child, who had 'only asthma', as all the ragweed allergic children had rhinitis. The characteristics of baseline data of the included children can be seen in Table 1. In the 'rhinitis only' group ($n = 17$) the children had allergic rhinitis without asthma. In the 'asthma + rhinitis' group everybody had symptoms of asthma and rhinitis caused by ragweed pollen. The two groups were pooled ('ragweed sensitive' $n = 33$) also, for the analysis of the seasonal changes.

Methods

The forced expiratory volume in the first second of expiration (FEV_1) was measured using a Custo med instrument in asthmatic children. 'In-check' portable inspiratory flow meter (Clement Clarke International Limited, Harlow, Essex, UK) was used for measurement of peak nasal inspiratory flow (PNIF) rate.

The following parameters were measured: the absolute eosinophil blood count (count/mm^3) using May Grünwald Giemsa staining. Serum total IgE determination (AMX) was made using our routine method. Serum sFas and sFasL levels

Table 1. Characteristics of the subgroups of 33 ragweed pollen allergic children

	Rhinitis only (n = 17)	Asthma + rhinitis (n = 16)
Age (mean $\pm$ s.e.m, yr)	10.3 $\pm$ 0.01	10.8 $\pm$ 0.86
BMI (mean $\pm$ s.e.m)	18.3 $\pm$ 0.79	18.6 $\pm$ 0.80
Male/female (total: 25/8)	10/7	15/1
Rhinoconjunctivitis severity symptom score (mean $\pm$ s.e.m)		
During season	9 $\pm$ 1.31	12.7 $\pm$ 3.08
Out of season	1.2 $\pm$ 0.51	1.8 $\pm$ 0.55
Asthma severity score (n)		Intermittent: 11, mild persistent: 3, moderate persistent: 2
Medication at baseline (n)		
Oral antihistamine	5	7
Intranasal corticosteroid	3	3
Topical antiallergic, eye drops	1	1
Topical antiallergic, nasal	0	0
Only short acting betamimetic (prn)	0	11
Inhaled corticosteroid	—	5
Long acting beta2 agonist	—	2
PNIF, l/min, mean $\pm$ s.e.m		
During season	*58.1 $\pm$ 8.38	82.3 $\pm$ 12.82
Out of season	*76.3 $\pm$ 5.69	92.3 $\pm$ 12.46
FEV ₁ (percent of predicted norm) mean $\pm$ s.e.m.		
During season		**85.9 $\pm$ 7.77
Out of season		**98.8 $\pm$ 4.1
Eosinophil count, pc/mm ³ , median (25–75% interquartile range)		
During season	***694 (363–867)	****876 (379–1020)
Out of season	***204 (0–354)	****250 (197–404)
Total IgE, kU/l, median (25–75% interquartile range)		
†During season	182 (102–269)	939 (227–1352)
Out of season	267 (116–514)	967 (243–1365)

*The peak nasal inspiratory flow (PNIF) is significantly higher out of the season in the 'rhinitis only' group $p = 0.02$ (Paired t -test).

**The forced expiratory volume (FEV₁) is significantly higher out of the season in the 'asthma + rhinitis group' $p = 0.018$ (Paired t -test).

The eosinophil count is higher during the season than out of season in both of the groups (Wilcoxon matched pairs test).

***In the 'rhinitis only' group $p = 0.002$.

****In the 'asthma + rhinitis' group $p = 0.04$.

†Total IgE is higher in 'asthma + rhinitis' group, than in 'rhinitis only', during season, $p = 0.03$ (Mann–Whitney U -test).

were measured by sandwich enzyme-linked immunosorbent assay (ELISA; Immunotech, Heidelberg, Germany). In the young patients interest only one blood sample was taken at the peak of the symptoms and one after the pollen season in symptom free period.

General findings. The peak nasal inspiratory flow (Table 1) was lower at the time of symptoms of allergic rhinitis compared with the symptom-free condition for all examined patients. The allergic 'rhinitis only' group showed a significant increase in PNIF values ($p = 0.02$) after the season. The increase of FEV₁ was significant after the pollen season in 'asthma + rhinitis' patients ($p = 0.018$).

The eosinophil count (Table 1) decreased significantly in the symptom-free state in both groups ('rhinitis only' group $p = 0.002$, 'asthma + rhinitis' group $p = 0.04$). Serum total IgE was significantly ($p = 0.03$) higher in season

in asthmatic patients than in the group without asthma, but did not change with the season.

Statistics

The results are described by mean and standard error of mean (s.e.m) or by median and 25–75 percentile for non normal values. The Statistica statistical package was used to perform the analysis. Comparisons between groups were analysed using paired an unpaired t -test, the Mann–Whitney U -test. Paired data were analysed using the Wilcoxon signed ranked test. The results were considered as significant if $p < 0.05$.

Results

Seasonal changes in sFas and sFas ligand

There was no difference in sFas levels during (1941 ± 68 pg/ml) and after the pollen season

(1963 ± 83 pg/ml) in the 'ragweed sensitive' group. In the group of 'asthma + rhinitis' the sFas level also did not change with the season, however it was somewhat lower during the symptomatic than in the symptom-free state (Fig. 1).

The sFasL level showed seasonal changes. It was significantly higher ($p = 0.0086$) in the 'ragweed sensitive' group when symptoms were present compared with the symptom-free period (99 ± 13 and 53 ± 16 pg/ml, respectively). In the group 'asthma + rhinitis' the FasL changed according to the season (Fig. 2), it was significantly higher in the symptomatic than in the symptom-free phase of the disease (125 ± 20 and 32 ± 20 , respectively, $p = 0.000017$), as was the case in the 'ragweed sensitive' group.

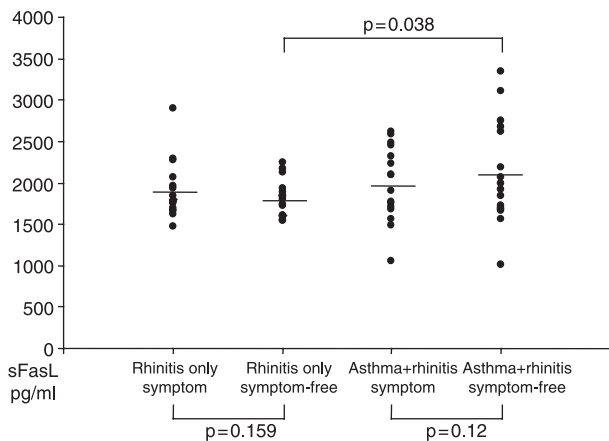


Fig. 1. Soluble Fas (pg/ml) levels in the different allergy groups ('rhinitis only', and 'asthma + rhinitis') in symptomatic and in symptom-free periods.

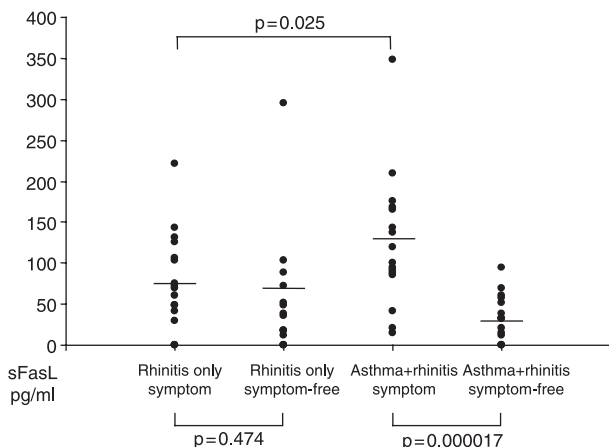


Fig. 2. Soluble Fas ligand levels in the different groups ('rhinitis only', and 'asthma + rhinitis') in symptomatic and in symptom-free periods.

The level of sFasL diminished or did not change out of the season in 16 of 16 cases in the subgroup of 'asthma + rhinitis' and in 13 of 17 cases in the subgroup of 'rhinitis only' patients.

'Asthma + rhinitis' compared with 'rhinitis only'

We found differences in the levels of sFas and sFasL between 'asthma + rhinitis' and 'rhinitis only' groups. Children in the 'asthma + rhinitis' group had higher sFas ($p = 0.03$) during the symptom-free state than the 'rhinitis only' group did (2115 ± 156 and 1820 ± 52 pg/ml, respectively), and they had higher ($p = 0.02$) levels of sFasL during the symptomatic period than the 'rhinitis only' group (125 ± 20 and 75 ± 14 pg/ml, respectively, Figs 1 and 2). There was no difference in the sFasL level between asthma and rhinitis in the symptom-free phase (Fig. 2).

Discussion

The cell surface and soluble factors involved in programmed cell death have been examined in adult allergic rhinitis, whereas, to our knowledge they have not been characterized in children yet.

T-helper 2 biased peripheral immune response appears to be an important pathogenetic factor in allergic inflammation. The persistent allergic inflammation could originate not only from the delayed eosinophil apoptosis, but the Th2 predominance may also be due to the preferential apoptosis of circulating memory/effector Th1 cells, as described by Akdis et al. in atopic dermatitis (9).

Similarly to Kato et al., we did not find seasonal changes in sFas levels in children with allergic rhinitis (22). Kato et al. found a significant upregulation in sFas levels during the stable phase and downregulation during the attack phase in patients with bronchial asthma. The sFas levels in our patients with asthma were also lower in the pollen season than in the pollen-free season, however, the difference was not significant.

The seasonal changes in cell surface FasL has been examined in several studies (14–20). Krug et al. found elevated cell surface FasL on T cells in bronchoalveolar lavage fluid after allergen challenge, he was unable to detect sFasL by the ELISA technique in bronchoalveolar lavage fluid in mild asthma patients (18). In our examination the serum levels of sFasL were significantly higher in the pollen season than after the season in 'asthma + rhinitis' children. We have taken these measurements once during the pollen season, at peak of the symptoms and once after

the pollen season. Analysing the changes of each of the well known allergic rhinitis cases, the history, the time of symptoms, the high pollen count, the physical examination, the blood tests, and nasal smear and the highly significant difference in sFas ligand show a reliability excluding other relevant causes such a viral infection which could interfere with the result. The seasonal elevation is present in all of the 'asthma + rhinitis' children and most of the 'rhinitis only' patients. The elevation of the sFasL shows that apoptosis plays a role in allergic response of children.

It seems plausible to compare these parameters of apoptosis in different organ manifestations of the allergic reaction, therefore we compared the two different manifestations of airway allergy, namely, asthma and rhinitis. There are inherent limitations to the number of allergic subgroups because of the rare occurrence of a pollen allergic child without rhinitis. In our examined ragweed allergic population there was no asthmatic child without allergic rhinitis. Therefore, we could not examine an isolated ragweed allergic 'asthma only' group.

Kato et al. suggests differences in cellular apoptosis between rhinitis and bronchial asthma. He examined patients with isolated bronchial asthma. The sFas level was also higher in our asthmatic patients than in children with rhinitis only, but the difference was statistically significant only in the symptom-free state. It is possible that the disease burden in 'asthma + rhinitis' is higher than that of rhinitis alone. It will be interesting to test sFas on the target organs such as bronchoalveolar lavage or nasal washings. In the present study the serum sFasL level was significantly higher in bronchial asthma than in isolated allergic rhinitis at the time of allergic symptoms, however, in the symptom-free period there was no statistical difference between asthma and rhinitis.

Interestingly enough, in the asthma subgroup with the higher sFasL we measured lower sFas levels, although it was not statistically significant. It will be interesting to further examine asthmatic patients, although one might have to use a different allergen which causes only asthma without rhinitis.

Conclusion

The higher level of sFasL during the pollen season may signal its role in the pathogenesis of allergic asthma. The deviation in the apoptotic reaction, change in cell death could explain the persistent allergic inflammation mainly in the

lower airways. The results showed that the level of sFas in the symptom-free state and sFasL in the symptomatic period were higher in allergic asthmatic children compared with the 'rhinitis only' children. The deviations of the apoptotic function may be related to the development of chronic asthmatic symptoms and persistent inflammation. Apoptosis might be different in upper and lower allergic airway diseases. Our findings show that it would be worthwhile to further examine apoptosis in isolated asthma.

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