

Short communication

Changes in serum haptoglobin level after allergen challenge test in asthmatic children

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Bronchial asthma is characterized by airway inflammation, which underlies the phenomenon of bronchial hyperresponsiveness. Previous studies have shown that this correlates with the serum concentration of haptoglobin. The occurrence of the late asthmatic response (LAR) after an allergen challenge test is associated with airway inflammation. The objectives of this study were to examine serum levels of haptoglobin during the 24 h after allergen challenge and to compare changes between the subjects with and without LAR. We studied two groups of children with perennial asthma who developed the early asthmatic response (EAR) only (group I: $n=14$), and EAR but also LAR (group II: $n=14$) after an allergen (*Dermatophagoides pteronyssinus*) challenge test. Serum concentrations of haptoglobin were measured at baseline, at EAR, and at 2 h (recovery), 8 h (LAR), and 24 h after the challenge. Baseline levels were similar in the two groups (group I: 128 ± 57 mg/dl; group II: 129 ± 50 mg/dl). In group I, there was no significant change in the level at any time point; in contrast, the subjects in group II showed a relative fall (92 ± 40 mg/dl) at 8 h, and an increase (161 ± 79 mg/dl) at 24 h after the challenge. Our results indicate that the serum concentration of haptoglobin decreases at the time of LAR and is subsequently replenished during the ensuing time. Although further studies are needed, we think that haptoglobin may be infused into the airways during the inflammatory process associated with LAR, and that this may be followed by "overshooting" production.

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Haptoglobin is a hepatic acute-phase reactant, and its plasma level is greatly elevated in response to stress, infection, acute inflammation, tissue necrosis, or neoplastic diseases, probably by the stimulation of synthesis (1).

It has now become clear that asthma is a chronic inflammatory disease of the airways involving many interacting cells and various chemical mediators. This inflammation underlies the phenomenon of bronchial hyperresponsiveness (BHR), which is the hallmark of asthma (2). Previous studies have shown a relationship between the concentration of haptoglobin and BHR (3, 4). This suggests that airway inflammation is associated with increased levels of haptoglobin in bronchial asthma. In our recent study, haptoglobin levels at acute exacerbation inversely correlated with the degree of response to initial bronchodilator therapy

(5). This finding supports the above hypothesis, since the extent to which bronchoconstriction or airway inflammation contributes to airflow obstruction during exacerbation is theoretically presumed to determine the responsiveness to bronchodilator therapy (6).

Allergen challenge tests have been employed most productively as an investigative tool in studies of the pathophysiology and mechanism of asthma (7). The early asthmatic response (EAR) probably comprises a combined effect of airways smooth-muscle contraction and mucosal edema. In contrast, the late asthmatic response (LAR) more closely resembles clinical asthma in its characteristic slow onset, prolonged time-course, and subsequent increase in BHR. Accumulating evidence suggests that LAR is associated with airway inflammation (8).

This study aimed to investigate the time course of haptoglobin concentration in serum during the 24 h after an allergen challenge test. In particular, we wished to study serum levels of haptoglobin at the times of EAR and LAR after this test and to compare their changes between the subjects with and without LAR.

Material and methods

Subjects

Children with mild to moderate atopic perennial asthma at the allergy clinic of Seoul National University Children's Hospital (Korea) were enrolled in this study. They underwent skin prick tests for reaction to 20 common airborne allergens and were positive to house-dust mite (*Dermatophagoides pteronyssinus*: D.p.). At the time of the study, all patients had been free of respiratory infections for 4 weeks. Asthma was controlled by a β_2 -agonist on an as-needed basis alone, or by inhaled cromolyn sodium or corticosteroids. Before the study, all patients had to stop using the β_2 -agonist or other medications for 24 h, oral theophylline for 48 h, and inhaled cromolyn sodium or corticosteroids for 7 days, respectively. The parents of the patients gave informed consent to the study, and the protocol was approved by the hospital ethics committee.

Allergen challenge test

Allergen challenge tests were performed with a simple modification of the method described by Chai et al. (9). Lung function was measured with a computerized spirometer (Microspiro-HI298, Chest, Japan), and the study was continued only if the forced expiratory volume in 1 s (FEV_1) before each test was at least 70% of the predicted value (10). The largest value of triplicate FEV_1 on each occasion was used for analysis.

Extracts of D.p. were obtained from Bencard, UK, and serial alternative five- and twofold dilutions were prepared with buffer phosphate (10^{-3} , 2×10^{-4} , 10^{-4} , 2×10^{-5} and 10^{-5} w/v concentrations). Allergen concentrations were inhaled after a control inhalation of buffer phosphate, starting with the dilution which produced a 2-mm wheal reaction in the skin prick test. Aerosols were generated by a nebulizer operated by compressed air at 20 psi (AP-50, Model 646, DeVilbiss, Somerset, PA, USA) and an inhalation-triggered dosimeter (Rosenthal-French, Model 2A, Laboratory for Applied Immunology, Fairfax, VA, USA). FEV_1 was measured at 10 min after the inhalation of five inspiratory breaths of each concentration. Inhalations were continued at 15-min intervals until there was a fall

of 20% or more from baseline FEV_1 . If the fall in FEV_1 was between 15% and 20% during the procedure, the next increment was at half the usual concentration. After the last concentration, FEV_1 was measured at hourly intervals for 10 h. Changes in FEV_1 after allergen inhalation were expressed as a percentage of the baseline.

Response was calculated as ΔFEV_1 (percent fall in FEV_1 from baseline), measured at 10 min after the last concentration of allergen (magnitude of early response), and the maximal ΔFEV_1 between 3 and 10 h after allergen inhalation (magnitude of late response). The EAR or LAR was defined as when the magnitude of the early or late response exceeded 20%. Fourteen subjects who showed EAR and no LAR (magnitude of late response <5%) (group I), and the other 14 subjects who showed EAR and LAR (group II) were entered consecutively into the study. To test haptoglobin levels, blood were taken at baseline (immediately before allergen challenge), at EAR, on recovery (2 h after inhalation of the last concentration of allergen), and at 8 and 24 h after the challenge.

Methacholine challenge test

To determine whether haptoglobin levels changed due to possible diurnal variation or due to bronchoconstriction *per se*, eight subjects in group II underwent the methacholine challenge test. This was performed several weeks after the allergen test, and methacholine was inhaled at the same time of day as allergen. As described previously (11), the concentrations of methacholine were inhaled until FEV_1 fell from the postbuffer saline value by more than 20%. FEV_1 was measured at hourly intervals for 10 h, as in allergen challenge tests. Blood specimens for haptoglobin were drawn at baseline and 8 and 24 h after the challenge.

Measurement of haptoglobin

The serum haptoglobin level was measured by the single radial immunodiffusion method (12) with the NOR Partigen® immunodiffusion plate (Behring Werke AG, Germany). Sera from sampled blood were immediately stored deep-frozen and checked for hemolysis. On each plate, patient sera were applied undiluted with the control serum to check the accuracy of measurement. After 2 days of diffusion, a device to measure the diameters of the precipitates to an accuracy of 0.1 mm, namely, a scaled magnifying glass against a black background with lateral illumination, was used. Haptoglobin concentrations were obtained by reading the values from the appended table of calibration values for the precipitate ring diameter measured. The accuracy

of this assay system was also checked by means of control serum.

Statistical analysis

Comparison of the values between the groups was made by the unpaired Student's *t*-test. Differences between means for paired data were tested by the paired Student's *t*-test. All analyses were performed with Stat-View II (Abacus Concepts, Inc, Berkeley, CA, USA) on a Macintosh computer (Apple Computer, Inc, Cupertino, CA, USA). In each case, statistical significance was accepted when the *P* value was less than 0.05.

Results

Clinical characteristics and allergen bronchoprovocation data of the studied subjects are shown in Table 1. There was no significant difference in sex, age, height, and the severity of asthma between the two groups. Subjects with mild asthma were being managed with an inhaled β_2 -agonist as needed, and those with moderate asthma with inhaled cromolyn ($n=2$ in group I, $n=4$ in group II) or inhaled corticosteroids ($n=3$ in each group), added to the β_2 -agonist.

Baseline FEV₁ in group I ($92.0 \pm 6.6\%$ of predicted) was comparable to that in group II ($91.6 \pm 8.1\%$ of predicted). In group I, FEV₁ decreased to $72.5 \pm 5.0\%$ of baseline during the early phase, and this initial fall had recovered ($>90\%$ of baseline) within 2 h in all except one subject. No subsequent decreases in FEV₁ were observed. In group II, FEV₁ decreased to $69.6 \pm 6.7\%$ of baseline during the early phase, which was not significantly different from that of group I. The pattern or magnitude of recovery from the EAR was also similar to group I. The time of onset of LAR varied among the subjects. Late-phase FEV₁ was recorded between 5 and 8 h after

the challenge, with a mean of $67.2 \pm 5.1\%$ of baseline. At 8 h, when blood was drawn to check haptoglobin concentration for the late-phase level, all subjects showed a fall in FEV₁ of $>20\%$. Thereafter, there was a gradual increase in FEV₁, so that mean FEV₁ at 24 h after the challenge was $92.0 \pm 5.7\%$ of baseline. The cumulative provocation dose of allergen causing a 20% fall in FEV₁ expressed as breath unit showed no significant difference between the two groups.

Changes in serum haptoglobin concentration after allergen challenge in group I are depicted in Fig. 1. Baseline levels varied between 51 and 238 mg/dl (mean 128 ± 57 mg/dl). After the challenge, there was no significant difference from baseline values at any time point up to 24 h, nor was there a significant variation in any individual. There was some tendency for the 8-h value (122 ± 61 mg/dl) to be lower and for the 24-h value (133 ± 61 mg/dl) to be higher than the baseline value, but there was no statistical significance.

Changes in serum haptoglobin concentration after allergen challenge in group II are depicted in Fig. 2. Baseline levels (129 ± 50 mg/dl) were also variable, but were not significantly different from those in group I. At the times of EAR and recovery, the values remained unchanged. In contrast to group I, there was a significant decrease at 8 h (92 ± 40 mg/dl, $P < 0.01$) and a significant increase at 24 h (161 ± 79 mg/dl, $P < 0.01$) after the challenge, compared to baseline value. When the changes in each individual were examined, 12 subjects showed a fall in haptoglobin concentration at 8 h, and 11 subjects a rise at 24 h. The two subjects with an increase at 8 h were found to have a decreased level at 24 h.

Eight randomly sampled subjects in group II underwent the methacholine challenge test. The mean percent fall in FEV₁ after this challenge was $28.2 \pm 7.3\%$, which was comparable to the magnitude of EAR in group II. During the 10 h after

Table 1. Clinical characteristics and allergen bronchoprovocation data of studied subjects

	Sex (M/F)	Age (years)	Height (cm)	Severity of asthma	Allergen bronchoprovocation test					
					Baseline FEV ₁ §	EAR* FEV ₁ ¶	2-h FEV ₁ ¶	Late-phase† FEV ₁ ¶	24-h FEV ₁ ¶	PD ₂₀ ‡ (breath unit)
Group I (n= 14)										
Mean	9 M/	10.8	137	9 Mild/	92.0	72.5	96.2	96.6	100.5	16.2#
SD	5 F	1.8	10	7 Moderate	6.6	5.0	4.1	1.0	2.0	(10.0–26.3)
Group II (n= 14)										
Mean	8 M/	10.6	138	7 Mild/	91.6	69.6	95.0	67.2	92.0	13.8#
SD	6 F	2.2	11	7 Moderate	8.1	6.7	5.2	5.1	5.7	(8.5–22.4)

* Early asthmatic response; † lowest FEV₁ between 3 and 10 h after challenge; ‡ cumulative provocation dose of allergen causing 20% fall in FEV₁ expressed as breath unit (1 breath unit = 1 breath of 2×10^{-4} w/v of *Dermatophagoides pteronyssinus*); § % predicted for height (10); ¶ % of baseline; # geometric mean (range of 1 SD).

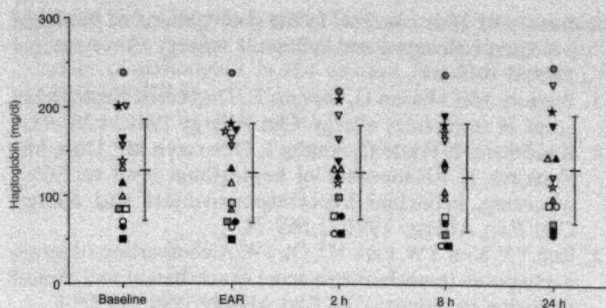


Fig. 1. Changes in serum haptoglobin level (mean $\pm$ 1 SD) at various time intervals after allergen challenge test in group I. Each symbol represents one individual. There was no significant change at any time interval.

challenge, FEV₁ showed no phasic response. The mean levels of serum haptoglobin at 8 and 24 h were 133 ± 56 mg/dl and 124 ± 41 mg/dl, respectively, values of which were not significantly different from that of the baseline (130 ± 55 mg/dl) (data not shown).

Discussion

In this study, most subjects who developed LAR (group II) showed a relative fall in serum haptoglobin concentration at the time of this response. The fall was followed by an increase at 24 h after allergen challenge. In contrast, in subjects who displayed EAR only (group I), there was no significant change in serum haptoglobin concentration at any time point. Our findings are somewhat surprising, as LAR is considered to reflect airway inflammation in asthma (8), and this is presumably associated with a higher concentration of serum haptoglobin (4, 5).

Changes in serum haptoglobin concentrations induced by the allergen challenge test could have reflected lipopolysaccharide contamination of allergen extracts (13). This is unlikely, however, since serum haptoglobin levels were unchanged in group I, which was exposed to a similar amount of inhaled allergen as group II (data not shown). The present study included a control day after methacholine inhalation, which caused no change in haptoglobin concentration at either 8 or 24 h after allergen challenge; the decrease at the time of LAR and the 24-h increase in serum haptoglobin concentration in group II subjects were thus not due to diurnal variation, and did not appear to be related to the effects of bronchoconstriction or the withdrawal of medications. In this study, haptoglobin levels were determined by radial immunodiffusion (12); some samples were also examined by rate nephelometry (14), but choice of method did not affect the levels recorded and did not, therefore, appear to have any influence on the overall results of this study. The

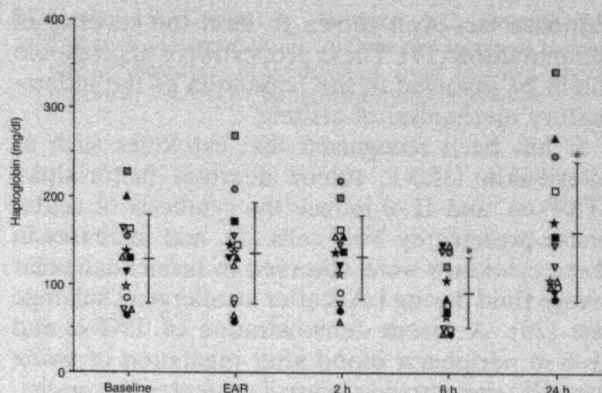


Fig. 2. Changes in serum haptoglobin level (mean $\pm$ 1 SD) at various time intervals after allergen challenge test in group II. Each symbol represents one individual. *Statistically significant ($P < 0.01$) compared to baseline value.

development of LAR may occur as a continuum from no response to one that is moderate or severe (15). We arbitrarily selected subjects with virtually no LAR and with definite LAR, so that changes in serum haptoglobin concentration could be compared according to the existence of LAR.

The mechanism by which a significant decrease in serum haptoglobin levels occurred during LAR and was followed by an increase at 24 h is not clear. The profile of serum haptoglobin is analogous to eosinophil counts in peripheral blood after an allergen challenge test. A relative fall in eosinophil count was noted during LAR, but the count increased at 24 h after the challenge (16). The interpretation of these findings was that eosinophils are recruited to the lung in parallel with LAR and the increase seen at 24 h may represent an eosinophilopoietic bone-marrow response. The level of serum haptoglobin at a given time point may be governed by the rate of production of the proteins and the rate of their elimination. Whether the decrease in serum haptoglobin at the time of LAR arises secondarily to decreased production or to enhanced elimination cannot be determined from the present study. However, the coincidence of the fall with the timing of LAR and the lack of this phenomenon in group I subjects suggest that it may be related to local inflammatory events associated with LAR. The presence of haptoglobin in bronchoalveolar lavage fluid has been reported, and it has been assumed that it is probably derived from serum (17). We suggest that haptoglobin may be infused into airways during LAR, although further study, including bronchoalveolar lavage, is needed to test this hypothesis. It may be significant that haptoglobin has been found to inhibit inflammatory mediator synthesis in animal tissue (18). The prior initiation of acute-phase

response has been shown to limit the severity of inflammation (19). These properties of haptoglobin might be involved in the regulation of the inflammatory mechanism of asthma.

It has been recognized that cytokines such as interleukin (IL)-1, tumor necrosis factor- α (TNF- α), and IL-6 induce the synthesis of acute-phase proteins by liver cells (1), and increases in these cytokines were observed in bronchoalveolar lavage fluid during LAR after an allergen challenge test (20). A recent demonstration of TNF- α and IL-6 in peripheral blood after inhalation of swine dust (21) may provide indirect evidence of a mechanism by which a local inflammatory reaction in the lung may induce the hepatic production of haptoglobin. In an *in vitro* study (22), IL-6 was found to upregulate the production of haptoglobin in a time-dependent manner; the increase was already detectable 2 h after IL-6 treatment began, and for a period of at least 16 h the haptoglobin level continued to rise linearly. The increase in serum haptoglobin levels seen at 24 h after allergen challenge could thus be explained by the kinetics of hepatic cellular response to cytokines, such as IL-6, released during LAR and production which "overshot".

LAR is associated with acute airway inflammation (23) and with an increase of BHR, which can persist for days or even weeks after the initial exposure to the allergen (8); these features mimic asthma exacerbation induced by natural exposure to the allergen (24). However, there have been discrepancies in recorded serum haptoglobin data. On acute exacerbation of asthma, serum haptoglobin increased (5), but at the time of LAR after allergen challenge in the present study, it showed a relative decrease. This phenomenon may be due to temporal lags between haptoglobin production and progression from one compartment to another, as illustrated by the eosinophil count in peripheral blood after allergen challenge (16). The present results are one example of those which must be interpreted with an understanding of the overall kinetics of biologic variables.

In conclusion, the serum concentration of haptoglobin shows a decrease at the time of LAR and is subsequently replenished during the 24 h after an allergen challenge test. Although further studies on the mechanism of these changes are needed, we speculate that haptoglobin may be infused into the airways during the inflammatory process associated with LAR and that this may be followed by "overshooting" production.

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