Biochemical Evaluation of Bronchial Asthma.

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SUMMARY In order to identify a true antigen responsible for the induction of the asthmatic attack, antigen provocation tests were performed, and the correlation between the pulmonary functions and the changes in plasma lysosomal enzyme activities was obtained.

When DSCG was administered prior to the provocation, release of the lysosomal enzyme activities was significantly prevented, and the asthmatic attack was suppressed or a slight, even if induced.

The lysosomal enzyme activities were lower in the adrenal steroid hormone-dependent patients. Measurement of the plasma lysosomal enzyme activities may give promising methods in evaluating a true antigen in place of the pulmonary function tests, and in addition, dependency on the steroidal hormone may be judged by the present biochemical method.

When the antigen, an extract of mites, induced a positive skin test but negative provocation test, lysosomal enzymatic activities did not change significantly in a preliminary test, and the result was excluded from the present report.

Relationship between bronchial asthma and any related changes in activities of lysosomal and other enzyme systems are not well documented, and some reports dealt with the change of enzymatic activities during the asthmatic attack. However, such changes in enzymatic activity during various periods of attack are not known.

It is understood currently that the SRS-A (slow reacting substance A) and histamine are the most influential and significant factors in the early response type allergy, and any possible intervention of the lysosomal enzymes in the inflammation and autolysis is not examined yet. One of the etiologic factors of the bronchial asthma may be allergic reactions in the lung and the airway, and there is a possibility that the lysosomal enzyme system may be related to the aggravation of the inflammation.

Skin sensitivity tests for various allergens have been used to identify the causative allergen in the atopic type of
asthma (called asthma in this paper), but some non-atopic healthy subjects would show positive reaction, and some intradermal allergen inducing positive reaction would not be an antigen when inhaled in many cases.

Thus, in order to identify the true allergen, a particular allergen inducing the positive intradermal reaction was enforced to inhale for a certain period of time (for 3 min) under strictly controlled conditions, and the true allergen can be determined by knowing the change of the functions of lung before and after (or during) the antigen inhalation provocation test (provocation test in this paper). However, such antigenic challenge for children is difficult in that the functions of the lung are difficult to assess, and then well reproducible results can not be obtained practically, and the determination of the lung functions during the asthmatic attack may give considerable suffering to the patients.

The authors noted that asthmatic attack is a kind of inflammatory expression, and the blood specimens were collected during the provocation tests. Lysosomal enzyme activities were determined intending to find any alternative functional test to the lung function determinations.

Changes in lysosomal enzyme activities was also examined in the present study when DSCG (disodium cromoglycate), used for the treatment of asthma, was administered.

**Materials and Methods**

1. **The subjects** used in the present study were out- and in-patients at the Department of Pediatrics, National Children's Hospital in Tokyo. There were 23 patients (14 male and 9 female), aging from 5 to 15 years old, and they were classified according to the degree of severity of asthma.
   a) Moderate group (A group).

   According to the classification defined by the Children's Allergy Study Group, subjects with the moderate severity were 12 (7 male and 5 females).

   b) DSCG administered group before the test (B group).

   The threshold value of the antigen was obtained in the preliminary provocation test in 5 patients (2 male and 3 females) in this group, and then DSCG (Intal, Fujisawa Pharm. Ind.), 20mg, was administered by means of a spinhaler 15 min prior to the provocation test.

   c) adrenal steroid hormone dependent group (C group).

   Severe steroid hormone dependent bronchial asthma patients, 6 (5 male and 1 female), were involved in this group.

2. The antigen inhalation provocation test.

   The antigen used for the inhalation provocation test was that having induced a positive intradermal reaction to the particular subject concerned.

   Allergen (Allergen Extract, Torii Pharm. Co.) was diluted with physiological saline to $1 \times 10^4$ to $1 \times 10^5$, and the solution was inhaled by the patient using a Porta Bird (Bird Co., U.S.A.) with the intermittent positive pressure breath (IPPB) method to perform the provocation test. Duration of the inhalation of the antigen was 3 min at the maximum, and the pulmonary functions were measured at the indicated time intervals. When PFR $\cdot$ FFV1.0 (peak flow rate and forced expiratory volume in a second) was reduced to less than 85% of the pre-test value, or when wheezing was
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heard clinically, the antigen was judged to be positive one at the dilution. The antigen which induced a positive inhalation provocation test, was an extract of mites at 10,000 dilution in the present study. Such cases as individuals showing positive skin test but negative inhalation provocation response were excluded from the present report for the evaluation of the change of the lysosomal enzymatic activity change because the enzymatic activities in such subjects were not altered significantly.

Prior to the provocation test, administration of prednisolone and antihistamine drugs was not allowed for 48 hrs, and inhalation of becromethasone and ingestion of any bronchodilators for 12 hrs, and inhalation of DSCG for 8 hrs.

   a) PRF (Peak flow rate)
      PFR was measured using a peak flowmeter (Wright, U. S. A.).
   b) FEV₁₀ (Forced expiratory volume in a second)
      FEV₁₀ was measured using a Uni-Spiro MK II (Chest, U. S. A.).

      These measurements were performed before, immediately after 5 min, 15 min, and 30 min after the antigen inhalation.


      Blood specimens were collected before, 5 min and 30 min after the onset of the asthmatic attack after the antigen inhalation from the brachial medial vein with heparin. The specimen was centrifuged at 3,000 rpm for 10 min immediately after the collection and the plasma was stored at −70°C until analysis was performed.

| Table 1 Assay of lysosomal enzyme activities by fluorometry. |
|-----------------|-----------------|-----------------|-----------------|
|                | Blank           | Enzyme blank    | Test soln.      | Std. Std.       |
| Plasma          |                 |                 | 30µl            |                 |
| H₂O             | 90µl            | 30µl            | 40µl            | 60µl            |
| 4-MU Std        |                 |                 | 50µl            | 30µl            |
| Enz. substrate  | 60µl            | 60µl            |                 |                 |
| Incubation      |                 |                 | 37°C for 60min  |                 |
| Stop wlp 0.2M Glycine buffer (pH 10.7), 800µl |                 |                 |                 |                 |
| Assay           | λex 350–360nm   | λem 450nm       |                 |                 |

5. Determination of the plasma lysosomal enzyme activity.

      The lysosomal enzyme activity was determined according to the method reported by Griffins. 4-Methyllumbelliflorone (4-MU) formed by enzymatic decomposition of 4-methylumbelliferyl-2-acetamide-2-deoxy-β-D-glucopyranoside, 4-MU-α-D-mannoside, or 4-MU-α-D-fucoside (Nakarai Chem.) was fluorometrically determined on Hitachi RF-502 Spectrofluorometer. Conditions are shown in Table 1. Acid phosphatase activity was determined with Acid phosphatase B Kit Wako (Wako Pure Chem.) on Hitachi 101 Type Spectrophotometer.

**Results**

When PFR × FEV₁₀ was reduced to less than 85% of the pre-test value, or when wheezing was heard clinically, the asthmatic attack was judged to be induced by the antigen. Difference between male and female subjects was negligible, and the total changes are described in the following:

1. Pulmonary functions:
Fig. 1 Changes in the FEV\(_{1.0}\) figures when a clear wheezing is found through an antigen inhalation provocation test.

- A group
  - FEV\(_{1.0}\) (Mean±SE): before 1,583±100ml
  - after 5min 1,194±152ml (p<0.01)
  - after 30min 1,228±166ml (p<0.01).

After the antigen provocation test, FEV\(_{1.0}\) was significantly reduced. Changes in FEV\(_{1.0}\) at each time of the test are shown as percent values according to the following equation:

\[
\frac{\text{FEV}_{1.0} \text{ after inhal.} - \text{FEV}_{1.0} \text{ before inhal.}}{\text{FEV}_{1.0} \text{ before inhal.}} \times 100 = \text{FEV}_{1.0}(\%)
\]

and it was -28±6.7% after 5min and -23±8.3% after 30min.

B group
- FEV\(_{1.0}\) (Mean±SE): before 1,725±179ml
  - after 5min 1,400±249ml (p>0.05)
  - after 30min 1,663±359ml (p>0.05),
  - showing no significant change.

C group
- FEV\(_{1.0}\) (Mean±SE): before 1,800±230ml
  - after 5min 1,350±320ml (p<0.1)
  - after 30min 1,438±347ml (P<0.1),
  - showing a tendency of reduction.

b) PFR • PFR(%) (Fig. 2)
A group
- As in the case of FEV\(_{1.0}\), FEV\(_{1.0}\) (%), PFR was expressed as follows:
  - PFR\(_{1.0}\) (Mean±SE): before 213±16.3 l/min
    - after 5min 161±23 l/min (P<0.01)
    - after 30min 174±27.3 l/min (P<0.05),
    - showing significant reduction.
  - PFR\(_{1.0}\) (%) -25.5±6.9% after 5min and -20.5±9.9% after 30min.
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B group

PFR_{1.0} (Mean±SE): before 193±28.5 l/min
after 5min 163±26 l/min (P>0.05)
after 30min 178±41.5 l/min (P>0.05),
showing no significant change.

PFR (%) −14.9±6.8% after 5min and
±5.5±3.4% after 30min.

C group

PFR_{1.0} (Mean±SE): before 353±24 l/min
after 5min 275±72.5 l/min
after 30min 255±62.5 l/min,
showing tendency of reduction.

PFR (%) −24.4±19% after 5min and
−29.9±15.6% after 30min.

2. Plasma lysosomal enzyme activities:

a) Change in the plasma lysosomal en-
zyme activities in the 3 groups (A, B, and C groups).

As in the case of the preceeding se-
tion, changes in 4 enzyme activities at
various periods of the provocation test are
expressed as Mean±SE n mol/h/ml plasma.

Then, the statistical significance at
each test period was examined in each
group according to the following equation:

Activity after the provocation
− Activity before the provocation
= Amount of change

A group

α-Fucosidase activity
+24.3±11.2 n mol/h/ml (P<0.1) after 5min,
and
+21.8±10.1 n mol/h/ml (P<0.1) after 30min,
showing an increasing tendency.

N-acetyl-β-glucosaminidase
+7.5±7.0 n mol/h/ml (P<0.1) after 5min,
and
+13.1±5.5 n mol/h/ml (P<0.05) after 30min,
showing an increasing tendency after 5min
and increased activity after 30min.

Acid phosphatase
+0.14±0.05 BL unit (Bessy Lowry) (P<0.05)
after 5min, and
+0.06±0.04 BL unit (P>0.05) after 30min,
showing increased activity after 5min.

α-Mannosidase
+1.80±0.81 n mol/h/ml (P<0.1) after 5min,
and
−0.66±0.53 n mol/h/ml (P>0.1) after 30min,
showing increased activity.

B group

α-Fucosidase
+7.8±7.8 n mol/h/ml (P>0.1) after 5min,
and
+6.6±10.6 n mol/h/ml (P>0.1) after 30min,
showing no change in activity.

N-acetyl-β-glucosaminidase
−1.9±7.1 n mol/h/ml (P>0.1) after 5min,
and
+3.3±3.6 n mol/h/ml (P>0.1) after 30min,
showing no change in activity.

Acid phosphatase
+0.03±0.06 BL unit (P>0.1) after 5min,
and
+0.14±0.09 BL unit (P>0.1) after 30min,
showing no change in activity.

α-Mannosidase
+0.02±0.61 n mol/h/ml (P>0.1) after 5min,
and
+0.3±0.51 n mol/h/ml (P>0.1) after 30min,
showing no change in activity.

C group

α-Fucosidase
−48.0±19.2 n mol/h/ml (P<0.1) after 5min,
and
−44.2±20.5 n mol/h/ml (P<0.1) after 30min,
showing decreasing tendency.
N-acetyl-β-glucosaminidase

\[-13.2\pm 12.4 \text{ n mol/h/ml} (P>0.1) \text{ after 5 min, and}
-9.6\pm 4.4 \text{ n mol/h/ml} (P>0.1) \text{ after 30 min, showing no change in activity.}\]

Acid phosphatase

\[-0.21\pm 0.06 \text{ BL unit} (P<0.05) \text{ after 5 min, and}
-0.16\pm 0.05 \text{ BL unit} (P<0.1) \text{ after 30 min, showing significant decrease in activity after 5 min, and tendency of decrease after 30 min.}\]

α-Mannosidase

\[+1.82\pm 1.31 \text{ n mol/h/ml} (P>0.1) \text{ after 5 min, and}
+3.90\pm 1.18 \text{ n mol/h/ml} (P<0.02) \text{ after 30 min, showing increasing tendency after 30 min.}\]

b) Comparison among groups
(Figs. 3—6).

Statistical significance of the difference between the enzyme activities in the groups B and C to those in the group A at each time of the provocation test was examined. No statistical significance between the groups A and B was found for any enzyme activities, but the difference
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of the enzyme activities in the groups A and C was statistically well correlated as follows:

- α-Mannosidase: increased after 30 min (P<0.01)
- α-Fucosidase: decreased at any time (P<0.01).
- N-Acetyl-β-glucosaminidase: decreased after 30 min (P<0.03)
- Acid phosphatase: decreased at any time, after 5 min (P<0.01) after 30 min (P<0.02).

The above results are summarized in Table 2.

Discussion

In order to identify any specific antigen to an asthmatic patient, numbers of antigens are applied by scratching skin, and the positively reacted antigen(s) after 15 min (early reaction) is identified as the specific antigen responsible for the asthmatic attack, and it is used for the desensitization therapy. However, such skin positive antigen is not necessarily the antigen inducing the asthmatic attack by

![Fig. 6 Changes of α-mannosidase activity in three groups of antigen inhalation provocation test](image)

- ●—● provocation group
- ○—○ DSGC premedication group
- ×—× steroid dependent group

Table 2 Changes in the mean±[S.E.] of lysosomal enzyme activities, FEV₁₀, and PFR in each time span

<table>
<thead>
<tr>
<th></th>
<th>FEV₁.₀</th>
<th>FEV₁₀(%)</th>
<th>PFR</th>
<th>PFR(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min B</td>
<td>1583.0</td>
<td>1725.1</td>
<td>1800.0</td>
<td>275.0</td>
</tr>
<tr>
<td>30 min C</td>
<td>1194.0</td>
<td>1400.0</td>
<td>1350.0</td>
<td>28.0</td>
</tr>
<tr>
<td><strong>group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min B</td>
<td>1752.1</td>
<td>1900.0</td>
<td>230.0</td>
<td>67.0</td>
</tr>
<tr>
<td>30 min C</td>
<td>1438.0</td>
<td>1663.0</td>
<td>359.0</td>
<td>8.3</td>
</tr>
<tr>
<td><strong>group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min B</td>
<td>1800.0</td>
<td>2000.0</td>
<td>230.0</td>
<td>9.5</td>
</tr>
<tr>
<td>30 min C</td>
<td>1438.0</td>
<td>1663.0</td>
<td>359.0</td>
<td>8.3</td>
</tr>
</tbody>
</table>

*Group A*: provocation group, *group B*: DSGC premedication group, *group C*: steroid dependent group

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inhalation, and some healthy subjects (approx. 10%) show positive skin test to many antigens.

It is the reason that the true antigen responsible for asthma should be determined by enforced inhalation of the antigen to cause asthmatic attack, as expressed by the reduction of $FEV_{1.0}$ before and after the inhalation. However, the measurement of the pulmonary functions is painful for patients, particularly for children and during the attack in many cases, and the reproducibility of the pulmonary function determination is difficult. It should be noteworthy that the airway is much more sensitive in the asthmatic patients than in the healthy subjects, and enforced respiration makes wheezing in the patient, thus the judgement of the attack is difficult and subjective.

Therefore, some objective index, such as biochemical indices, is required to judge any change evoked by the antigen and the related asthmatic attack.

In addition to the chemical mediators related in the early response of the asthmatic attack in the antigen inhalation provocation test, lysosomal enzymes are released in the blood stream in relation to the disposition of the antigen–antibody complex by the leukocyte (above all, neutrophils). Then, when the lysosomal enzyme activities may be determined as the index to know the antigen–antibody (allergen–asthmatic attack) reaction in place of the determination of the pulmonary functions, if the pulmonary functions and lysosomal enzyme activities are well correlated.

It was found in this study that a moderately severe group of the patient (group A), in which the control of the asthma can be made only with the bronchodilator, without using adrenal steroid hormone, showed tendency of increasing lysosomal enzyme activities after the antigen provocation trial. All enzymes tested showed increased in activity after 5 min of inhalation, but the attitude of the change in plasma was not uniform after 30 min. This fact may suggest that there is a probability of releasing the lysosomal enzymes in parallel with the degranulation of SRS–A and histamine from the mast cell and basophil in the early reaction. Generally, lysosomal enzymes are involved in the inflammatory disease together with such local inflammatory substance as histamine, and then release of ECF–A (eosinophil chemotactic factor of anaphylaxy) in blood to mobilize local acidphils, and the acidphil might release NCF–A (neutrophil chemotactic factor of anaphylaxy). Thus, released neutrophils digest the phagocytes (such as the antigen–antibody complex) and release the digestions together with the lysosomal enzymes, which aggravate the inflammation of the tissue as the secondary mediator. In the case of asthma, lysosomal enzymes may be involved in the early reaction phase.

The above description may be rational based on the experimental results obtained in the group B. DSCG suppresses degranulation of the mast cell and basophils, so that it is used for the anti-allergic drug. When DSCG was inhaled prior to the provocation test, lysosomal enzyme activities were not altered after the antigen provocation. Kowal–Gierizak et al. reported that when asthma was mitigated by DSCG administration, but leukocyte lysosomal enzyme activities increased, and when DSCG was administered in vitro, leukocyte of the atopic subjects induced the activity of the lysosomal enzyme, which was not observed in the non-atopic subjects. Their
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findings do not necessarily agree with the present results. The reason may be that the subjects used in the present study would not induce the activity of lysosomal enzyme rapidly because the system, induced by the antigen provocation, was so mildly controlled that the attack was none or a slight as judged by the results of FEV$_{1.0}$ (%). Therefore, when the antigen-antibody reaction complex might be formed, but the lysosomal membrane is stabilized by the pre-administered DSCG and then suppression of degranulation and of release of lysosomal enzymes was effective. Mobilization of leukocyte and phagocyte were also prevented, so that enzymatic activities in leukocyte were not leaked although they were elevated within the cell.

However, in the group C in which adrenal steroid hormone must be administered in addition to the bronchodilator because of severe asthmatic attack, quite opposite tendencies to those of the group A were found in the change of lysosomal enzyme activities except $\alpha$-mannosidase. Stabilization of the lysosomal membrane was possibly still effective after 12 hrs of discontinuation of the steroid administration, or other complexed allergic factors than the antigen might be related to such severe subjects with asthma.

As Selye$^8$) stated that the larger stress to the body would cause the reaction of the adrenal gland in addition to the sympathetic nervous system, and the sympathetic nervous functions are lowered when the symptom is more severe$^9$), and the adrenal system might compensatorily participate to the sympathetic function. When the administration of adrenal steroid hormone is chronic, stabilization of the lysosomal membrane and defensive mechanism might be established in the body. Such defensive mechanism might involve the intrinsic production of the inhibitory substance to the release of the lysosomal enzyme activities. However, these are still speculations, and need more examinations. The reason that only $\alpha$-mannosidase activity being increased is not known. It might be locally situated.

References

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