Characteristics of Two Lines of Guinea Pigs (BHS and BHR) Differing in Bronchial Sensitivity to Acetylcholine and Histamine Exposure

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In a previous study, we reported that as model animals to be used in the study of bronchial asthma in humans, two lines of guinea pigs were developed by ourselves: bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR) as a control. Studies on biological characteristics in guinea pigs of two lines were undertaken, and the following results were obtained. 1) Airway resistance of guinea pigs of the two lines to intravenously administered acetylcholine, histamine and leukotriene D4 was found to be different between BHS and BHR. Airway resistance of BHS to the chemicals was increased compared with those of BHR. 2) The number of muscarinic acetylcholine receptors in lung membrane preparation and its affinity increased significantly in BHS compared with those of BHR. In β-adrenergic and histamine H1 receptors, there was observed no difference between BHS and BHR. 3) No difference in IgE antibody production to ovalbumin was observed between BHS and BHR. 4) When total leukocytes and differential leukocyte count (percentage, %) in peripheral blood of BHS and BHR were examined, relative percentage of lymphocytes and eosinophils was significantly higher in BHS than in BHR, while percentage of neutrophils was significantly lower in the former than in the latter. — KEY WORDS: airway resistance, differential leukocyte count, guinea pigs, IgE antibody production, receptors

The determination of bronchial hypersensitivity is of clinical importance for diagnosis and therapy of bronchial asthma. The degree of bronchial hypersensitivity is also related to the severity of bronchial asthma and the coreresponding type of treatment needed for controlling the asthmatic symptoms [9]. From these reasons, increasing attention has been paid to bronchial hypersensitivity in asthma in recent years [10,18]. Models of guinea pigs or dogs induced by inhalation of citric acid, ozone, platelet activating factor (PAF) or infected with Influenza virus etc. [11,12,15,30] were used in these experiments. However, these models using otherwise normal animals are all artificial products. Therefore, development of model animals with spontaneous bronchial hypersensitivity has been required.

We have already reported [22] that guinea pigs of two lines, namely a bronchial hypersensitive line (BHS), and a bronchial hyposensitive line (BHR) as control were developed for serving as model animals of bronchial asthma. In the following the characteristics of guinea pigs of the two lines will be reported.

Materials and Methods

Experimental animals: Ten to 15 week-old guinea pigs of either sex in F4-F1 generation of bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR), both devel-
oped by ourselves [19-22], were used in groups of each 5 to 9 animals. In addition, 8 week-old specific pathogen-free (SPF) male Hartley guinea pigs were purchased from Japan SLC, Hamamatsu, Japan, to be used for passive cutaneous anaphylaxis (PCA).

Measurement of airway resistance: Airway resistance was measured by the overflow technique of Konzett and Rössler [16] and Goto et al. [7] which was modified for electrical recording via an air pressure transducer (TP-220 T, Nihon koden, Tokyo, Japan). The animals were anesthetized with 1.35g/kg of urethan (Kishida Chemical Co., Osaka, Japan) given intraperitoneally and 10mg/kg of gallamine triethiodide (SIGMA Co., Saint Louis, USA) given intravenously. An endotracheal tube was inserted into lower trachea and connected to an air pump in order to perform an artificial ventilation which had a constant tidal volume of 3 to 4 ml and a frequency of 50 strokes/minute. Tracheal pressure was recorded via a side-arm of the cannula connected to a pressure transducer. The resting pressure of the airway for control period was recorded for 5 minutes to ensure consistency, and to produce a tracing of 15 to 20mm in height and maximum pressure (obtained by clamping off the trachea) of 90 to 100mm in height. The chemicals were given by intravenous injection as solution in physiological saline through a cannulated jugular vein at intervals of 10 to 20 minutes. Airway resistance with maximum bronchoconstriction was calculated as remitting pressure by a method of computation shown later.

\[
\text{Increase in airway resistance (\%) = \left[ \frac{\text{remitting pressure after the chemical's injection} - \text{remitting pressure before the chemical's injection}}{\text{maximum remitting pressure} - \text{remitting pressure before the chemical's injection}} \right]}
\]

Acetylcholine chloride (ACh, Daiichi Pharmaceutical Co., Tokyo, Japan), histamine dihydrochloride (Hist, Nakarai Chemical Co., Kyoto, Japan) and leukotriene D₄ (LTD₄, Wako Pure Chemical Co., Osaka, Japan) were dissolved either in physiological saline (in cases of ACh, Hist) or phosphate buffered saline (LTD₄) just prior to use.

Receptor binding assay: Lung membrane preparation [3, 23]: The animals were sacrificed by exsanguination under ether narcosis. Lungs of guinea pigs of two lines were removed. Lungs were twice homogenized with a polytron (PT 10/35, KINEMATICA Co., Bern, Switzerland) for 20 seconds in ice-cold assay buffer (50 mM sodium potassium phosphate buffer, pH 7.5), and filtered through a layer of nylon mesh to remove undisrupted cells. The total membrane fraction pellet, which contained mainly the cytoplasmic membrane, was obtained by centrifugation at 50,000 g for 10 minutes. The resulted pellet was washed four times in ice-cold assay buffer by resuspension and centrifugation at 50,000 g for 10 minutes each. The final pellet was resuspended and diluted in assay buffer at a concentration of approximately 0.7 to 1.0 mg protein/ml. The protein was assayed by the method of Lowry et al. [17].

Muscarinic acetylcholine (mACh) receptor assay: According to the method of Yamamura et al. [31], binding experiments were performed by incubating 400 μg of lung membrane suspension (150 μl) for 60 minutes at 25°C with various concentrations (finally 0.1-3.2 nM) of ³H-quinucilidinyl benzilate (QNB, 100 μl, Amersham Laboratories, Buckingham, England) in the absence or presence of 10 μM atropine sulfate (total 1000 μl, SIGMA Co., Saint Louis, USA). Incubations were terminated by diluting the samples with 5 ml of ice-cold assay buffer, immediately followed by vacuum filtration through Whatman GF/C glass fiber filters (Whatman Bio Systems Ltd., Maidstone, England). Subsequently, the filters were rapidly washed twice with 6 ml of the same buffer. After washing, the filters were dried and then counted in 5 ml of Aqueous Counting Scintillant II scintillation fluid (SIGMA Co., Saint Louis, USA) by a liquid scintillation counter (TRI-CARB 4640, Packard Japan, Osaka, Japan). Specific binding was defined as the difference between the total binding and the non-specific binding determined in the presence of 10 μM atropin. The number and the affinity were calculated by Scatchard analysis [26].

β-adrenergic (β-Adr) receptor assay: According to the method of Barnes et al. [4], binding experiments were performed by incubating 200 μg of lung membrane suspension (100 μl) for 15 minutes at 20°C with various concentrations (finally 0.1-3.2 nM) of ³H-dihydroalprenolol (DHA,100 μl, SIGMA Co., Saint Louis, USA) in the absence or presence of 1 μM DL-propranolol (total 250 μl, Wako
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Pure Chemical Co., Osaka, Japan). After the same procedure as for mACh receptor assay was followed, specific binding was calculated as the difference between the total binding and the nonspecific binding determined in the presence of 1 μM DL-propranolol (Wako Pure Chemical Co., Osaka, Japan).

Histamine H₁ (Hist H₁) receptor assay: According to a method of Tran et al. [29], binding experiments were performed by incubating 200 μg of lung membrane suspension (150 μl) for 30 minutes at 25°C with various concentrations (finally 0.1–3.2 nM) of ³H-pyriramine (100 μl, Dupont Co., Boston, USA) in the absence or presence of 2 μM triprolidine (total 450 μl, Dupont Co., Boston, USA). After the same procedure for mACh receptor assay was followed, specific binding was determined as the total binding detracted by the nonspecific binding determined in the presence of 2 μM triprolidine.

IgE antibody production: IgE antibody production in guinea pigs of BHS and BHR to ovalbumin (OA, Grade V, SIGMA Co., Saint Louis, USA) was studied in the method of Graziano et al. [6]. Animals were injected intraperitoneally with 10 μg of OA mixed with 4 mg of aluminium hydroxide (Wako Pure Chemical Co., Osaka, Japan) gel. A similar immunization was repeated every 30 days, 5 times in total. Sera were obtained 7 days after the last immunization and stored at −70°C until use. PCA were carried out as described by Ovary [24]. Serial two-fold dilutions from 100 to 3200 folds of guinea pig anti-OA sera were prepared with saline. Normal guinea pigs weighing approximately 550 to 600g were sensitized intracutaneously with the dilutions of the guinea pig antisera. Eight days thereafter, the guinea pigs were challenged intravenously with 1 ml of the saline solution containing 1mg of OA and 1% Evans blue (Wako Pure Chemical Co., Osaka, Japan). Thirty minutes later, the guinea pigs were killed, and the diameter of blue spots on the inner side of the skin was measured. A blue spot larger than 5 mm in diameter was judged as positive. Three recipients were injected with 6 dilutions (1 : 100 to 1 : 3200) of an antiserum. Therefore, PCA titers of each of the 5 sera could be determined in 3 recipients. The PCA titers were presented as the geometric mean of PCA titers obtained in each of the 3 recipients. These procedures were taken to minimize the differences in sensitivity of individual recipients.

Differential leukocyte count (blood was drawn from abdominal vena cava): Total leukocytes were counted using a micro cell counter (CC-15, Sysmex Co., Tokyo, Japan). Differential leukocyte count was done by light microscopy of Giemsa-stained blood smears.

Analysis of data: The data are expressed as the mean ± S. E.. Statistical significance of difference was determined by Student’s t-test or Welch’s test.

Results

ACh or Hist were administered intravenously to the guinea pigs of BHS and BHR at the doses (ACh : 10, 20 and 40 μg/kg, Hist : 1, 2.5 and 5 μg/kg) indicated in Figs. 1 and 2. Bronchoconstriction to both of the chemicals increased significantly with dose dependence (P < 0.05, P < 0.01). Bronchoconstriction of BHS was increased compared with those of BHR. Thus, there was obvious difference between two lines.

Fig. 3 shows the results of bronchoconstriction to LTD₄ (0.2 μg/kg, i. v.). The same result as those shown in Figs. 1 and 2 was obtained. Bronchoconstriction of BHS (48 ± 4.5

Fig. 1. Acetylcholine-induced bronchoconstriction in guinea pigs of bronchial hypersensitive line (BHS) and bronchial hyporesitive line (BHR). * **: significantly different from BHR (P < 0.05, P < 0.01, respectively). Values represent the mean ± S. E. of five male animals of F₄-generation.
Fig. 2. Histamine-induced bronchoconstriction in guinea pigs of bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR). N. S.: not significantly different *. **: significantly different from BHR (P<0.05, P<0.01, respectively). Values represent the mean ± S. E. of five male animals of F4 generation.

Fig. 3. Leukotriene D4 (0.2 μg/kg, i. v.)-induced bronchoconstriction in guinea pigs of bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR). **: significantly different from BHR (P<0.01). a): values represent the mean ± S. E. of nine female animals of F6 generation.

% was increased significantly compared with those of BHR (26±3.9%, P<0.01).

Table 1 shows the number of the binding sites (Bmax) and affinity (Kd) of receptors on lung membrane preparation of guinea pigs of two lines. Of mACh receptors, the number and affinity of BHS (130±9.5 fmol/mg protein, 0.565±0.054 nM) increased significantly compared with those of BHR (89±6.8 fmol/mg protein, 0.233±0.037 nM, P<0.01). There were no significant difference observed between the animals of two lines in the number and affinity of β-Adr and Hist H1 receptors.

Fig. 4 shows the results of IgE antibody production to OA in actively sensitized guinea pigs of BHS and BHR. Mean ± S. E. of PCA titer of BHS and BHR was 880±196 in the former and 1160±286 in the latter, respectively. Thus, no difference in IgE antibody production was observed between the animals of BHS and BHR.

Table 2 shows total leukocyte and differential leukocyte count in animals of BHS and BHR. Lymphocytes (86.3±1.4%) and eosinophils (3.6±0.6%) in BHS were significantly higher compared with those of BHR (76.3±3.3% and 1.4±0.2%, respectively, P<0.01). Reversely, neutrophils (7.4±0.9%) in BHS were significantly lower compared with those of BHR (19.5±3.4%, P<0.01).

Discussion

Bronchial hypersensitivity is a prerequisite for diagnosis of bronchial asthma patients [1]. Generally, guinea pigs have been widely used as allergic and asthmatic model animals.
Table 1. Changes in various receptors of lung membrane preparation in bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR)

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Group</th>
<th>B max (fmol/mg protein)</th>
<th>Kd (nM)</th>
</tr>
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<tbody>
<tr>
<td>Muscarinic</td>
<td>BHS</td>
<td>130±9.5**</td>
<td>0.565±0.054**</td>
</tr>
<tr>
<td></td>
<td>BHR</td>
<td>89±6.8</td>
<td>0.233±0.037</td>
</tr>
<tr>
<td>β-adrenergic</td>
<td>BHS</td>
<td>698±34</td>
<td>0.801±0.053</td>
</tr>
<tr>
<td></td>
<td>BHR</td>
<td>700±56</td>
<td>0.805±0.043</td>
</tr>
<tr>
<td>Histamine H₁</td>
<td>BHS</td>
<td>138±17</td>
<td>0.453±0.086</td>
</tr>
<tr>
<td></td>
<td>BHR</td>
<td>117±8</td>
<td>0.311±0.033</td>
</tr>
</tbody>
</table>

Values indicate mean±S.E. **: Significantly different from BHR at P<0.01.

Seven male animals of F₄ generation were used as one group.

Table 2. The total leukocytes and differential leukocyte count in guinea pigs of bronchial hypersensitive line (BHS) and bronchial hyposensitive line (BHR)

<table>
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<tbody>
<tr>
<td>BHS</td>
<td>1</td>
<td>78</td>
<td>9</td>
<td>82</td>
<td>5</td>
<td>0</td>
<td>4</td>
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<tr>
<td></td>
<td>2</td>
<td>61</td>
<td>4</td>
<td>88</td>
<td>5</td>
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<tr>
<td></td>
<td>3</td>
<td>61</td>
<td>11</td>
<td>80</td>
<td>6</td>
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<tr>
<td></td>
<td>4</td>
<td>56</td>
<td>9</td>
<td>85</td>
<td>4</td>
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<td>2</td>
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<td></td>
<td>5</td>
<td>90</td>
<td>5</td>
<td>91</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>100</td>
<td>5</td>
<td>91</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td></td>
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<tr>
<td></td>
<td>7</td>
<td>59</td>
<td>8</td>
<td>85</td>
<td>4</td>
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<td></td>
<td>8</td>
<td>67</td>
<td>8</td>
<td>88</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>71.5</td>
<td>7.4**</td>
<td>86.3**</td>
<td>3.6**</td>
<td>0</td>
<td>2.8</td>
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<tr>
<td>± S.E.</td>
<td></td>
<td>5.7</td>
<td>0.9</td>
<td>1.4</td>
<td>0.6</td>
<td>0</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

BHR

|        | 1         | 75                     | 16                            | 81     | 1      | 0    | 2    |
|        | 2         | 76                     | 19                            | 77     | 2      | 0    | 2    |
|        | 3         | 86                     | 5                             | 90     | 1      | 1    | 4    |
|        | 4         | 74                     | 23                            | 72     | 2      | 0    | 3    |
|        | 5         | 86                     | 21                            | 73     | 2      | 0    | 4    |
|        | 6         | 49                     | 22                            | 74     | 1      | 0    | 3    |
|        | 7         | 92                     | 12                            | 84     | 1      | 0    | 3    |
|        | 8         | 108                    | 38                            | 59     | 1      | 0    | 2    |
| Mean   |           | 80.8                   | 19.5                          | 76.3   | 1.4    | 0.1  | 2.9  |
| ± S.E. |           | 6.0                    | 3.4                           | 3.3    | 0.2    | 0.1  | 0.3  |

Abbreviations: Neutr.: neutrophils Lymph.: lymphocytes Eos.: eosinophils Baso.: basophils Mono.: monocytes. Values represent the mean±S.E. of eight male animals of F₄ generation. a) : ×10⁹/μl **: significantly different from BHR (P<0.01, respectively).

[2,14]. Therefore, in order to establish model animals with constant bronchial hypersensitivity, and with hyposensitivity as a control, Hartley guinea pigs have been selected to two ways using their reactivity to ACh and Hist exposure as parameters. We have reported [22]
that two lines have already been separated onto F<sub>6</sub> generation. In this report, a study was undertaken on the characteristics in animals of the two lines.

The bronchial reactivity in guinea pigs to intravenously administered ACh and Hist was found to be obviously different between the animals of the two lines, i.e., it was considerably higher of BHS, compared with that of BHR.

Bronchoconstriction induced by ACh is known to occur directly through mACh receptors [27], while Hist is known to act through the vagus nerve in addition to its direct action on Hist H<sub>1</sub> receptors [14]. It is feasible that constitutional change might have occurred to distinguish animals of BHS and BHR.

It is well known that LTD<sub>4</sub> has strong bronchoconstrictive effects on human or guinea pig airway [8]. To confirm this assumption, it was studied whether difference in bronchoconstriction induced by LTD<sub>4</sub> (i.v.) could be observed between the two lines. Bronchoconstriction of BHS was found to have increased significantly compared with those of BHR.

Next, the receptors and the affinity on lung membrane preparation of guinea pigs of two lines were assayed. The number of mACh receptors and its affinity was significantly increased in BHS compared with BHR. This finding is in agreement with the intensity of bronchoconstriction caused by intravenous injection of ACh. On the other hand, no difference was observed in the number and affinity of β-Adr receptors and Hist H<sub>1</sub> receptors between BHS and BHR animals. It is known that the number of β-Adr receptors decreases in experimental asthma in sensitized guinea pigs [4], and in asthmatic patients with attack of asthma [28]. However, since our guinea pigs were not sensitized, there could be no difference in numbers of the β-Adr receptors. As mentioned above concerning Hist, bronchoconstriction in these guinea pigs must have been induced via vagus nerve in addition to its direct action on Hist H<sub>1</sub> receptors. From this reason, no difference could have been observed between the animals of two lines.

It has been well known that IgE antibody in asthmatic patients with atopy is higher than in normal subjects [5]. Therefore, we investigated IgE antibody production in guinea pigs of two lines. No difference in antibody production was observed between the animals of BHS and BHR. It is clinically known that there is no correlation between bronchial hypersensitivity and antibody production [25]. Popa [25] studied bronchial responsiveness to Hist and allergen in 26 subjects with ragweed allergy. He reported that specific IgE level was not correlated with the intensity of bronchial sensitivity to allergen. Thus, there was no correlation between bronchial sensivity and IgE production in guinea pigs of BHS and BHR. These results suggest as if genetic control of IgE antibody production and that of bronchial sensitivity are operating independent by to each other in guinea pigs of BHS and BHR.

The patients with atopic allergy are well known to have larger number of eosinophils compared with normal subjects in peripheral hematological examination [13]. Leukocytes and eosinophils in animals of BHS were significantly large number, compared with BHR. Reversely, neutrophils in BHS were significantly small number, compared with BHR. From these results, it seems to us that these differences between BHS and BHR are genetically determined. However, it is still unclear whether there is any correlation between bronchial sensitivity and differential leukocyte count in guinea pigs of the two lines.

In future, when our guinea pigs were clarified bronchial hypersensitivity, we think to be advantage for elucidation of pathogenesis of bronchoconstriction or development of new drugs.

References

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アセチルコリンおよびヒスタミン暴露に対する
気道感受性を異にするモルモット二系統
(BHS, BHR) の特性

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気管支喘息モデル動物として、気道過敏系 (BHS) や
びその対照として気道非過敏系 (BHR) のモルモット
二系統が著者らによりすでに開発されている。今回、こ
れらモルモット二系統の生物学的特性について調べ、以
下の結果を得た。1）アセチルコリン、ヒスタミンおよ
びロイコトリエン D4 の静脈内投与による BHS の気道
抵抗は BHR より高かった。2）肺膜における BHS の
ムスカリシン受容体数および親和性は BHR と比較して、有意に増加した。しかしながら、β
アドレナリン、ヒスタミン H1 受容体数および親和性は
二系統間に差を認めなかった。3）卵白アルブミンに対
する IgE 抗体産生能は二系統間に差を認めなかった。
4）BHS および BHR の末梢血における白血球数、白
血球分類について調べた。BHS のリンパ球、好酸球は
BHR よりも有意に多く、逆に BHS の好中球は BHR よ
りも有意に少なかった。