● 原 著

宿主因子がBAL-cellグルココルチコイドレセプターに及ぼす影響について

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Host conditions that affect the glucocorticoid receptors of the bronchoalveolar lavage cells

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【Summary】

Using rabbits, this study was undertaken to ascertain whether various physical conditions of the host, as well as pretreatment with prednisolone, affect the glucocorticoid receptor (GR) content of the bronchoalveolar lavage (BAL) cells. Our results have shown that BAL-cells from rabbits of various ages were found to exhibit an age-dependent reduction of the GR content. As for the effect of nutritional deprivation, the GR content tended to increase during the initial phase of malnutrition (days 4 to 8), and then return to the control level after day 12. Most significantly, the GR content of the BAL-cells from malnourished rabbits showed a limited response to cellular activation. Further, chronological changes were seen in the GR content of BAL-cells from non-treated rabbits and from sensitized rabbits that were given an intravenous injection of prednisolone (2 mg/kg). In both instances, the GR content showed a transitory decrease after 3 hours, and a rapid increase by day 4, followed by a return to the control levels. In contrast, no significant changes in the dissociation constant were seen among the BAL-cells from each experimental procedure. These results indicate that the GR content of BAL-cells are greatly influenced by the conditions of the host, such as aging, cellular activation from a sensitization, malnutrition, and pretreatment with a steroid, so that these factors should be considered before deciding on a regimen of steroid therapy.

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glucocorticoid receptor,
host conditions,
pretreatment with prednisolone

【概　要】

呼吸器疾患におけるステロイドの効果の指標として家兎気管支肺胞洗浄細胞（以下BAL-cell）のグルココルチコイドレセプター（以下GR）をとりあげ，宿主因子（加齢，活性化，絶食）およびステロイド投与が及ぼす変化を観察した．その結果，加齢では若年群ほど高値を示した．

絶食では一過性に増加した後，減少傾向を示し，さらにheat-killed BCGによって活性化されたBAL-cellのGRの上昇を抑制した．一方，プレドニゾロン1回投与によるGRの推移をheat-killed BCG感作群と非感作群において観察したところ，両群とも3時間後に低下し，2日から4日後に一過性に上昇を示したが7日後に前値に復した．

以上の結果より，ステロイド治療を施行する際は，これらの宿主因子およびステロイド投与がBAL-cellのGRを変化させることを十分考慮に入れる必要があると考えられた．

I. Introduction

Since the use of glucocorticoids for the clinical management of a respiratory disease is now widespread[1], to have a precise indicator of the body's response to the steroid would be of great benefit. Recent investigations[2] have demonstrated that alterations in the composition and function of the bronchoalveolar lavage cells (BAL-cells) from patients with an immunological lung disease are associated with the initiation and/or intensity of the alveolar inflammation, and with the patient's prognosis. Further, the effects of glucocorticoids on target cells are said to be mediated by glucocorticoid receptors[3]. Hence, a clearer knowledge of factors that affect the receptors is vital to understand the overall response to a glucocorticoid.

Therefore, we have measured the glucocorticoid receptors in BAL-cells obtained from rabbits under conditions such as aging, malnutrition, and pretreatment with prednisolone, so as to clarify the physiological changes to the glucocorticoid receptors caused by such conditions.

II. Materials and methods

1. Rabbit treatment regimen (Fig. 1)

Male New Zealand white rabbits, weighing 1.5 to 3.5 kg, were used for this study. They were housed in metal cages under a normal day-night cycle. So as to obtain the required BAL-cells for each host condition to be examined, we divided the rabbits into 5 groups. Each group then received one of the numbered procedures described below.

1) Aging

BAL-cells were obtained from rabbits under 2 months old (infants), from 5 to 10 months old (adults), and over 24 months old (aged rabbits).

2) Malnutrition

Malnourished rabbits were prepared by restricting food intake for 4, 8, and 12 days.

3) Cellular activation and malnutrition

Rabbits were sensitized intravenously with 500 μg of a heat-killed BCG (gift from Dr. Q.N. Myrvik) suspended in 0.1 ml of light mineral oil[4]. Three weeks after this the rabbits were malnourished for 8 and 12 days.

4) Pretreatment with prednisolone

After a venous administration of prednisolone (2 mg/kg ; Shionogi Co., Osaka), rabbits were sacrificed at 3 h, 6 h, and at the end of days 1, 2, 4, and 7.

5) Pretreatment with prednisolone after a sensitization

Rabbits that had been sensitized by heat-killed

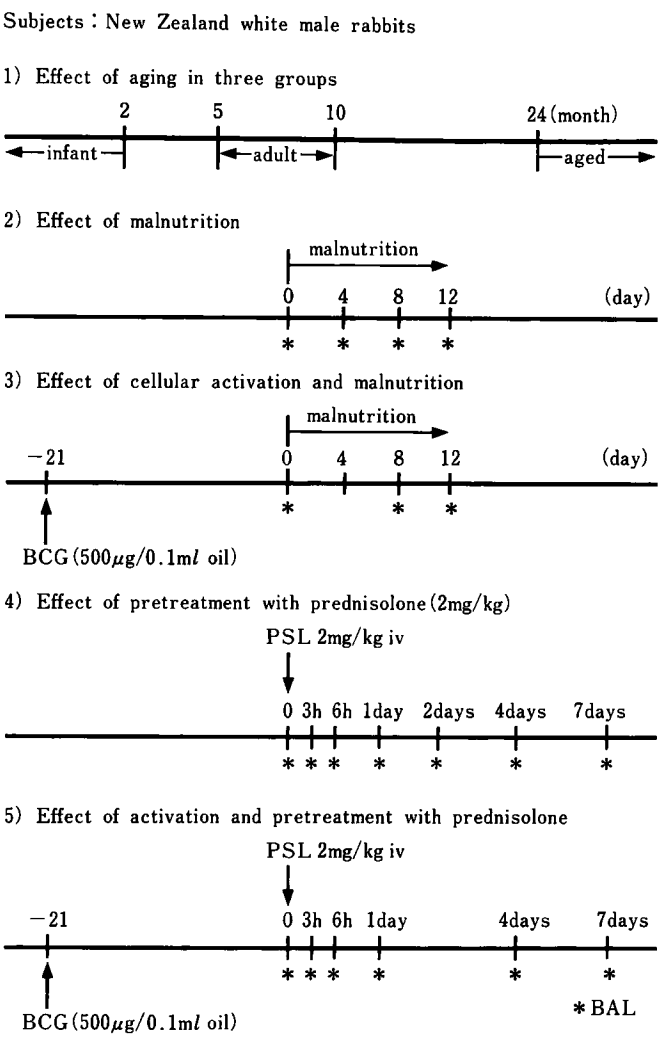

Fig. 1 Experimental design. The number of the experimental protocol corresponds to the number and description given in materials and methods.

BCG 3 weeks previously were administered the same dose of prednisolone described in 4) and sacrificed at 3 h, 6 h, and at the end of days 1, 2, 4, and 7.

Normal rabbits were used as a control for a procedure given in 2) and 4).

2. Preparation of cells

On being subjected to one of the procedures described above, each rabbit was anesthetized by an intravenous injection of sodium pentobarbital (100 mg per kg) at 10 a.m. The abdominal cavity was then opened, and the rabbit sacrificed by descending aorta exsanguination. The lungs were lavaged by an endotracheal instillation of saline until a total of 200 m*l* of the lavage fluid was collected. Following lung lavage, the cells were washed three times in phosphate buffered saline (PBS, pH 7.4) and suspended in PBS at a concentration of 1.0×10^7/m*l*. After rinsing, the total cell number was counted by routine hemocytometry. Cell differentials were also performed by cytocentrifuged smears stained with May-Gruenwald-Giemsa.

3. Receptor content

The count of the glucocorticoid receptors (GR)

in the 1.0×10^6 BAL-cells per tube was measured by using ^{3}H-prednisolone (18.5 MBQ/2 ml) (Radiochemical Center, Amersham Corp., Arlington Heights, IL). The BAL-cells were then incubated with 1, 2, 4, 10, 20 and 40 nM of ^{3}H-prednisolone in the presence of absence of approximately a thousand-fold excess of prednisolone for 2 h at 37°C under gentle shaking. The mixture was then filtered through a glass fiber filter (Whatman GF/B), after which cells that had been trapped by the filter were washed 4 times with PBS. Following this, the filter was dried, and its radioactivity measured by a scintillation counter. The number of binding sites per cell and the dissociation constant were then calculated from Scatchard plots[5,6].

Statistical comparisons were made by ANOVA, followed by Tukey's multiple comparison procedure.

III. Results

1. Cell populations of BAL

Fig. 2 illustrates the percentage of macrophage and lymphocyte in BAL from rabbits of various conditions. BAL-cell from BCG+pretreatment with PSL, compared with BCG-vaccinated BAL-cells, the percentage of macrophage increased. Aging and fasting did not affect the BAL-cell population.

2. Aging

The age-dependent reduction of the GR content showed significant differences, i.e., between the infant vs the adult rabbit ($p<0.05$), and the infant vs the aged rabbit ($p<0.05$) (Fig. 3). However, aging did not affect the dissociation constant (K.D. mean±SE infant : 17.6±5.6, adult : 8.46±2.5, aged : 10.5±2.1 not statistically significant).

3. Malnutrition and activation

Fig. 4 illustrates the fluctuation of the GR content in the BAL-cells from rabbits with or without malnourishment. The GR content tended to increase during the initial phase of malnutrition, and then return to the control level after day 12. An important finding is that the GRs in the BAL-cells from the malnourished, sensitized rabbits showed a far greater decrease at day 12 (mean : 2,175±197 binding sites/cell, $p<0.01$), when compared with the GR content of the BAL-cells of rabbits subjected to malnutrition alone (K.D. mean±SE day 4 : 16.0±7.5, day 8 : 13.1±6.3, day 12 : 9.7±2.7, BCG : 19.7±6.3, BCG and day 8 : 24.6±5.0, BCG and day 12 : 13.8±3.3, not statistically significant).

4. Pretreatment with prednisolone and sensitization

The chronological changes in the GR content of the BAL-cells from the rabbits pretreated solely with an intravenous injection of prednisolone are indicated by the dotted line in Fig. 5, where in the GR content reached its lowest GR value at 3 h after injection (mean : 2,444±545 binding sites/cell, not statistically significant) and peaked on day 2 (mean : 9,770±1,147 binding sites/cell, $p<0.01$), followed by a return to the GR level of the controls on day 7 (mean : 2,994±538 binding sites/cell, not statistically significant).

As for the BAL-cells from the BCG-sensitized, prednisolone-pretreated rabbits, the GR content also showed a similar pattern, as can be seen by the solid line in Fig. 5, with the greatest GR decrease occurring at 3 h and the highest GR increase on day 1 (mean : 9,676±1,429 binding sites/cell, not statistically significant). Interestingly, the decrease of the GR content at day 7 was greater in the rabbits given a pretreatment of prednisolone than in the rabbits given prednisolone only. However, no significant change in the dissociation constant was noted in any of the experimental procedures (K.D. mean±SE PSL 3h : 4.0±2.5, PSL 6h : 7.7±2.2, PSL day 1 : 22.1±10.4, PSL day 2 : 18.2±4.2, PSL day 4 : 30.1±7.5, PSL day 7 : 10.7±1.6, BCG and PSL 3h : 11.7±2.2, BCG and PSL 6h : 33.8±6.1, BCG and PSL day 1 : 34.3±4.5, BCG and PSL day 2 : 18.6±3.0, BCG and PSL day 4 : 17.3±1.8, BCG and PSL day 7 : 11.6±2.7 not statistically significant).

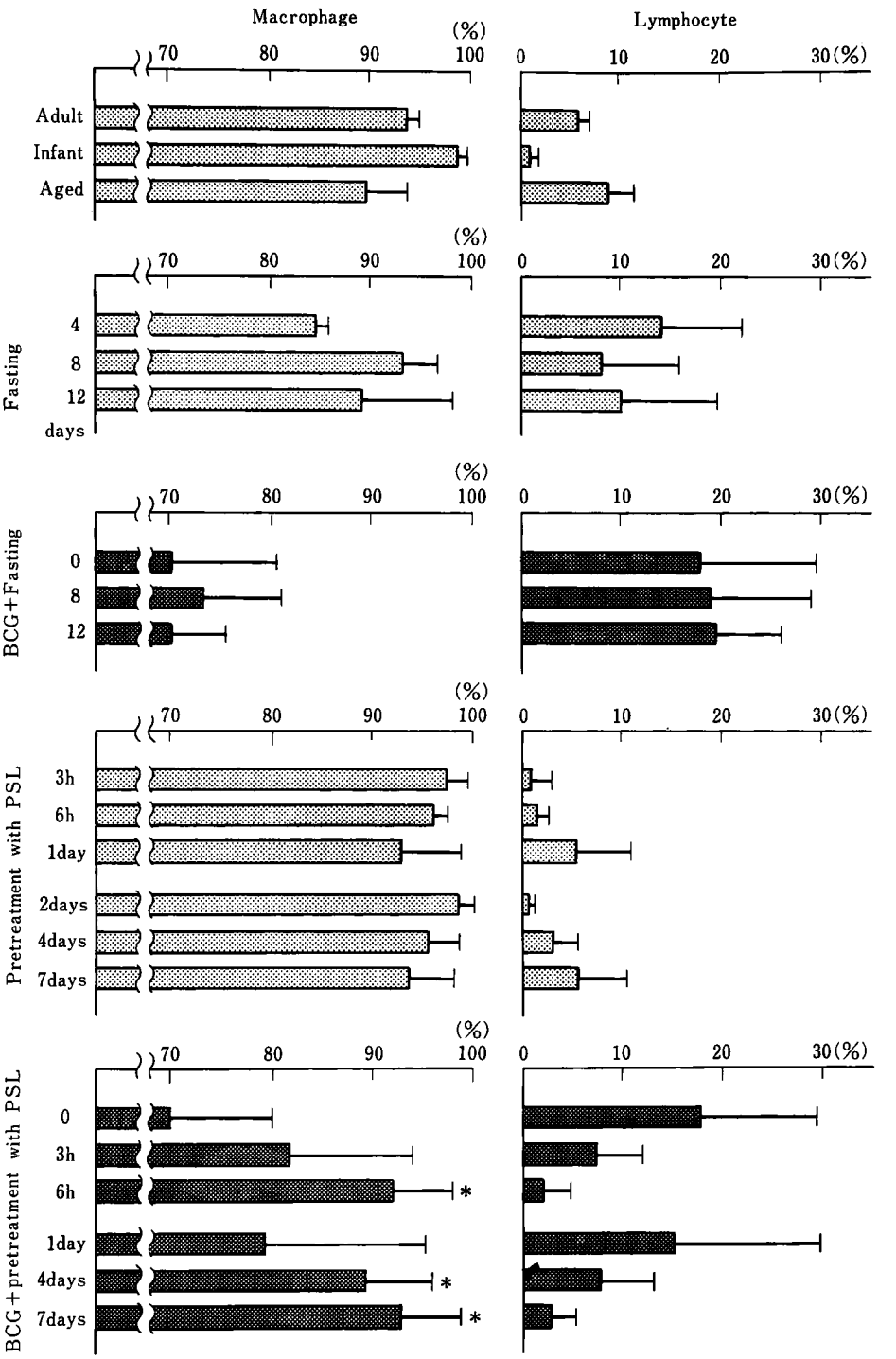

Fig. 2 Cell populations of BAL from rabbits of various conditions. In BAL-cells from BCG+pretreatment with PSL, compared with BCG-vaccinated BAL-cells, the percentage of macrophage increased. Fasting did not affect cell population of BAL. *$p<0.05$ ▨ without BCG ■ with BCG

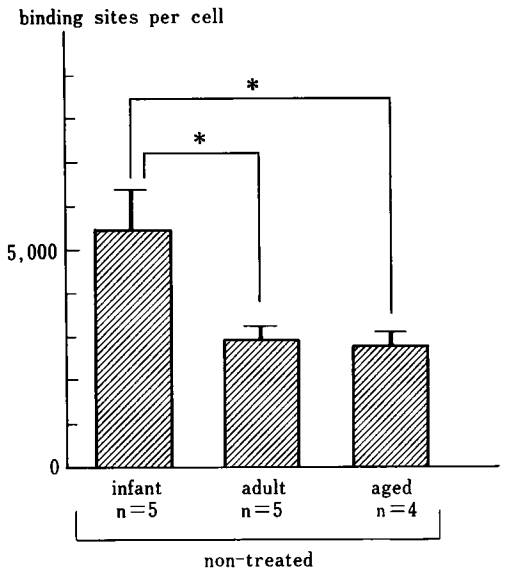

Fig. 3 The glucocorticoid receptor (GR) count in the bronchoalveolar lavage cells (BAL-cells) from various aged rabbits. The GR content showed a decrease with advancing age. Each value is expressed as a mean±SE. *p<0.05

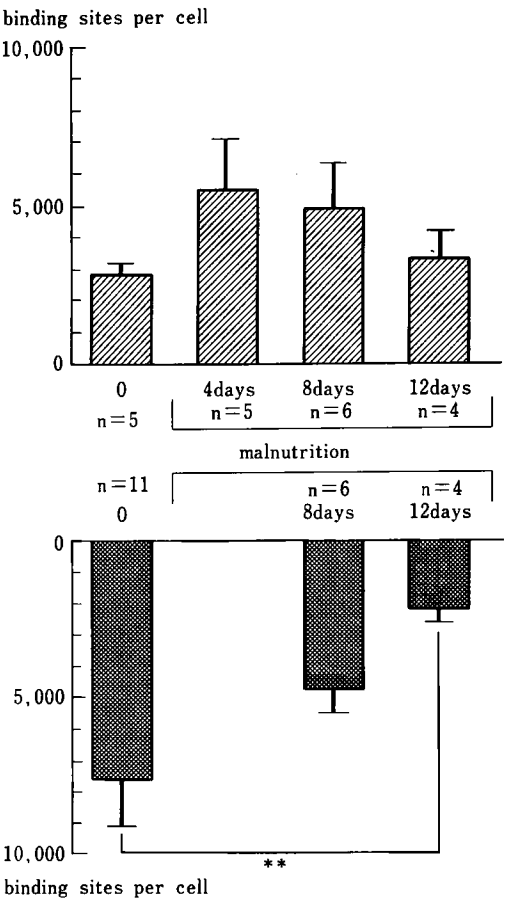

Fig. 4 Changes in the GR count in BAL-cells from malnourished rabbits (upper, hatched column) and the vaccinated, malnourished rabbits (lower, gray column). Note the marked decrease in the GR number in rabbits subjected to a vaccination and malnutrition (See Fig. 1 for the experimental procedure). Each value is expressed as a mean±SE. **p<0.01

IV. Discussion

This study was designed to determine whether host conditions that are encountered in clinical practice influence the GR content of BAL-cells. Also, as much attention has been focused on analyzing the possible prognostic indicators of steroid responsiveness. Munk and his colleague reported that the relation between receptor number and sensitivity of a cellular response to glucocorticoids is rather complicated[3]. It seems that it is a more important and direct analysis to know the changes in the GR content of the BAL-cells in terms of the first step of steroid action. As to the GR content of BAL-cells, especially the influence of host conditions remains unclear.

Our data has confirmed that the GR content of BAL-cells show an age-dependent reduction. Giannopoulos G, et al. have also reported the same results, having found that the GR content in fetal rabbit tissue was higher than the GR content in the adult rabbit[7]. This finding may account for the fact that younger patients with idiopathic pulmonary fibrosis tend to respond to glucocorticoids[8]. Little is known, however, about the mechanisms of this age-dependent reduction, but a recent investigation has disclosed that the cell cycle is closely related to the GR content[3], so that this same mechanism may be involved in an age-related GR reduc-

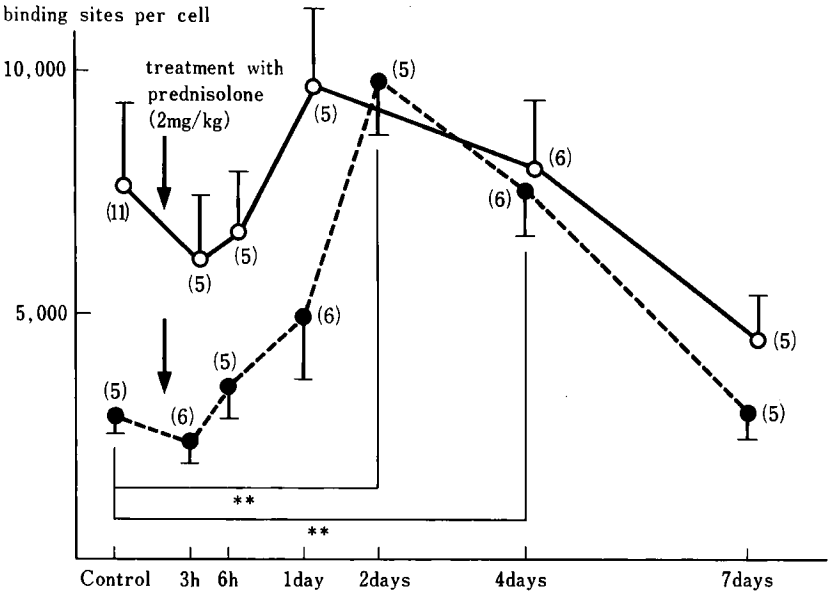

Fig. 5 The fluctuations of GRs in BAL-cells from the prednisolone-treated rabbits with or without a vaccination pretreatment (solid line and dotted line, respectively). Note the initial increase in number of GRs in both groups, whereas the subsequent decrease in the GR content of BAL-cells from the vaccinated, prednisolone-treated rabbits was much greater than from rabbits given prednisolone only. Each value is expressed as a mean±SE. n=( ). **$p<0.01$

tion.

George S.C. et al. have reported that the presence of activation is associated with a large increase in the number of specific glucocorticoid binding sites per cell[1]. Our data showed that the GRs in the BAL-cells from the malnourished, sensitized rabbits were far fewer than those in the GR content of BAL-cells from rabbits subjected to activation only, which suggests that malnutrition exerts more influence on the GRs in activated BAL-cells than on the GR content of normal BAL-cells. Also, Krasznai et al. have reported that in patients with a malignant tumor, the preoperative GR values were significantly lower in inoperable cases than in the operable cases[9]. Neifeld et al.[10] have shown that both protein and an RNA synthesis are necessary for an increase in the glucocorticoid-binding activity. Therefore, the mechanism underlying this lowering could include a decrease in the GR synthesis due to malnutrition. Therefore, glucocorticoid therapy may be more effective during the early phase of the disease than at the late phase, since patients with a long clinical history are often malnourished.

It is generally accepted that exogenous glucocorticoids regulate their receptors downwards in various tissue[11~13], and that this lowering of the GR content is one of the mechanisms leading to an increased tolerance to glucocorticoids. Thus, to achieve the greatest benefit with a minimal side effect, it would seem preferable to determine the changes that may have occurred to the GR content and then decide on the timing of a glucocorticoid administration. There have been, however, few reports about GR changes after a lowering of the GRs *in vivo*[14,15].

In our data, the GR content showed an initial increase just after a transitory down-regulation, followed by a decrease at day 7. On the other hand, Janet et al. have found that an administration of a glucocorticoid (prednisolone) resulted in a 30% decrease in the GR content after 1 week. They further reported no changes in the GR content

during the first 1 week. These slightly conflicting findings may be attributed to a difference in the administration schedule of the prednisolone. In both instances, however, the initial increase in the number of GRs which were observed after a single administration of a glucocorticoid would appear to support the clinical usefulness of high-dose steroid pulse therapy.

Our data has revealed that conditions such as aging, cellular activation, malnutrition, and the regular use of steroids greatly influence the GR content of the BAL-cells. Therefore, these common clinical conditions should be taken into account when considering the use of a steroid therapy.

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