Research Notes

Chicken lines selected for their primary antibody response to sheep red blood cells show differential hypothalamic-pituitary-adrenal axis responsiveness to mild stressors

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ABSTRACT The interaction between the neuroendocrine system and the immune system is well established and supports their mutually affecting relationship. Many animal selection lines have been created according to individual behavioral or neuroendocrine responses to stress. Here we present 2 chicken lines selected for 25 generations for their primary antibody response to immunization with SRBC, as well as the control line from the same parental strain. In the first experiment, the blood-sampling procedure caused a mild stress response, with the expected increase in plasma corticosterone levels. In a second experiment, group housing caused the expected increase in corticosterone levels. In both experiments, the hens of the low line showed the greatest increase in corticosterone levels to our 2 mild stressors. Our results show that birds selected throughout 25 generations for an immune parameter show different HPA axis responsiveness.

Key words: corticosterone, selection line, hypothalamic-pituitary-adrenal axis

INTRODUCTION

It is widely accepted that there is a 2-directional relationship between the neuroendocrine system and the immune system, and the complexity of this relationship is still being unraveled (Beishuizen and Thijs, 2004). The genetic background and environmental factors determine the phenotype of the individual (Ellenbroek and Cools, 2002). Within this interplay, there is a profound and complex relationship, which is reflected in stress handling and disease resistance in the individual (Elenkov and Chrousos, 2006). The influence of genetic background on disease resistance is often investigated in animal model species using selection lines that are created based on different behavior in experimental settings or on different endocrine responses to stress, or both (Lindqvist et al., 2007; Owen et al., 2008). The extremes of such selection lines have shown similarities in typical behavior and their endocrine responses that have been translated into 2 different coping styles. Animals showing a proactive coping style show active behavior when given this possibility and a low hypothalamic-pituitary-adrenal (HPA) axis response as the proactive behavior requires the activation of the sympatho-adrenomedullary system. On the other hand, the animals showing a more passive style of coping, also known as reactive coping, show less behavioral activity with a high HPA axis response. This dichotomy has been found in many species, such as rodents, chicken, and fish (Koolhaas et al., 2007; Øverli et al., 2007).

Disease resistance has been postulated to be a multigenic trait, regulated by the immune system and its modulations by interactions with physiological and environmental factors (Zekarias et al., 2002; Fulton, 2004; Owen et al., 2008). Here we present a selection line of layer hens that has been selected for 25 generations for their primary antibody (Ab) response to immunization with SRBC. Three lines were selected: the high responders, which produce high Ab levels to SRBC after immunization; the low responders, which produce low Ab levels to SRBC after immunization; and a control line from the same parental stock (Parmentier et al., 1994). In most selection lines, the lines are inbred so there is genetic uniformity. In the used selection lines, the selection is done randomly, keeping the maximum genetic variation intact, thereby causing the lines not to be exclusive to the selection criteria. For example, within the group of high responders, birds can be found that still show a low specific Ab formation and vice versa in the group of low responders. Interestingly, almost all immunological parameters of these lines differ, in-

Recently, neuroendocrine differences in responsiveness were found in these selection lines in response to cold stress (Hangalapura et al., 2004), suggesting a difference in HPA axis reactivity. Siegel et al. (1992) presented the first differences in corticosterone responses to immunization in these birds. Here we present data in which the differences in immune competence appear to be related to the same dichotomy as is seen in selection lines based on behavioral or endocrine criteria, or both.

**MATERIALS AND METHODS**

**Chickens, Housing, and Feed**

In this experiment, ISA Brown (Warren) medium heavy layer hens from 3 lines were used. The selection lines were selected for 25 generations before this experiment for either high or low primary Ab responses at 5 d after i.m. immunization (without the use of adjuvant) with SRBC at the age of 35 d. The randomly bred third line is the control line, originating from the same parental stock. The first experimental group consisted of 72 hens evenly divided over the 3 selection lines, but not immunized with SRBC. The second experimental group consisted of 150 hens evenly divided over the 3 selection lines and without immunization with SBRC.

The birds of the first experiment were kept according to routine procedures for layer hens in individual battery cages. The hens of the second experimental group were kept in a floor housing system with a total of 6 birds per cage, 2 birds of each line. The light regimen was 14 h of light (0500 to 1900 h), and temperatures were between 16 and 21°C. The birds were fed ad libitum with a commercial diet (180 g/kg of CP, 3,460 kcal/kg) with free access to water. Birds were weighed weekly, before the sampling procedure, therefore making them accustomed to handling.

All experiments were approved by the Animal Welfare Committee of Wageningen University.

**Blood Sampling and Corticosterone Determination**

All blood samples were taken by wing venipuncture between 0800 and 1000 h, and the sampling procedure took a maximum of 2 min per bird. The selection lines were sampled at random so all lines were sampled evenly over the whole sampling period. Blood samples of the first experimental group were taken at 9 wk of age, and the sampling procedure was considered to be comparable to a mild stressor (Webb and Mashaly, 1984). The second experimental group was group-housed, which has previously been shown to elevate corticosterone levels, and was sampled at the age of 8 wk (Mashaly et al., 1984). Plasma from heparinized blood was collected after centrifugation of the samples and then stored at −20°C until further analysis. Plasma corticosterone was determined using a RIA kit (IDS Inc., Bolton, UK; Buyse et al., 1987).

**Statistical Analysis**

All data not normally distributed were omitted from analysis. The mean differences due to blood sampling and line were tested with Bonferroni’s correction. Differences between the selection lines were determined by multiple comparison of the means. All data are presented as mean ± SEM. All analyses were according to SAS procedures (SAS Institute, 2003).

**RESULTS AND DISCUSSION**

In the present study, the modulatory effects of 2 mild stressors, a standard blood sampling procedure and social housing, were examined in 2 selection lines of chicken, together with the control line, on the reactivity of the HPA axis as measured by the plasma corticosterone levels.

The selection lines showed differential corticosterone responses to the blood-drawing procedure as is presented in Figure 1. The variance of the corticosterone levels increases, respectively, from high, control, to low line hens, with the low line showing the greatest variance. The high and control line birds showed comparable mean corticosterone levels, and the low line birds showed the highest mean levels of plasma corticosterone (Table 1). The differences in corticosterone levels between the low line birds and the control and high line and low line birds were significant.

The variance in corticosterone levels was similar between the selection lines in the second experimental group (Figure 2). The data obtained from the second experimental group, with social housing plus blood drawing as mild stressor, showed significant differences between the low line birds when compared with the control and high line birds (Table 1).

To date, the neuroendocrine relationship with the immune system has mostly been examined in selection lines based on either behavioral or neuroendocrine criteria, or both (Owen et al., 2008). Here, we examined the HPA axis responses to 2 mild stressors (the blood-sampling procedure and social housing coupled with blood sampling) in a selection line of chicken based on an immunological criteria, the primary Ab response to SRBC. In this experiment, it was of crucial importance that we used noninbred divergently selected chicken lines, causing larger variance in the measured results as is comparable to neuroendocrine measurements of outbred animals (de Boer et al., 2003). The corticosterone response to the sampling procedure was different between the lines, wherein the low line birds with a low Ab response showed the highest plasma corticosterone levels in both experimental settings. Because the birds
were accustomed to weekly handling, the sampling procedure was considered to be comparable to a mild stressor. Mild stress only elevates corticosterone levels slightly (Salomé et al., 2006; Hazard et al., 2008).

Our data together with the data from Siegel et al. (1992) and Hangalapura et al. (2004) showed that the low line birds have increased HPA axis reactivity in different experimental settings and when compared with the high line and control line birds. The measured corticosterone responses during cold stress and immunization clearly showed that the high line birds showed a rapid and lower corticosterone response. These results indicate that although selected for an immune trait, these selection lines show differential sensitivity to different stressors as is well known in coping styles. Within coping styles, the reactive animals are more environmentally sensitive and the proactive animals are more intrinsically regulated (Koolhaas, 2008). In the previously discussed context of coping styles, our data suggest that the low line birds may be considered to be reactive copers and the high line birds may be considered to be proactive copers. Therefore, selection based on immunological criteria results in the same dichotomy as is seen with selection based on either behavioral or neuroendocrine differences (van Hierden et al., 2002; Flisikowski et al., 2008).

In conclusion, chickens selected for their differential Ab response show the same dichotomy in HPA axis responsiveness as is seen within coping styles. Because these selection lines have already been extensively inves-

**Figure 1.** Scatter plot of the measured corticosterone levels (ng/mL) in plasma in all of the individual birds of the first experiment with the blood-drawing procedure as mild stressor in the high (H), control (C), and low (L) line hens. n = 23 to 24 birds per line. Arrows indicate data omitted from the analysis. *P < 0.05; **P < 0.01.

**Figure 2.** Scatter plot of the measured corticosterone levels (ng/mL) in plasma in all of the individual birds of the second experiment with the blood drawing and social housing as mild stressor in the high (H), control (C), and low (L) line hens. n = 46 to 50 birds per line. Arrow indicates data omitted from the analysis. *P < 0.05.

<table>
<thead>
<tr>
<th>Line</th>
<th>Blood sampling</th>
<th>Group housing</th>
</tr>
</thead>
<tbody>
<tr>
<td>High line (H)</td>
<td>46.1</td>
<td>57.8</td>
</tr>
<tr>
<td>Control line (C)</td>
<td>28.2</td>
<td>57.7</td>
</tr>
<tr>
<td>Low line (L)</td>
<td>81.3</td>
<td>76.3</td>
</tr>
<tr>
<td>SEM</td>
<td>12.2</td>
<td>6.5</td>
</tr>
<tr>
<td>H vs. L</td>
<td>0.0286</td>
<td>0.0437</td>
</tr>
<tr>
<td>C vs. L</td>
<td>0.0060</td>
<td>0.0457</td>
</tr>
<tr>
<td>Line</td>
<td>0.0144</td>
<td>0.0658</td>
</tr>
</tbody>
</table>

1Data are group (least squares) mean: blood sampling n = 22 birds per line; group housing n = 44 to 49 birds per line.

2Line = line effect.

3Line contrasts.
tigated for various immune traits, they present a suitable model for investigating immune traits and coping styles (i.e., stress reactivity and disease resistance).

REFERENCES


