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Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses

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Abstract

In the present study, depriving chickens of foraging material was shown to induce stress. The impact of this type of stress on the immune response was compared with feeding of corticosterone (1.5 mg per bird per day), a hormone known to be immunosuppressive and to be the major stress hormone of chickens. Corticosterone feeding induced stress as revealed by higher heterophil/lymphocyte (H/L) ratios, longer tonic immobility (TI) reaction, reduced body weight gain and reduced egg production. Blood corticosterone levels were increased. Corticosterone feeding decreased the antibody response to tetanus toxoid and SRBC, DTH to PPD from *Mycobacterium tuberculosis* and the inflammatory response to PHA. Housing chickens on slats also induced chronic stress, as evidenced by increased H/L ratios, prolonged TI duration and decreased egg production. Corticosterone levels were slightly but not significantly enhanced. This novel form of chronic stress strongly suppressed humoral and cellular immune responses as evidenced by lower antibody titers to sheep red blood cells (SRBC) and tetanus toxoid (TT) decreased DTH reaction to PPD and inflammatory reaction to PHA in the skin. In contrast, the antibody response to human serum albumin (HSA) was neither influenced by corticosterone feeding nor by keeping the birds on slats. Even the combination of corticosterone feeding and housing the birds on slats did not significantly impair antibody responses to HSA. In conclusion, the present study showed that chronic stress induced by depriving the birds of foraging material led to a similar impairment of humoral and cell-mediated immunity as did feeding with corticosterone. More importantly, it showed for the first time that depending on the antigen tested, there are stress-resistant and stress-susceptible antigen responses.

Keywords: Chicken; Corticosterone; Stress; Immune response, humoral; Immune response, cellular; Feather pecking

1. Introduction

The immune response to a given antigen or pathogen is subject to modulation by both genetic or environmental factors. For example, it has been put forward that stress downregulates an immune response, thereby

Abbreviations: H/L, heterophil:lymphocyte; TI, tonic immobility; DTH, delayed-type hypersensitivity; HAS, human serum albumin; TT, tetanus toxoid; AU, arbitrary units; ANOVA, analysis of variance

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jeopardizing anti-microbial resistance (Berczi, 1998; Dantzer, 1997; Dohms and Metz, 1991; Glaser et al., 1998; Magnusson et al., 1998; Sheridan et al., 1998). Stress induces a complex neuroendocrine response with an activation of the hypothalamus–pituitary–adrenal gland axis, and involvement of the sympathetic nervous system. The supporting evidence, however is based to a large extent on the use of artificial stress models. Moreover, in most studies, a humoral response to a single antigen or pathogen was measured, and the impact of stress upon cell-mediated immunity has not been addressed (Boa-Amponsem et al., 2000; Hester et al., 1996). Although stress was induced experimentally, it has not been verified in most models that the desired effects on stress were indeed achieved, as evidenced by hormonal, behavioral or physiological parameters.

In this study, a novel way to induce stress has been compared with corticosterone treatment of birds with regard to its immunosuppressive properties. Birds of defined genetic background were housed on slats without access to litter material, and a variety of stress parameters were compared with responses induced by chronic corticosterone feeding, and with those of control birds housed on litter. That both corticosterone feeding and housing birds on slats induced stress was verified by determining four established indicators of stress in birds; increase in heterophil:lymphocyte (H/L) ratios, blood corticosterone level, tonic immobility (TI), and impaired general performance (e.g. egg production). Humoral immune responses to three defined antigens were determined at various intervals after immunization. Cell-mediated immunity was determined by a DTH reaction to mycobacterial antigens following immunization, and by the swelling of wattles upon injection of PHA. The latter response was shown to represent cutaneous basophil hypersensitivity (Corrier and DeLoach, 1990; McCorkle et al., 1980). Five out of six parameters representing adaptive immunity were strongly impaired by stress induction, whereas one response (antibody formation to human serum albumin) was not influenced. Thus, while providing solid evidence in support of the concept that stress impairs adaptive immunity in chicken, this study reveals for the first time the existence of both stress-resistant and stress-susceptible antibody responses.

2. Materials and methods

2.1. Animals, housing conditions and corticosterone treatment

At 11 weeks of age, a total of 251 white laying hens (Lohman Selected Leghorn hybrids) were randomly assigned to groups of 15 or 16 individuals and distributed among 16 pens of identical size (265 cm × 90 cm, height 235 cm) built side by side along a corridor.

Housing conditions and the application of corticosterone in the feed were varied between the pens (2 × 2 factorial design, Fig. 1A). In eight pens, part of the floor was covered with deep litter consisting of wood shavings, chaff and long-cut straw, as foraging material ('litter' condition). In the other eight pens, the whole floor area was made of slats (width 1 cm, 1.5 cm apart; 'slats' condition). From the onset of the experiments, the birds had for 10 h light per day throughout. In four of the slats pens and four of the litter pens dietary corticosterone (Sigma; 1.5 mg per bird per day, 'corticosterone' condition) was offered from 11 to 19 weeks of birds age. In the remaining eight pens, the birds received a diet devoid of corticosterone ('no corticosterone' condition) (for more details see El-Lethey et al., 2002). The chosen rearing and feeding conditions resulted in four different treatments ('litter/corticosterone', 'litter/no corticosterone', 'slats/corticosterone', 'slats/no corticosterone'), each randomly assigned to four pens (Fig. 1A). The experiment was subjected to the Swiss authorization procedure prescribed by the Swiss Animal Legislation (Application No. 85/99).

2.2. Blood sampling

At 14 weeks of age (Fig. 1B), blood samples (1.5 ml) were taken from the right wing veins of eight birds per pen for determination of both blood corticosterone concentrations and heterophil/lymphocyte ratios (H/L). For the latter, one drop of blood was smeared onto a glass slide using a cover glass technique (Campbell, 1988). The smears were stained using Diff-Quik (Dade AG, Duedingen, Switzerland). One hundred leukocytes, including both granular (heterophils, eosinophils, basophils) and non-granular (lymphocytes, monocytes) cells, were counted once

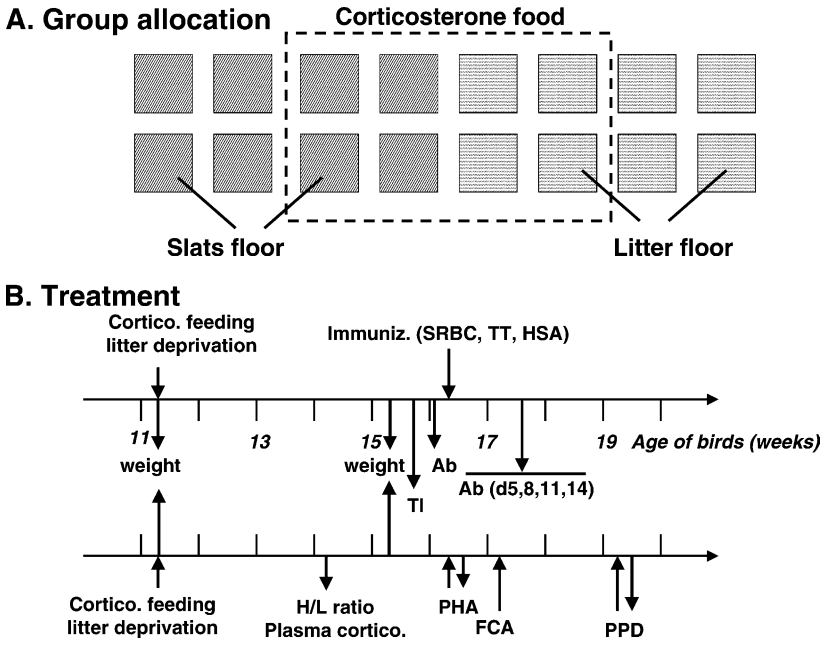

Fig. 1. Diagram showing the study design both with regard to allocation of the birds to the four treatment groups, each group consisting of four pens of 15–16 birds (panel A), and sequential experimental procedures (panel B). The vertical arrows indicate the approximate time after birth (in weeks) at which a treatment was applied. The horizontal arrows are the time axes for two subgroups of birds subjected to different experimental procedures. The blocks were randomized, and the graphical representation is designed to illustrate the experimental outline rather than the geographical arrangement of the pens. The abbreviations are as follows: Cortico.: corticosterone feeding; SRBC: immunization with sheep red blood cells; TT; tetanus toxoid; HSA: human serum albumin; Ab: antibody determination prior to immunization and at the indicated days after immunization; H/L ratio: determination of heterophil/lymphocyte ratio; PHA: PHA injection, followed by measurement of skin inflammation 24 h later; FCA: Freund's complete adjuvant; PPD: PPD injection, followed by measuring delayed-type hypersensitivity reaction 48 h later. Not shown: monitoring of egg production (daily between weeks 16 and 20 in both procedural subgroups).

on each slide using a light microscope and 1000× magnification. One milliliter of blood was immediately transferred to 4 °C, centrifuged (4500 × *g* for 5 min), and sera were aspirated and stored at −20 °C until assessment of corticosterone content. This was performed by a radioimmunoassay (RIA) (Beuving and Vonder, 1977) at the laboratory of ID-Lelystad, Institute for Animal Science and Health, Lelystad, The Netherlands.

2.3. Tonic immobility (TI reaction)

At 15 weeks of age (Fig. 1B), the TI reactions of each of 123 hens (i.e. the remaining non-bled birds) were measured. This test is based on measuring the time lag between relief of restraint and righting response, which is delayed in stressed chickens. The experimenter tested each bird in a separate room individually and once only by placing the bird on its back in a U-shaped wooden cradle and restraining it for 45 s (El-Lethey et al., 2000). The time until the bird showed a righting response was recorded. Failure to obtain TI reaction lasting at least 10 s resulted in exclusion of the bird from the analysis. If the bird showed no righting response over the 15 min test, a maximum score of 900 s was given.

2.4. General performance

Body weight of all birds was recorded individually at 11 and 15 weeks of age. Total egg production in weeks 16–20 was calculated for each pen as a percentage of the maximum number of eggs that would have been produced if every hen had laid one egg per day.

2.5. Immunization

At 16 weeks of age, blood samples (1 ml) were obtained from the right wing veins of each of 123 hens (the remaining non-bled birds) to determine antibody concentrations before immunization. Two days later (Fig. 1B), an antigen cocktail (0.25 ml per bird, containing 4.5 mg human serum albumin (HSA) and 8 IU tetanus toxoid (TT)) was injected intramuscularly into the left breast muscle. Concomitantly, 0.1 ml of 20 vol.% of SRBC in PBS was injected into the right breast muscle.

2.6. Determination of antibody titers

Blood samples (1 ml) were collected from the left wing veins at 5, 8, 11, and 14 days post-immunization. Antibody titers to TT and HSA antigens of sera stored frozen were determined by ELISA, whereas antibody titers to SRBC were quantified using an indirect hemagglutination assay.

For determination of antibody titers to HSA and TT, ELISA plates (Immunoplates, Starwell, Nunc, Maxisorp) were coated overnight at room temperature in a humid chamber with 100 μl per well of HSA (10 μg/ml) or TT (20 LF antigen from Berna, Swiss Serum and Vaccine Institute, Bern, Switzerland), diluted 1:1000 in 0.1 M sodium carbonate–bicarbonate buffer, pH 9.6. Plates were washed with washing solution (0.9% sodium chloride and 0.25% Tween 20) using an ELISA washer. One hundred microliters of a 1:300 dilution of serum samples in ELISA buffer (0.25 M sodium chloride, 20 mM Tris pH 7.5, and 0.125% Tween 20) was added to antigen-coated and negative control wells followed by treatment with 100 μl of 1000-fold diluted goat anti-chicken IgG (Fc-portion-specific) conjugated with horse radish peroxidase (Bethyl, Montgomery, TX) and finally with 100 μl of *ortho*-phenylene-diamine·2HCl and 0.02% of H_2O_2 substrate. The optical densities of the wells were read at 450 nm in an ELISA reader and converted into arbitrary units (AU), using a calibration curve run on the same plate. One hundred AU corresponded to the activities of sera containing high concentrations of anti-TT and anti-HSA antibodies, respectively as determined in pilot experiments.

For determination of SRBC antibodies, an indirect hemagglutination assay was used. One hundred microliters of serum samples were serially diluted in PBS (10 mM phosphate pH 7.4) in two-fold steps in round bottom microtest plates (Becton-Dickinson, Basel, Switzerland), and 10 μl of SRBC (10 vol.% in PBS) was added to each well. The plates were incubated for 2 h at 37 °C, followed by 20 min at 4 °C. Then, 50 μl out of 110 μl was transferred to new microtest plate, and 100 μl of 500-fold diluted goat anti-chicken IgG (heavy and light chain-specific, Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD) was added per well. Agglutination titers were read at least 2 h after the addition of anti-IgG and was expressed as the $\log_2$ of the reciprocal of the highest dilution showing 50% agglutination.

2.7. Phytohemagglutinin-elicited skin reaction

At 16 weeks of age, 200 μg of PHA (phytohemagglutinin-P; Difco, Detroit, MI) in 0.1 ml of sterile pyrogen-free physiologic saline or saline only was injected intradermally (ID) into the right or left wattle, respectively. Wattle thickness of each bird was measured with a constant-tension dial micrometer (Mitutoyo Co., Tokyo, Japan) just before the injection and again 24 h later. The response was recorded in millimeters as the difference between PHA response (right wattle) and the saline response (left wattle) 24 h after injection.

2.8. Induction of DTH

After a 3-day recovery period from the PHA reaction, the same eight birds per pen were sensitized to *Mycobacterium tuberculosis* by subcutaneous (SC) injection of Freund's complete adjuvant (Sigma) containing 0.5 mg of the organisms into several sites of the pectoral muscle. Two weeks after sensitization, the birds were injected ID into the right or left wattle, respectively, with 0.1 ml of PPD (Statens Serum Institute, Copenhagen, Denmark) two-fold diluted with sterile pyrogen-free saline or saline only. The delayed-type hypersensitivity (DTH) reaction in terms of thickness of wattles was measured by a micrometer before injection to assure equality and 24 h later. The intensity of the DTH reaction was expressed as the difference between the PPD and the saline site 24 h after injection.

2.9. Statistical analysis

To minimize handling of individual birds, animals from only one of four equally treated pens were bled after each of the post-immunization intervals. All laboratory analyses and skin tests were performed in a blinded fashion. Order of blocks and of pens within a block was randomized throughout the experimental procedures. The 16 pens were treated as independent units in all analyses. Mean values for the behavioral, physiological, and immunological parameters were calculated for each pen. The analyses were performed using Systat (Wilkinson, 1992) and Microsoft Excel. All statistical tests were two-tailed with an α level of 0.05. The data were subjected to analysis of variance (ANOVA) with housing on slats and corticosterone feeding as main factors. When there was a significant interaction in the 2×2 ANOVA, the significance of treatment differences were determined by the Tukey–Kramer honestly significant differences test. Adjusted antibody titers were derived by subtracting the pre-immunization titers from the respective post-immunization titers of each individual bird. Square root transformation was applied to serum corticosterone concentrations to achieve a normal distribution of the residuals. However, untransformed data are given in the figures and Table 1.

3. Results

3.1. Effect of treatments on established stress parameters

Feeding corticosterone to the birds led to a general state of stress as evidenced by increased H/L ratios, serum corticosterone concentrations and TI duration along with reduced body weight gain and egg production (Table 1). Housing the birds on slats resulted in higher H/L ratios, longer TI duration and slightly but not significantly altered corticosterone levels and body weight gains. However, the effects on corticosterone levels and body weight gain were more than additive when altered housing and feeding with corticosterone were combined.

Feeding corticosterone to hens reared on slats had no additional effect on the duration of TI reaction or egg production. This was reflected in a significant interaction between the two main factors "housing on slats" and "corticosterone feeding" (Table 1). The H/L ratios were significantly higher in groups of hens housed on slats and fed corticosterone than in those housed on litter only, and those fed with corticosterone only, although the effects of the two treatments were less than additive.

3.2. Antibodies against SRBC

Before immunization, the hens had no detectable antibody titers to SRBC regardless of the presence or absence of stressors. After immunization, antibody titers increased, reaching a maximum at day 8 and decreased again in control birds. Both "housing on slats" ($F_{(1,12)} = 7.24$, $P < 0.05$) and "corticosterone feeding" ($F_{(1,12)} = 10.75$, $P = 0.01$) significantly decreased post-immunization titers, reducing these to barely detectable levels between days 8 and 14 (Fig. 2). When corticosterone feeding and housing on slats were combined, there was no further decrease than when these stressors were applied as single treatments. This was reflected in a non-significant interaction between the two factors ($F_{(1,12)} = 3.65$, $P = 0.08$).

3.3. Tetanus toxoid (TT) antibodies

Tetanus toxoid behaved as a recall antigen, as the hens showed antibody titers prior to immunization. Corticosterone had a significant influence on these pre-existing antibody titers ($F_{(1,12)} = 106.5$, $P < 0.0001$), which were decreased from 15.1 AU in control hens to 5.4 AU in hens fed corticosterone (Fig. 3a). After immunization, antibody titers increased, reaching a maximum between days 8 and 11. In animals kept on slats ($F_{(1,12)} = 31.9$, $P = 0.0001$) and in animals fed with corticosterone ($F_{(1,12)} = 66.85$, $P < 0.0001$), the titer increases were significantly lower (Fig. 3b). The reduction in antibody titers in animals exposed to both stressors was not significantly higher than in birds fed with corticosterone only. This was reflected in a significant interaction between the two factors "housing on slats" and "corticosterone feeding" ($F_{(1,12)} = 14.54$, $P < 0.01$).

3.4. Human serum albumin (HSA) antibodies

There were low, but detectable antibody titers to HSA before immunization, and they were not significantly

Table 1
The influence of feeding chickens with corticosterone and depriving them of foraging material on established stress parameters

Stress parameter	Treatment[a]				*P*-value[b]		
	S/C	S/N	L/C	L/N	Housing on slats	Corticosterone	Interaction
H/L ratios	3.45a (3.21–3.59)	2.5b (2.44–2.55)	3.07c (2.93–3.16)	1.04d (0.94–1.14)	<0.0001	<0.0001	<0.0001
Corticosterone levels (ng/ml)	10.24a (6.27–19.06)	4.63b (4.03–5.43)	7.35a (7.12–7.34)	3.31b (2.96–5.56)	NS	<0.01	NS
TI duration (s)	701.8a (511.6–863.1)	635.8a (560–746.5)	620.9a (416.3–786.5	196.4b (133–273.29)	0.001	0.001	0.01
Body weight gain per week	287.29a (270–303.8)	309.2b (302.5–321.9)	269.2a (261.47–282.93)	322.5b (297–347.2)	NS	0.001	NS
Egg production	14.7a (11.9–18.9)	18.4a (15.2–20.7)	18.5a (16.6–19.5)	38.3b (33.2–43.8)	<0.0001	<0.0001	0.0001

S/C: slats/corticosterone; S/N: slats/no corticosterone; L/C: litter/corticosterone; L/N: litter/no corticosterone.

[a] Means and ranges (in brackets) are indicated; groups carrying the same letters are not statistically different.

[b] *P*-value calculated by ANOVA are shown for housing on slats, corticosterone feeding and the interactions between the two main factor.

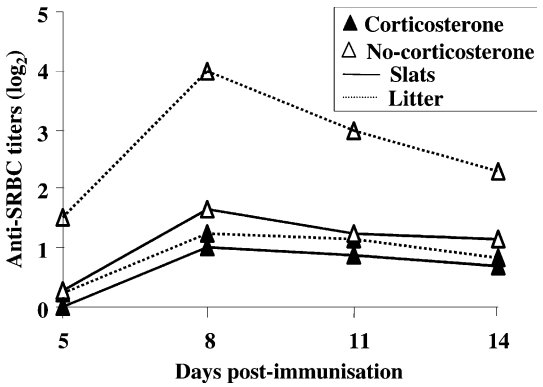

Fig. 2. The influence of stress on the antibody response to SRBC. Mean antibody titers to SRBC at various times after immunization are displayed on a $\log_2$ scale. Four groups experiencing the same treatment are subsumed to mean values.

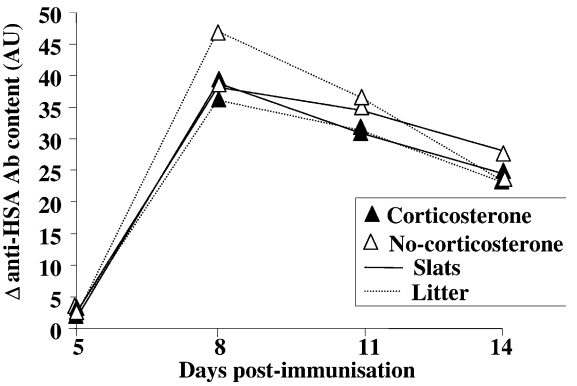

Fig. 4. The influence of stress on the antibody response to HSA. Mean anti-HSA Ab contents in AU at various times after immunization are shown as differences (Δ) to pre-immunization titers. Four groups experiencing the same treatment are subsumed to mean values per pen.

affected by the treatments applied. Following immunization, there was a strong increase in the anti-HSA titers regardless of the treatments, reaching a maximum at day 8 post-immunization (Fig. 4). Neither "housing on slats" ($F_{(1,12)} = 0.01$, $P = 0.91$) nor "corticosterone feeding" ($F_{(1,12)} = 0.09$, $P = 0.77$) had a significant effect on the increase in antibody titers to HSA. Even when the two stressors were combined, there was no influence on anti-HSA titers at any time between days 5 and 14 after immunization. This was reflected in a non-significant interaction between these two factors ($F_{(1,12)} = 0.03$, $P = 0.86$).

3.5. Cutaneous response to phytohemagglutinin-P (PHA)

Both "housing on slats" ($F_{(1,12)} = 17.43, P < 0.01$) and "corticosterone feeding" ($F_{(1,12)} = 52.38$, $P < 0.0001$) significantly decreased the hens' skin reaction to PHA (Fig. 5a). When corticosterone feeding and housing on slats were combined, there was no further decrease in the skin response than when these stressors were applied individually. This was reflected in a significant interaction between the two main factors ($F_{(1,12)} = 13.91$, $P < 0.01$).

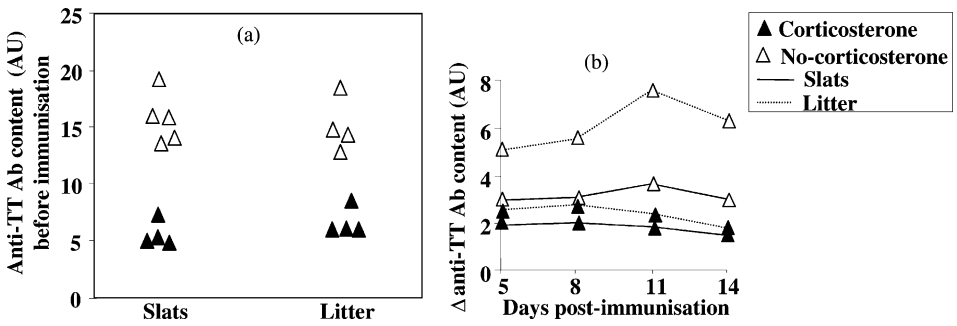

Fig. 3. The influence of stress on the antibody response to TT. Panel (a): antibody (Ab) content to TT in AU prior to immunization expressed as group means. Panel (b): mean anti-TT Ab contents in AU at various times after immunization are shown as differences (Δ) to pre-immunization titers. Four groups experiencing the same treatment are subsumed to mean values per pen.

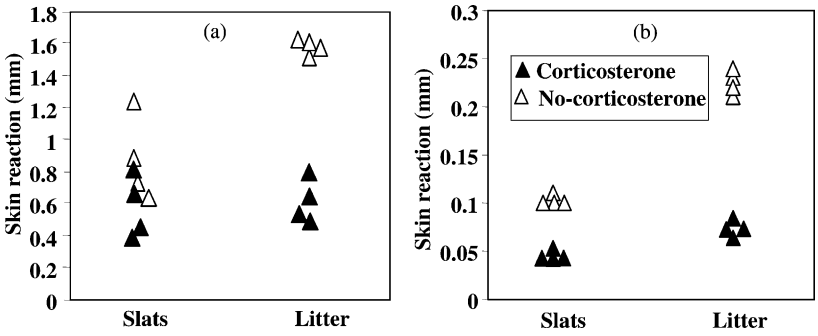

Fig. 5. The influence of stress on cellular immunity. DTH and the inflammatory response to PHA are shown for groups held under the indicated conditions. Panel (a): cutaneous response to phytohemagglutinin-P (PHA) expressed as difference in wattle thickness to control (saline injection). Panel (b): delayed-type hypersensitivity reaction to purified protein derivative (PPD) expressed as difference in wattle thickness to control (saline injection). In both panels, group means for the birds of four pens receiving the same treatment are shown.

3.6. Cell-mediated immune response to mycobacterial antigens

Both "housing on slats" ($F_{(1,12)} = 317.65$, $P < 0.0001$) and "corticosterone feeding" ($F_{(1,12)} = 652.59$, $P < 0.0001$) had a significant effect on the DTH reactions elicited in the wattle. The DTH reaction in hens both housed on slats and fed corticosterone was significantly weaker than those exposed to one of these treatments only, although the effect was less than additive. This was reflected in a significant interaction between the two factors ($F_{(1,12)} = 127.41$, $P < 0.0001$, Fig. 5b).

4. Discussion

The goal of this study was to assess the effects of chronic stress induced by suboptimal housing conditions, on both humoral and cell-mediated immune responses in laying hens. The effect of this type of stress on the immune response was compared with feeding the animals with corticosterone, the primary glucocorticoid secreted by the avian adrenal gland and reported to be increased in stressful situations (Curtis et al., 1980; Siegel, 1980). Glucocorticoids both induce immunosuppression and the metabolic and behavioral changes associated with stress. An array of established physiological, behavioral and performance parameters has been used in our study as indicators of stress. These included an increase in H/L ratios, circulating corticosterone concentrations (Gross and Siegel, 1983), and TI duration (Gallup, 1979), along with reduced body weight and egg production (Donker and Beuving, 1989; Hughes and Black, 1976). Whereas all these parameters were influenced by corticosterone feeding, depriving of foraging material impaired body weight gain slightly but not significantly. Similarly, a rise in blood corticosterone levels was detectable but not significant. Since the other three stress parameters were severely altered, this suggests that there is a hierarchy of stress indicators, some of which may not be influenced by lighter forms of stress such as depriving the animals of foraging material.

The impact on both humoral and cell-mediated immunity was measured in birds fed corticosterone and/or deprived of foraging material. The results were confirmatory in several regards (Corrier and DeLoach, 1990; Davison and Misson, 1987; Murray et al., 1987), yet they were remarkable in two ways. First, four out of six immune response parameters were altered regardless of whether birds were housed on slats or chronically fed with corticosterone which is not only a strong stressor but also directly immunosuppressive. Although not all stress-related parameters were influenced by housing the birds on slats, it led to a strong immunosuppression. The humoral immune responses to two out of three antigens, and two types of cellular responses were profoundly impaired by both treatments. Combined application of the two stressors did not lead to additive effects. The only immune

parameter influenced by corticosterone feeding, but not by depriving of foraging material, was the pre-existing level of anti-TT antibodies.

The second unexpected finding was that the humoral immune response to one out of the three antigens tested, human serum albumin (HSA) was not modulated by housing the birds on slats as a stressor, or feeding them with corticosterone. This was all the more surprising as HSA was injected simultaneously with two other antigens which showed profoundly impaired immune response when the birds were exposed to stressors. Moreover, the recall antigens TT and HSA were injected at the same site, yet the modulation by stress was completely different. This novel finding suggests that immune mechanisms operate in a strictly antigen-dependent manner, and they call for caution when extrapolating from studies in which the response to one single antigen has been analyzed under stress conditions. To which extent such a lack of modulation by stressors is relevant to natural pathogens remains to be determined. The finding nevertheless calls for extended studies with more than a handful of antigens.

Our findings raise the question: what distinguishes HSA from the other antigens used? We suspect that the anti-HSA response in chickens is a ''recall'' response. The exact antigen inducing a primary reaction is probably distinct from HSA and unknown at the moment. However, it is likely that bovine serum albumin admixed to vaccines the birds were given at early age is the original primary antigen, thereby making the anti-HSA response to a secondary response. That the behavior towards HSA is not typical, for all recall antigens is illustrated by the immune response to TT, another protein type ''recall'' antigen. Again, immune responses to a broader array of antigens at various concentrations need to be tested in order to allow firm conclusions. Another possible explanation for the variable modulation by stress of antibody responses is the amount of antigen given. In the case of albumin, one order of magnitude less antigen was used in this study than in an earlier report (Thaxton et al., 1968). The chosen dose nevertheless was sufficient to produce a stress-resistant antibody response. It will be of interest to study this issue in more detail as it obviously has implications for vaccine design in chickens.

The role of corticosterone used in this study deserves a comment. It could be argued that upon feeding with corticosterone, a direct immunosuppressive effect is induced without involvement of stress. Interestingly, the metabolic and neurological changes observed suggest that this type of feeding induces chronic stress. It could also be argued that elevated corticosterone levels are the consequence rather than the cause of complex psychoneurological processes occurring in stressed chickens. However, plasma corticosterone levels were slightly but not significantly enhanced after depriving of foraging material, yet behavioral stress parameters were clearly elevated. The effect of stress on the immune system is mediated either by endocrinological changes, involving the hypothalamus–pituitary–adrenocortical (HPA) axis or by involvement of the sympathetic nervous system and neuropeptides. How stress induced by housing chickens on slats led to immunosuppression is not clear at the moment: Although no significantly elevated levels of corticosterone were measured in birds deprived of foraging material, a peak corticosterone response might have occurred at a time not covered by blood sampling. Supporting evidence is the finding that circulating corticosterone in the chickens peaked and then began to decline after only 70 min of heat stress (Edens and Siegel, 1975), and an initially high corticosteroid concentration is often seen to decrease when the animal is exposed to a stressor continuously or intermittently (Pearson and Mellor, 1976). Nevertheless, the method of inducing mild stress by depriving the bird of foraging material is a novel way to induce stress in the absence of artificial methods such as endocrine interference (Donker and Beuving, 1989; Gross et al., 1980; Puvadolpirod and Thaxton, 2000), or subjecting the birds to heat stress (Donker et al., 1990; Regnier et al., 1980).

The immunization schedule chosen is also noteworthy. Given the aim that animal usage and expected results should be optimized, the study was combined with a behavioral part reported elsewhere (El-Lethey et al., 2002); leaving the time after week 16 for immunological experimentation. It was furthermore attempted to avoid the possibility of antigenic competition, a phenomenon seen when antigens are given about 2 weeks apart (Taussig, 1973). Antigenic competition was reported to be mediated by naturally secreted glucocorticoids, following a neuroendocrine regulatory feedback (Besedovsky et al., 1979). This led to simultaneous application of antigens for which

humoral responses were to be determined. The disadvantage was the potential interference between antigens, some of which were given in a cocktail. The possibility of interference was regarded as remote, however, since to none of the antigens, adjuvant properties were attributed. Freund's adjuvant was given in the same week to different birds and only after the PHA skin reaction has subsided. Furthermore, a potential immunostimulatory effect would not explain the differential immune response to TT and HSA given as a cocktail. Further experiments, however, have to confirm that HSA given as a single antigen behaves as we have reported here.

In ecologically oriented research, the maintenance of a functioning immune system is regarded as an investment, subject to tradeoffs with other investments such as progeny nurturing or fecundity (Lochmiller and Deerenberg, 2000). Viewed in this way, stress may absorb ''energies'' no longer available for host defense. Why the immune response to certain, but not other antigens and delivery forms is not affected under stress remains, however, an open question.

5. Conclusions

In this study, a novel model for inducing stress in chickens is presented. It is based on depriving chickens of foraging material, a measure of high practical relevance in poultry. This model of inducing stress had a profound impact on both humoral and cellular immune responses, and was comparable in immunosuppression to effects induced by chronic corticosterone feeding. This points to the importance of providing sufficient foraging material to chickens for maintaining their resistance to microbial agents. For the first time, it was shown that certain antigen responses were resistant to stress, a finding of high relevance for vaccine design (Glaser et al., 1998). The properties distinguishing between antigens inducing a stress-resistant and a stress-susceptible response remain to be determined.

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