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Influence of experimentally induced endogenous production of cortisol on the immune capacity in swine

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Abstract

Field studies have suggested that 'stressors', such as transportation and mixing, might interfere with the immune competence of pigs. Therefore, an experimental model was established to study the influence of elevated concentrations of circulating cortisol on the immune capacity in swine. Three experimental groups, with six pigs in each, were immunized twice, 4 weeks apart, with *Mycoplasma hyopneumoniae* antigen. Endogenous production of cortisol was induced by intramuscular injection of adrenocorticotropic hormone (ACTH) twice daily. One group received ACTH during the week before and after the second immunization, one group during the week after the second immunization only, while one group served as untreated controls.

The treatment with ACTH induced high, but physiological, concentrations of cortisol in plasma. Simultaneously, the number of lymphocytes per milliliter blood decreased while the neutrophil number increased. The elevated concentrations of cortisol also coincided with reduced proliferation and interleukin-2 production by blood lymphocytes stimulated with the mitogens concanavalin A and phytohemagglutinin in vitro, while the responses to pokeweed mitogen were less affected. The suppression of mitogen responses was more pronounced in cultures of whole blood than in cultures of purified peripheral blood mononuclear cells (PBMC).

Antibody production, induced by *M. hyopneumoniae* in cultures of purified PBMC was

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also inhibited by ACTH treatment. Both the rate of increase and the magnitude of the antibody production induced by the primary immunization were reduced. In contrast, no effects of ACTH treatment were recorded for the response to the second immunization or on the serum levels of antibodies to *M. hyopneumoniae*.

The ability of blood leukocytes to produce interferon-α (IFN-α) at exposure in vitro to fixed pseudorabies virus adsorbed to porcine kidney cells increased in all animals shortly after the second immunization with *M. hyopneumoniae*. The influence of cortisol on the IFN-α-producing capacity was dependent on whether the test was carried out in whole blood cultures or in cultures with purified PBMC. This finding further emphasizes that the relevance of in vitro assays for measuring in vivo phenomena must be carefully scrutinized.

Abbreviations

ACTH, adrenocorticotropic hormone; Con A, concanavalin A; cpm, counts per minute; DELFIA, delayed fluoroimmunoassay; DX, dexamethasone; IFN-α, interferon-α; Ig, immunoglobulin; IL-2, interleukin-2; i.m., imtramuscular; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PK, porcine kidney; PRV, pseudorabies virus; PWM, pokeweed mitogen; SPF, specific pathogen free.

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1. Introduction

Modern swine management often includes the transfer of piglets from several producers to fattening units. In such newly established herds, there is a rapid spread of infections among the animals. Signs of reduced immune functions are also seen 1 week after allocation, possibly owing to stress of animals during transportation, mixing and establishment of new ranking orders (Artursson et al., 1989; Wallgren et al., 1993). Such an immunosuppression, coinciding in time with exposure to new micro-organisms, would be expected to increase susceptibility of the pigs to infections.

The most extensively studied mediators of stress-induced immunosuppression are the glucocorticoids (for overview, see Cohen, 1992). The effects of elevated concentrations of cortisol are evident in vivo as neutrophilia and lymphopenia, a result of redistribution of leukocytes. In the mouse, apoptosis of immature thymocytes also contributes to the lymphopenia (Ucker, 1987). The function of immunocompetent cells under the influence of corticosteroids has been studied mainly in vitro, either by addition of the steroid to cell cultures or by assessing reactivities of mononuclear cells purified from the blood of individuals treated with corticosteroids. Depending on method, contradictory results are obtained for some functional tests such as the in vitro production of antibodies, reviewed by Dupont (1988). There is, however, no doubt that the mitogen-induced proliferation of peripheral blood mononuclear cells (PBMC) is suppressed by corticosteroids. For example, Blecha and Baker (1986) demonstrated that cortisol, both in vitro and in vivo, reduced the proliferation of bovine PBMC, probably a result of suppressed production of interleukin-2 (IL-2). The suppressed IL-2 pro-

duction is most likely to be a result of the interference of glucocorticosteroids with the transcription of genes coding for cytokines (Northrop et al., 1992), as has also been shown for other immunoregulatory proteins (Helmberg et al., 1990). However, the response to stressors shows considerable inter-species differences, and swine are regarded as corticosteroid resistant, as reviewed by Griffin (1989).

In the present study, we therefore established a model to study the effect of corticosteroids in vivo on the function of porcine PBMC in vitro. High, but physiological, concentrations of corticosteroids were induced by treating pigs with adrenocorticotropic hormone (ACTH) at a second immunization with *Mycoplasma hyopneumoniae* antigen. The tests of PBMC functions were performed both with purified PBMC and in whole blood cultures, attempting to mirror more closely the in vivo situation. Since approximately 90% of the Swedish fattening pigs are affected by infections with *M. hyopneumoniae* (Wallgren et al., 1993), we studied the effect of ACTH treatments on the antibody response in vitro to that antigen. We also measured in vitro mitogen-induced proliferation and IL-2 production, as well as the ability of PBMC to produce interferon-α (IFN-α) after exposure to pseudorabies virus (PRV).

2. Materials and methods

2.1. Animals and experimental design

Eighteen specific pathogen free (SPF) pigs (Serogrisen, Ransta, Sweden), nine gilts and nine barrows, from four litters were included in the study. After arrival at the Animal Department at the National Veterinary Institute, Uppsala, Sweden, three experimental groups (A, B and C) were formed and the pigs were accustomed to each other for 4 weeks. To reduce the effect of litter origin, each experimental group consisted of six pigs from diverse litters (one gilt and one barrow from sow 1 and sow 2, respectively, one gilt from sow 3 and one barrow from sow 4). The pigs were 4 months old when the experiment began.

Blood samples were collected by jugular vein puncture using evacuated glass tubes (B-D vacutainer, Becton-Dickinson, Meylan Cedex, France) without additive or with heparin (143 USP units) or EDTA (0.12 ml 0.34 M) as additive. Blood samples were collected from all animals at 09:00 h on Days −11, 0, 7, 14, 21, 23, 25, 28, 30, 32, 35, 37, 42, and 49. Day 0 was the day on which the first immunization was administered. On Days 0 and 28, the blood samples were collected before the immunizations.

All pigs were immunized twice intramuscularly (i.m.) with 1.5 ml *M. hyopneumoniae* antigen (1.0 mg protein ml^{-1}), mixed with an equal volume of Freund's incomplete adjuvant (Day 0) or with aluminum hydroxide adjuvant (Day 28).

Pigs in Groups A and B were treated with porcine ACTH (Acton prolongatum; Ferring, Lund, Sweden). The treatment with 60 IU ACTH per 100 kg body weight was carried out twice daily (at 06:00 h and 16:00 h). Pigs in Group A were treated with ACTH during the week before and after the second immunization

with *M. hyopneumoniae*, i.e. from Day 21 (evening) until Day 35 (morning). Pigs in Group B were only treated with ACTH during the week after the second immunization, i.e. from Day 28 (evening) until Day 35 (morning). Pigs in Group C were not treated with ACTH at all.

2.2. Total and differential leukocyte counts and concentrations of plasma cortisol

Blood was collected with EDTA, and the total number of leukocytes determined by counting in a microcell counter (Sysmex F-800, Toa Medical Electronics Co., Kobe, Japan). Differential leukocyte counts were made on blood smears stained with Giemsa's and May Grünewald's solutions. The concentrations of cortisol were determined in plasma by a standard radioimmunoassay validated for pigs (Nyberg, 1988). These analyses were performed according to standard procedures at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

2.3. Isolation of peripheral blood mononuclear cells (PBMC)

PBMC were isolated from heparinized blood by centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden). The concentration of PBMC was adjusted to 4×10^6 cells ml^{-1} growth medium, i.e. RPMI 1640 medium with 20 mM HEPES buffer, supplemented with glutamine (2 mM), penicillin (200 IU ml^{-1}), streptomycin (100 μg ml^{-1}), 50 μM 2-mercaptoethanol and 5% FCS (Myoclone; Gibco, Paisley, UK).

2.4. Proliferation and IL-2 production by purified PBMC and in whole blood cultures

The proliferation and IL-2 production induced by concanavalin A (Con A; Pharmacia, Uppsala, Sweden; 5 μg ml^{-1}), phytohemagglutinin (PHA; Wellcome, Dartford, UK; 1 μg ml^{-1}) or PWM (10 μg ml^{-1}; pokeweed mitogen, Boehringer-Mannheim, Mannheim, Germany) was determined in cultures of purified PBMC (4×10^6 cells ml^{-1}) or in whole blood cultures (100 μl heparinized blood diluted 1:15 in growth medium) as described earlier (Magnusson and Fossum, 1992). The proliferation is expressed as mean counts per minute (cpm) value for quadruplicate cultures, while the IL-2 activity is expressed as percentage of a laboratory standard according to Edfors-Lilja et al. (1991).

2.5. Production of IFN-α by purified PBMC and in whole blood cultures

Glutaraldehyde-fixed, PRV-infected, porcine kidney (PK-15) cells were used for induction of IFN-α production as described in detail by Artursson et al. (1989). Either 4×10^5 PBMC in 200 μl growth medium or heparinized blood diluted 1:15 in growth medium were added to quadruplicate wells containing PRV-infected PK-15 cells and to duplicate control wells containing uninfected PK-15 cells. The supernatants were collected after 18 h at 37°C, and stored at −20°C until analysed.

The concentrations of IFN-α in the supernatants, as well as in serum samples, were measured by an immunoassay (Diaz de Arce et al., 1992), further modified

to utilize the principle of time-resolved fluorometry (delayed fluoroimmunoassay; DELFIA) with Europium lanthanide as label (Artursson, 1993).

2.6. M. hyopneumoniae antigen preparations

Three preparations of *M. hyopneumoniae* strain V 27 antigen were used. Whole *M. hyopneumoniae* was cultured in Friis broth with 10% SPF swine serum (National Veterinary Institute, Uppsala, Sweden) and mixed with adjuvant for immunization, a Tween 20 treated preparation for detection of serum antibodies and a sonicated whole cell antigen preparation for induction of antibody production in vitro and for detection of these antibodies. All antigen preparations were kindly provided by Dr G. Bölske, the National Veterinary Institute, Uppsala, Sweden and are described in detail by Bölske et al. (1987, 1990).

2.7. Antibody production to M. hyopneumoniae in vitro and in vivo

The in vitro production of antibodies to *M. hyopneumoniae* was determined as previously described in detail by Wallgren et al. (1992). In brief, 4×10^5 PBMC in 200 μl volumes of growth medium containing 2.5 ng PWM ml^{-1} were added to quadruplicate wells of microtiter plates (Dynatech, Chantilly, VA, USA) coated with *M. hyopneumoniae* antigen. After 4 days at 37°C in a humid atmosphere with 7% CO_2 in air, 150 μl of the culture supernatants was collected. The PBMC were discarded and the plate was washed three times in PBS with 0.05% Tween. The amount of antibodies to *M. hyopneumoniae* bound in the induction plate as well as the amount of antibodies bound to *M. hyopneumoniae* in the culture supernatants were determined by ELISA. The optical density (A_{450}) of medium controls was subtracted from the sample values and the mean optical density for the culture supernatants was added to that for the corresponding stimulated wells. This figure is used as a measure of the ability to produce antibodies to *M. hyopneumoniae* in vitro.

Serum antibodies to *M. hyopneumoniae* were detected by the ELISA initially described by Bölske et al. (1990) and further modified by Wallgren et al. (1992). The quantities of serum antibodies to *M. hyopneumoniae* are expressed as $\log_{10}$ ELISA titres calculated from ten-fold serial dilutions of the sera.

In both ELISA systems, positive and negative control sera were included and the measured absorbance values of samples were corrected to a standard value equal to 0.9 absorbance units for the positive control serum.

2.8. Polyclonal production of immunoglobulin (Ig) in vitro

The method of Magnusson and Fossum (1992) was used to estimate the polyclonally induced production of Ig in vitro. Briefly, 4×10^5 PBMC were cultured in 200 μl growth medium containing 0.25 ng PWM ml^{-1}. After 72 h, 100 μl supernatant was collected and the concentration of Ig was determined by an indi-

rect ELISA for porcine Ig. Two step titrations of the samples were performed in duplicate wells. As negative and positive references, growth medium and known amounts of purified swine IgG, respectively, were included. The mean concentration of Ig produced (ng ml^{-1}) was calculated from a standard preparation of porcine Ig using an universal assay calculator program (Assay Zap; Biosoft, Cambridge, UK).

2.9. Statistical analysis

The significance of difference between means of a group at various sampling occasions was tested by the paired Student's *t*-test, Stat View 512 (Version 1.0, Abacus Concepts, Calabasas, CA).

Effects of litter and sex on the ability to produce antibodies to *M. hyopneumoniae* in vitro were tested with the non-parametric Mann–Whitney *U* test, Stat View 512 (Version 1.0, Abacus Concepts, Calabasas, CA, USA).

Differences between experimental groups were tested by an aligned rank test (Lehmann, 1975), blocking for sex and litter origin, and the non-parametric Mann–Whitney *U* test.

3. Results

3.1. Influence of litter and sex

In a recent study (Edfors-Lilja et al., 1993), various degrees of heritability and/ or litter effects were demonstrated for several immune parameters in swine, including total and differential white blood cell counts, mitogen-induced proliferation and IL-2 production and IFN-α production. Similarily, clearcut influence of litter, as well as the influence of sex, was found on the ability to produce antibodies to *M. hyopneumoniae* in vitro. As exemplified by the results of Day 21 (i.e. 3 weeks after the first immunization, but before initiation of the treatment with ACTH), significant differences ($P<0.05$) were obtained despite the low number of individuals (Fig. 1). Consequently, data for all immune parameters were blocked for effect of litter and sex when comparing experimental groups.

3.2. Elevation in plasma cortisol levels induced by treatment with ACTH

The concentrations of cortisol in plasma are shown in Fig. 2 as mean values (nmol l^{-1} plasma) for the three groups of animals. Already at the first sampling, during the period of treatment with ACTH (43 h after initiating the treatment), the concentrations of cortisol were significantly increased (Group A: Day 23 compared with Day 21, $P<0.05$; Group B: Day 30 compared with Day 28, $P<0.01$). The cortisol concentrations remained elevated throughout the period of ACTH treatment, but dropped below the normal level when the treatment was
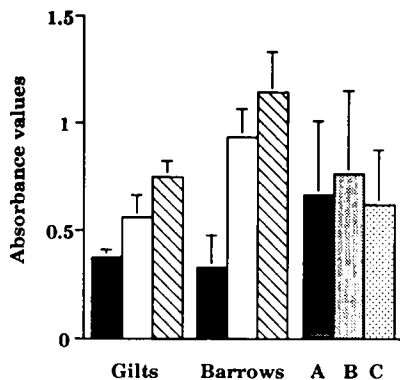
Fig. 1. Effects of litter and sex on the ability to produce antibodies to *M. hyopneumoniae* in vitro. PBMC were isolated Day 21, i.e. 3 weeks after the first immunization but before any treatment with ACTH, and tested for their ability to produce antibodies in vitro as described in Material and methods. The bars represent mean values (+SD) for gilts and barrows, respectively, originating from litter 1 (filled), litter 2 (open) or litter 3 and 4 (hatched). The mean values for the three experimental groups on that day are also given in the figure.
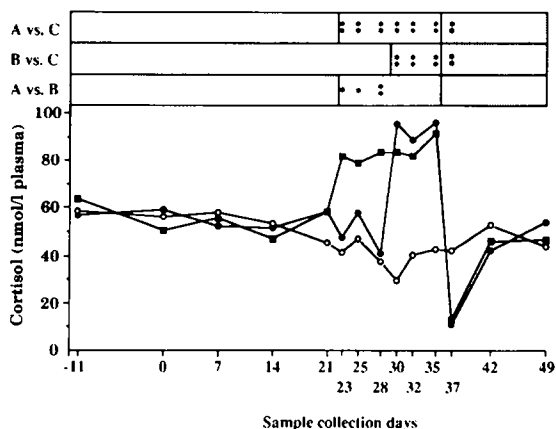
Fig. 2. Mean plasma levels of cortisol (nmol per litre plasma) for pigs in Groups A (■), B (●) and C (○), respectively. Significant differences ($*P<0.05$; $**P<0.01$) between groups, calculated as described in Materials and methods, are shown at the head of the figure. Shaded areas indicate periods when ACTH was not administered to any of the compared groups.

terminated (Day 37). Thereafter, the plasma concentrations of cortisol returned to normal levels. Significant differences between groups are shown in Fig. 2.

3.3 Alterations in total and differential leukocyte counts

The number of lymphocytes (Fig. 3(A)) decreased significantly in blood obtained from the ACTH-treated animals, simultaneously with the increase in

Fig. 3. Mean number ($\times 10^9$ l^{-1}) of (A) lymphoyctes and (B) neutrophilic granulocytes for pigs in Groups A (■), B (●) and C (○), respectively. Significant differences (*$P < 0.05$; **$P < 0.01$) between groups, calculated as described in Materials and methods, are shown at the head of the figure. Shaded areas indicate periods when ACTH was not administered to any of the compared groups.

plasma cortisol concentrations. This lymphopenia seemed to end when the ACTH treatment was completed (Day 37). However, the number of lymphocytes was significantly lower in blood from pigs in Group A than in blood from pigs in Groups B and C, 1 and 2 weeks after termination of the ACTH treatment.

The number of neutrophilic granulocytes (Fig. 3(B)) increased during the ACTH treatment. At all sampling occasions during this period, the neutrophil numbers were significantly higher in blood from the ACTH-treated pigs than in blood from the control pigs. For all animals, including the control animals, a marked increase ($P < 0.01$) in the total number of neutrophilic granulocytes was found 2 days after the second immunization with *M. hyopneumoniae* (Day 30).

3.4. Effects of ACTH treatment in vivo on the in vitro mitogen-induced proliferation and IL-2 production of blood mononuclear cells

The mitogen-induced proliferation in whole blood cultures and in cultures of purified PBMC is shown as log$_{10}$ cpm values in Fig. 4. The proliferative responses
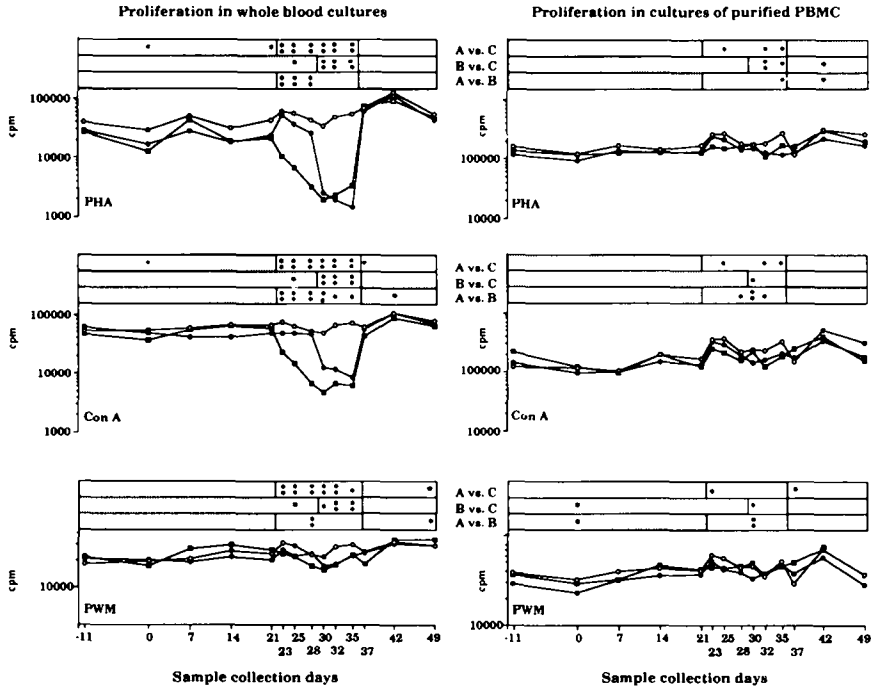
Fig. 4. Proliferation (mean cpm values) induced by the mitogens PHA, Con A and PWM in whole blood cultures and in cultures of purified PBMC. Blood was obtained at the indicated sample collection days from pigs in Groups A (■), B (●) and C (○) and was cultured as described in Material and methods. Significant differences (*P < 0.05; **P < 0.01) between groups, calculated as described in Material and methods, are shown at the head of the figure. Shaded areas indicate periods when ACTH was not administered to any of the compared groups.

to PHA and Con A were significantly suppressed in the whole blood cultures at all sampling occasions during ACTH treatment. Although significant, the suppression of the PWM-induced proliferation during the ACTH treatment was less evident.

Less clear cut results were obtained when the mitogen-induced proliferation was assessed in cultures of purified PBMC. Repeated significant differences between Groups A and C were only recorded at Days 32 and 35 for the PHA- and Con A-induced proliferation and between Groups B and C at Days 32 and 35 for the PHA-induced proliferation.

The ability to produce IL-2 in response to mitogen stimulation was assessed in whole blood cultures (Fig. 5) and in cultures of purified PBMC (results not shown). Clear suppressive effects of the ACTH treatment were seen, but only in whole blood cultures, and only with the mitogens PHA and Con A. In contrast, no inhibitory effects were noted in the cultures of purified PBMC.
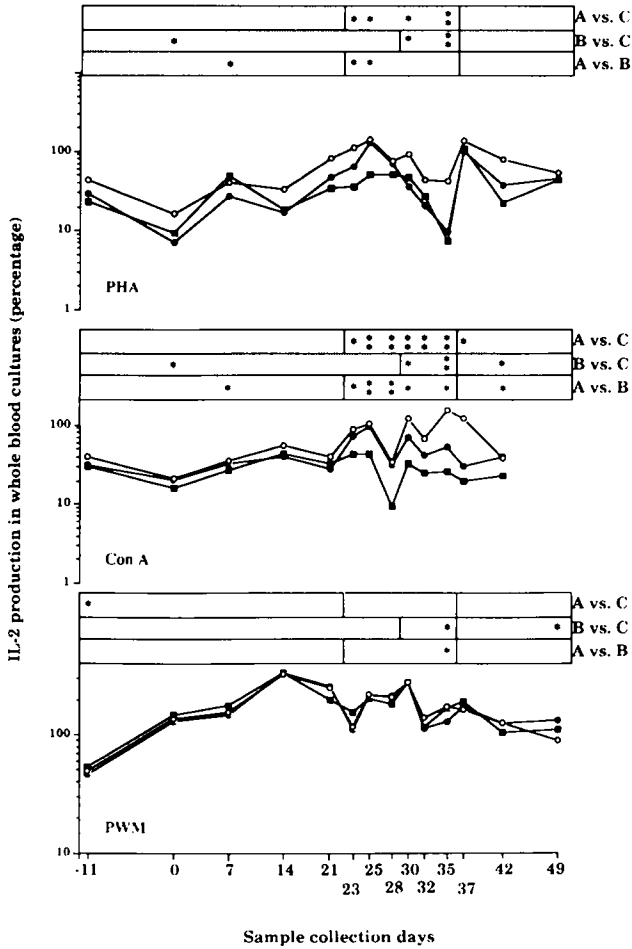
Sample collection days

Fig. 5. The amounts of IL-2 induced by the mitogens PHA, Con A and PWM in whole blood cultures. Blood was obtained at the indicated sample collection days from pigs in Groups A (■), B (●) and C (○), respectively. The concentration of IL-2 produced is expressed as percentage of an internal standard. Significant differences ($*P < 0.05$; $**P < 0.01$) between groups, calculated as described in Material and methods, are shown at the head of the figure. Shaded areas indicate periods when ACTH was not administered to any of the compared groups.

3.5. Effects of ACTH treatment on the ability to produce IFN-α

No pig displayed detectable concentrations of IFN-α (i.e. less than one unit ml^{-1}) in serum at any sampling occasion.

A marked increase in the IFN-α producing capacity was observed after the second immunization with *M. hyopneumoniae*, both in cultures of purified PBMC and in whole blood cultures (Fig. 6). In ACTH-treated pigs, this increase was evident earlier in cultures of purified PBMC, i.e. 2 days after the second immu-

Fig. 6. Effects of treatment with ACTH on the ability to produce IFN-α in cultures of whole blood and in cultures of purified PBMC. The values are mean values for pigs in Groups A (■), B (●) and C (○), respectively. Significant differences (*$P < 0.05$; **$P < 0.01$) between groups, calculated as described in Materials and methods are shown in the head of the figure. Shaded areas indicate periods when ACTH was not administered to any of the compared groups.

nization (Day 30), than in whole blood cultures (Day 37). With purified PBMC of the control pigs, the increased ability to produce IFN-α was not seen until Day 35, the same day as in cultures of whole blood obtained from these pigs.

Comparison of the treatment groups during the ACTH treatment demonstrated that, when assessed in whole blood cultures, pigs in the control group (Group C) produced significantly more ($P < 0.05$) IFN-α than ACTH-treated pigs (Group A) prior to the second immunization. Furthermore, pigs in Group C produced significantly more ($P < 0.01$) IFN-α than pigs in both Groups A and B at Day 35. The difference between Groups C and A remained significant ($P < 0.01$) throughout the experimental period. However, when assessed in cultures of purified PBMC, the ability to produce IFN-α was lower among control pigs (Group C) than among ACTH-treated pigs (Groups A and B) immediately after the second immunization.

3.6. Effects of ACTH treatment on the production of antibodies

The influence of elevated concentrations of cortisol in plasma on the ability to produce specific antibodies to *M. hyopneumoniae*, in vivo and in vitro, and the polyclonally induced Ig production in vitro was studied.

Initially, all animals were seronegative ($\log_{10}$ titre: A = 0.25 ± 0.13; B = 0.26 ± 0.12; C = 0.17 ± 0.11) to *M. hyopneumoniae* but seroconverted within 2 weeks after the first immunization ($\log_{10}$ titre: A = 2.47 ± 0.28; B = 2.72 ± 0.23;
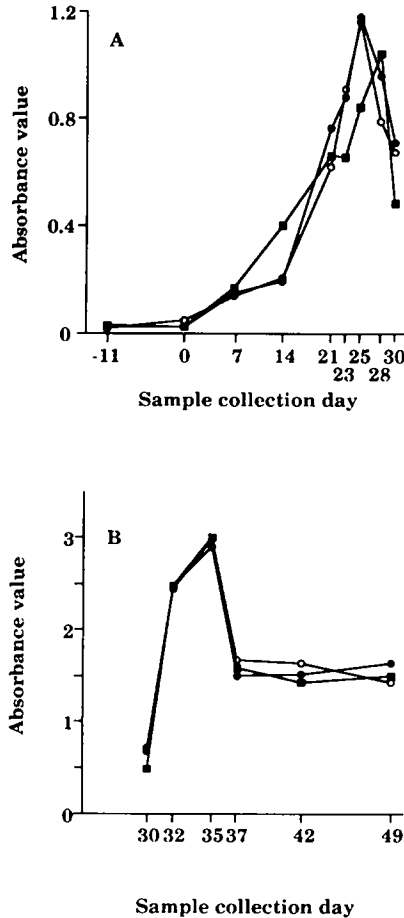
Fig. 7. Effects of treatment with ACTH on the ability of porcine PBMC to produce antibodies to *M. hyopneumoniae* in vitro during a (A) primary and (B) secondary immune response. The PBMC were obtained from pigs in Groups A (■), B (●) and C (○) and the levels of antibodies to *M. hyopneumoniae* was quantified using the ELISA technique described in Material and methods. The values are mean absorbance values for each group of animals.

C=4.47±0.28). A further increase in antibody titres was seen after the second immunization, reaching peak values on Day 37 ($\log_{10}$ titre: A=4.43±0.32; B=4.29±0.26; C=4.47±0.28). No significant differences between the experimental groups were found for the accumulated levels of antibodies to *M. hyopneumoniae* in serum.

As illustrated in Fig. 7(A), the quantities of antibodies produced in vitro at stimulation with *M. hyopneumoniae* antigen increased continuously until Day 25 for PBMC obtained from the non-treated pigs (Groups B and C). This increase was retarded for PBMC obtained from ACTH-treated pigs (Group A) and was significantly lower ($P<0.05$) at Days 23 and 25 for the ACTH-treated pigs than for the non-treated pigs (Groups B plus C). The response to the first immunization also decreased to a significantly lower level ($P<0.01$; Day 30) for animals in Group A than for those in Groups B and C, respectively. After the second immunization (Days 32 and 35), isolated PBMC of all pigs became increasingly able to produce specific antibodies to *M. hyopneumoniae* (Fig. 7(B)) and thereafter (at Days 37 to 49) decreased. During this period, no differences were noted between the experimental groups.

The ability to produce Ig at stimulation with a sub-mitogenic dose of PWM was tested with purified PBMC. The Ig production of PBMC from the various pigs ranged from 0.04 to 0.61 ng ml^{-1} culture medium during the experimental period, but no significant effects of treatment with ACTH in vivo were observed (results not shown).

4. Discussion

A model to study the effect of experimentally induced stress was established in swine. Daily treatments with ACTH elevated the endogenous production of cortisol to levels corresponding to those elicited in pigs by restricted ability to move, electric pulsation or heat stress (Becker et al., 1985). By this 'stress model', cortisol was found to influence the T-cell-dependent antibody production of PBMC to *M. hyopneumoniae*, but not the T-cell independent, polyclonally induced immunoglobulin production. Further, both the proliferation and IL-2 production were suppressed by cortisol when induced by the T-cell mitogens PHA and Con A, but not by PWM, which is believed to act both on T and B lymphocytes (Sharon, 1983). Therefore, it seems likely that cortisol, also in swine, interferes with the production of IL-2 as described earlier for human (Bettens et al., 1984) and bovine (Blecha and Baker, 1986) cells.

As genetic differences have been previously described for several immune parameters (Edfors-Lilja et al., 1991, 1993), pigs from four litters were alloted to the experimental groups to eliminate these differences. This procedure increased the individual variation within the groups, but made it possible to use a statistical method blocking for the effect of sex and litter origin when analysing data.

The ACTH-induced neutrophilia and lymphopenia resemble the alterations in differential white blood cell counts, normally achieved by sustained elevation of

cortisol levels (Quinn, 1990). Two days after the second immunization with *M. hyopneumoniae*, the highest neutrophil numbers were recorded for all pigs, including those in the control group. Consequently, both the ACTH treatment and the immunization could be mirrored in the differential white blood cell counts.

The proliferation and IL-2 production in response to mitogen stimulation were tested both in whole blood cultures and in cultures of purified PBMC. In accordance with earlier studies on oestradiol (Magnusson and Fossum, 1992), the most pronounced effects were found in the whole blood cultures stimulated with PHA or Con A. As hormones are known to alter the number of blood lymphocytes, this has to be considered when functional tests are carried out in whole blood cultures. In the previous study on oestradiol, no significant correlation between cpm values and the total number of PBMC per milliliter of blood in the samples was found. Further, no significant effects of cell concentration were seen on the magnitude of proliferation in a pilot study where whole blood from four pigs was serial diluted and stimulated with PHA (mean cpm values ± SD in blood diluted 1:10; 16 332 ± 9631, 1:15; 12 150 ± 4170, 1:20; 10 529 ± 7412, 1:25; 11 363 ± 8542). Further, the responses to stimulation with PWM (proliferation and IL-2 production) were not severely affected, despite the variation in the number of PBMC per unit blood. Therefore, it is most likely that the glucocorticoid not only reduced the number of mononuclear cells in the blood circulation but also affected their responsiveness to stimulation with the T-cell mitogens PHA and Con A.

In accordance with Nowacki et al. (1993), who described a transient increase of porcine natural-interferon-producing (NIP) cell frequency in connection with vaccination, an increase in the IFN-α-producing capacity was found after the second immunization for all animals. When analysed in whole blood cultures, this increase in IFN-α-producing capacity was not as high for pigs treated for 2 weeks with ACTH as for pigs in the other two experimental groups. In contrast, when analysed in cultures of purified PBMC, the increase in the IFN-α-producing capacity was more pronounced for ACTH-treated pigs than for non-treated pigs. One explanation for these discrepancies could be that the negative influence of cortisol seen in whole blood cultures is reduced when the PBMC are purified and cultured in medium free from cortisol. However, this is probably not the only explanation, as purified PBMC obtained from the ACTH treated pigs produced approximately twice the amount of IFN-α as PBMC obtained from the control pigs did. For example, if the number of a very efficient but rare circulating cell population, such as the porcine NIP-cell (one per 1000 PBMC), is unaltered despite the occurrence of lymphopenia, an enrichment of NIP cells in cultures with a fixed number of purified PBMC could account for the relatively higher amounts of IFN-α produced. In this context, it is notable that enrichment of porcine IFN-producing leukocytes increases the IFN yield per cell (Nowacki and Charley, 1993).

Despite this, a lower amount of antibodies to *M. hyopneumoniae* was produced in vitro at Days 23 and 25 and the time to reach the peak response to the primary immunization was delayed in the ACTH-treated animals, no differences between

the groups were seen in their in vitro or in vivo responses to the second immunization. These results agree with those of Oldham and Bridger (1992), who demonstrated that the time at which dexamethasone (DX) was administered to calves in relation to rotavirus infection was decisive as to the effect it had on their antibody production. When DX treatment started after primary, but before secondary, infection the systemic and local responses were not affected while they were inhibited when the DX treatment commenced prior to the primary infection. In the latter case, the clinical signs of disease were also greater. Consequently, the present experimental results suggest that 'stressors' that elicit an increased production of cortisol may interfere with the immune capacity of swine. This can be of clinical importance when pigs are exposed to new micro-organisms and external stressors simultaneously, e.g. when mixed and transported to new environments.

5. Acknowledgments

References

Artursson, K., 1993. Studies on the interferon α/β system of pigs. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Artursson, K., Wallgren, P. and Alm, G.V., 1989. Appearance of interferon α in serum and signs of reduced immune function in pigs after transport and installation in a fattening farm. Vet. Immunol. Immunopathol., 23: 345–353.

Becker, B.A., Nienaber, J.A., Christenson, R.K., Manak, R.C., DeShazer, J.A. and Hahn, G.L., 1985. Peripheral concentrations of cortisol as an indicator of stress in the pig. Am. J. Vet. Res., 46: 1034–1038.

Bettens, F., Kristensen, F., Walker, C., Schwuléra, U., Bonnard, G.D. and de Weck, A.L., 1984. Lymphokine regulation of activated (G1) lymphocytes. II. Glucocorticoid and anti-Tac-induced inhibition of human T lymphocyte proliferation. J. Immunol., 132: 261–265.

Blecha, F. and Baker, P.E., 1986. Effect of cortisol in vitro and in vivo on production of bovine interleukin 2. Am. J. Vet. Res., 47: 841–845.

Bölske, G., Strandberg, M.-L., Bergström, K. and Johansson, K.-E., 1987. Species-specific antigens of *Mycoplasma hyopneumoniae* and cross-reactions with other porcine mycoplasmas. Curr. Microbiol., 15: 233–239.

Bölske, G., Johansson, K.-E., Strandberg, M.-L. and Bergström, K., 1990. Comparison of the cross-reactions to different *Mycoplasma hyopneumoniae* antigen preparations in ELISA. Zbl. Bakt., Suppl. 20: 832–835.

Cohen, J.J., 1992. Glucocorticoids. In: I.M. Roitt and P.J. Delves (Editors), Encyclopedia of Immunology. Academic Press, London, pp. 616–617.

Diaz de Arce, H., Artursson, K., L'Haridon, R., Perers, A., La Bonnardière, C. and Alm, G.V., 1992. A sensitive immunoassay for porcine interferon-α. Vet. Immunol. Immunopathol., 30: 319–327.

Dupont, E., 1988. Immunological actions of corticosteroids and cyclosporine A. Curr. Opin. Immunol., 1: 253–256.

Edfors-Lilja, I., Bergström, M., Gustafsson, U., Magnusson, U. and Fossum, C., 1991. Genetic variation in Con A induced production of interleukin 2 by porcine peripheral blood mononuclear cells. Vet. Immunol. Immunopathol., 27: 351–363.

Edfors-Lilja, I., Wattrang, E., Magnusson, U. and Fossum, C., 1994. Genetic variation in parameters reflecting immune competence of swine. Vet. Immunol. Immunopathol., 40: 1–16.

Griffin, J.F., 1989. Stress and immunity: a unifying concept. Vet. Immunol. Immunopathol., 20: 263–312.

Helmberg, A., Fässler, R., Geley, S., Jöhrer, K., Kroemer, G., Böck, G. and Kofler, R., 1990. Glucocorticoid-regulated gene expression in the immune system. Analysis of glucocorticoid-regulated transcription from the mouse macrophage-like cell line P388D1. J. Immunol., 145: 4332–4337.

Lehmann, E.L., 1975. Nonparametrics: Statistical Methods Based on Ranks. McGraw-Hill, San Francisco, CA.

Magnusson, U. and Fossum, C., 1992. Effect of oestradiol 17-β treatment of gilts on blood mononuclear cell functions in vitro. Am. J. Vet. Res., 53: 1427–1430.

Northrop, J.P., Crabtree, G.R. and Mattila, P.S., 1992. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. J. Exp. Med., 175: 1235–1245.

Nowacki, W. and Charley, B., 1993. Enrichment of coronavirus-induced interferon-producing blood leukocytes increases the interferon yield per cell: a study with pig leukocytes. Res. Immunol., 114: 111–120.

Nowacki, W., Cederblad, B., Renard, C., La Bonnardière, C. and Charley, B., 1993. Age-related increase of porcine natural interferon-α producing (NIP) cell frequency and of interferon yield per cell. Vet. Immunol. Immunopathol., 37: 113–122.

Nyberg, L., 1988. Glucocorticoid binding proteins. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Oldham, G. and Bridger, J.C., 1992. The effect of dexamethasone-induced immunosuppression on the development of faecal antibody and recovery from and resistance to rotavirus infection. Vet. Immunol. Immunopathol., 32: 77–92.

Quinn, P.J., 1990. Mechanisms of action of some immunomodulators used in veterinary medicine. In: F. Blecha and B. Charley (Editors), Immunomodulation in Domestic Food Animals, Academic Press, San Diego, pp. 43–99.

Sharon, N., 1983. Lectin receptors as lymphocyte surface markers. Adv. Immunol., 34: 213–298.

Ucker, D.S., 1987. Cytotoxic T lymphocytes and glucocorticoids activate an endogenous suicide process in target cells. Nature, 327: 62–64.

Wallgren, P., Bölske, G. and Fossum, C., 1992. In vitro stimulation of antibody production to *Mycoplasma hyopneumoniae* by porcine peripheral blood mononuclear cells. Vet. Microbiol., 32: 363–374.

Wallgren, P., Artursson, K., Fossum, C. and Alm, G.V., 1993. Incidence of infections in pigs bred for slaughter revealed by elevated serum levels of interferon and development of antibodies to *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*. J. Vet. Med. B., 40: 1–12.