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Genetic variation in parameters reflecting immune competence of swine

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Abstract

Genetic variation in total and differential white blood cell (WBC) counts, phagocytic capacity of polymorphonuclear leukocytes (PMNL), virus induced interferon- α (IFN- α) production, mitogen induced proliferation and interleukin 2 (IL-2) production of mononuclear cells (MNC) in vitro was studied in blood collected from 124 Yorkshire piglets, aged 8 weeks. The piglets were the offspring from 12 sires and 31 dams. Data from an earlier experiment, including 96 piglets of seven sires and 24 dams, were added when estimating heritabilities for Con A induced proliferation and IL-2 production. The highest heritability ($h^2 = 0.87 \pm 0.41$) was estimated for the total number of PMNL. Medium high heritabilities ($h^2 = 0.3-0.4$) were estimated for the phagocytic capacity of PMNL, Con A induced proliferation and IL-2 production and the total number of WBC, while the heritability estimates were lower ($h^2 = 0.00-0.08 \pm 0.12$) for the total number of lymphocytes, serum concentrations of Ig and IFN- α production. Pronounced differences between litters from various dams were found for total number of lymphocytes, IFN- α production, Con A induced proliferation and IL-2 production. The Con A induced proliferation was positively correlated ($r = 0.48$, $P < 0.001$) with the IL-2 production and both these parameters were correlated ($r = 0.44$ and 0.37 , respectively, $P < 0.001$) to the virus induced IFN- α production. Despite these positive correlations, no parental offspring group was uniformly superior across all traits measured. However, the heritabilities estimated for the immune parameters are sufficiently high to be used as genetic markers in selection for general immune competence of swine.

Abbreviations

CL, chemiluminescence; CTLL, cytotoxic T lymphocyte line; IFN- α , interferon- α ; IL-2, interleukin 2; MDBK, Madin-Darby Bovine Kidney; MNC, mononuclear cells; NIP, natural interferon producing; PBS, phosphate buffered saline; PMNL, polymorphonuclear leukocytes; PPV, porcine parvo virus; SI, simulation index; WBC, white blood cell.

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Introduction

Genetic variation in immune responsiveness is documented in both laboratory and domestic animals. Resistance to infectious diseases might thus be improved by including immune responsiveness when selecting breeding animals. For that purpose relevant immune response traits have to be defined. So far, the most extensively studied immune response traits in pigs are those reflecting the antibody- and cell-mediated immunity such as antibody response to various antigens (Buschmann et al., 1974; Huang, 1977; Kräusslich et al., 1983; Rothschild et al., 1984a,b; Buschmann et al., 1985; Edfors-Lilja et al., 1985) and the proliferation of mononuclear cells induced by mitogens (Jensen and Christensen, 1980; Mallard et al., 1989a), while less attention has been paid to the innate immunity. In mice, selection for high antibody responders to a multideterminant antigen resulted in high line mice which also had an increased production of antibodies to other antigens than that selected for. However, these 'high responder' mice had a decreased resistance to intracellular microorganisms as a result of a poor lysosomal activity of their macrophages (Biozzi et al., 1984). To avoid such negative effects of selection for a single parameter, combined breeding values based on antibody response, mitogen or antigen induced lymphocyte blastogenesis, and monocyte uptake and killing of *Salmonella typhimurium*, have been used when selecting pigs for high and low immune responsiveness (Mallard et al., 1992). In this selection experiment, the parental animals grouped as high responders also produced antibodies with higher avidity than those of the control and low groups (Appleyard et al., 1992). These results indicate that selection for improved general immune competence of pigs might be possible.

Good markers for general immune competence might be found among essential parameters, such as the number of cells in the various leukocyte populations and/or the ability of the cells to produce cytokines. Genetic differences in total and differential number of circulating leukocytes (Mallard, personal communication, 1990; Edfors-Lilja and Fossum, 1991) and in the ability of mononuclear cells to produce interleukin-2 (Edfors-Lilja et al., 1991) have earlier been indicated in swine. In the present study we have therefore expanded on previous work and examined the genetic variation in the numbers of polymorphonuclear leukocytes (PMNL) and lymphocytes in porcine blood. Since the PMNL play a crucial role in the initial immune defence, especially against bacteria, we also measured their phagocytic capacity *in vitro*. Among the cytokines, we focused on the production of interferon- α (IFN- α), an important innate defence mechanism against viral infections, and interleukin-2 (IL-2) which activates a variety of cells in the immune system. In addition, we studied the genetic variation in previously used markers of immune responsiveness, i.e. mitogen induced proliferation of mononuclear cells and serum levels of immunoglobulins.

By using animals from a selection experiment with information from several generations and a statistical model including a relationship matrix, it was possible to include previously published data for some of the traits (Edfors-Lilja et al., 1991). Hence, it was possible to estimate heritabilities from the rather limited data set. By this measure, which is defined as the proportion of the total variation in a population that can be attributed to variation in genetic factors, it is possible to predict the usefulness of a certain trait as a genetic marker.

Materials and methods

Animals and experimental design

The piglets used in the present study were included in a selection experiment at the University Research Station, Lövsta, Uppsala, Sweden. From a random bred base population of Swedish Yorkshire pigs, two lines, each with two replicates, were selected for lean tissue growth rate on two protein diets, 18.5% and 13.1% crude protein, respectively. The male piglets were castrated at 3 weeks of age and the litters were weaned at 6 weeks of age. The housing and management of the animals as well as the experimental design of the selection experiment is described in more detail by Stern et al. (1993).

A total of 124 piglets from 31 litters were studied. Forty of the piglets after four boars and ten sows (Set 1) were born in the spring of 1989 and the remaining 84 piglets after eight boars and 21 sows (Set 2) in the spring of 1990. However, all 12 boars and 31 sows were from the same generation in the selection experiment. For some of the heritability estimates, previously published data on immune response from 96 additional animals after seven boars and 24 sows were included (Edfors-Lilja et al., 1991). These animals were from an earlier generation of pigs in the selection experiment.

Two weeks after weaning, four piglets of approximately the same weight were chosen from each litter. Blood was collected from the cranial vena cava, using evacuated glass tubes (B-D Vacutainer, Maylan, France) without additive or with 15 mg EDTA K₃ respective 143 USP units heparin as additive. Precautions were taken to standardize bleeding and assay procedures.

Serum analysis

The level of IFN- α and the total level of immunoglobulin were determined in serum collected from the experimental animals at the age of 8 weeks. Levels of IFN- α were determined by the bioassay described below and the immunoglobulin levels were determined by an indirect ELISA technique, according to Magnusson and Fossum (1992), using rabbit anti-swine immunoglobulins (Dakopatts, Copenhagen, Denmark). In addition, the an-

tibody titres to porcine parvo virus (PPV) were determined in serum from the piglets in Set 1 by a competitive ELISA (SVAnova, Uppsala, Sweden). The antibody titres to PPV were determined twice for each piglet, at 6 (immediately before weaning) and 8 weeks of age.

Total and differential white blood cell (WBC) counts

The number of WBC was determined in a celloscope and differential WBC counts were carried out using blood smears stained with Giemsa's and May Grünwald's solutions. These blood analyses were performed at the Department of Clinical Chemistry, Swedish University of Agricultural Sciences, according to standard procedures. From these data the numbers of PMNL and lymphocytes per litre blood were calculated.

Phagocytic capacity of blood polymorphonuclear leukocytes

The phagocytic capacity of blood PMNL was determined in whole blood from the piglets in Set 2, by a luminol enhanced chemiluminescence (CL) assay as described in detail for porcine leukocytes by Magnusson and Einarsson (1990). In brief, 100 μl of heparinized blood were added to a cuvette with 200 μl luminol, 200 μl opsonized zymosan particles and 500 μl phosphate buffered saline (PBS). Background CL was determined by replacing the zymosan particles with saline. The samples with zymosan were set up in triplicate and the control samples in duplicate. Light emission (mV) was recorded at intervals of 194 s until CL was close to background levels. The peak CL value was chosen as measure of the phagocytic capacity.

Isolation of peripheral blood mononuclear cells

The heparinized blood, diluted with an equal volume of PBS, was layered on Ficoll-Paque (Pharmacia, Uppsala, Sweden). After centrifugation at $550\times g$ for 30 min the MNC were collected and washed three times in PBS by centrifugation at $180\times g$ for 10 min. After the final washing, the cells were resuspended in RPMI 1640 medium with 20 mM HEPES buffer, supplemented with glutamine (2 mM), penicillin (200 IU ml^{-1}), streptomycin (100 $\mu\text{g ml}^{-1}$), 2-mercaptoethanol (5×10^{-5} M) and 5% FCS (Myoclone, Gibco) and the number of cells was adjusted to the desired concentration in growth medium.

Functional tests for blood mononuclear cells

The functional activity of MNC isolated from the piglets was demonstrated as mitogen-induced lymphocyte proliferation and IL-2 production, and as ability to produce IFN- α in vitro (Sets 1 and 2).

Lymphocyte stimulation test

One hundred μl cell suspension (2×10^6 cell ml^{-1}) was added to each well in round-bottomed microtiter plates (Nunc, Roskilde, Denmark). An additional 100 μl of growth medium or growth medium containing 10 μg (Sets 1 and 2) respective 2.5 μg (Set 2) Con A (Pharmacia) per millimetre, was added to quadruplicate wells. After 48 h culture at 37°C (5% CO_2), 50 μl culture supernatant was collected from each well for later determination (see below) of IL-2. Twenty five μl growth medium containing 0.5 μCi ^3H -thymidine (specific activity 5 Ci mmol^{-1} ; Amersham International, Amersham, UK) was added to each well. The cells were cultured for another 24 h before the radioactivity in each culture was determined in a liquid scintillation counter (betaplate counter; LKB Wallac, Turku, Finland). The mean cpm value for each quadruplicate was calculated and used in the further statistical analysis of data.

IL-2 production

The levels of IL-2 activity were determined in the supernatants collected after 48 h culture of the MNC in the presence or absence of the mitogen Con A. In brief, the bioassay measuring the proliferation (cpm-value) of an IL-2 dependent murine cytotoxic T lymphocyte line (CTLL), was used as described in detail for porcine cells by Edfors-Lilja et al. (1991). At every test occasion a porcine IL-2 laboratory standard was included and the IL-2 activity in the samples (expressed as percentage of the standard) was calculated according to Jordan (1972).

IFN- α production

Two hundred μl cell suspension (2×10^6 MNC ml^{-1}) were cultured for 17 h in microtiter plates with fixed, pseudorabies virus infected porcine kidney cells. One hundred and fifty μl of the culture supernatant was collected and the antiviral activity in the medium was measured by a conventional cytopathic effect inhibition bioassay as described by Artursson et al. (1989). Briefly, two-fold dilutions (from 1:100 to 1:4280) of the samples were added to confluent monolayer cultures of Madin–Darby Bovine Kidney (MDBK) cells in 96-well microtiter plates. After approximately 24 h, the samples were replaced by medium containing vesicular stomatitis virus in sufficient amounts to cause complete destruction of the cells in unprotected wells. After 24 h, residual MDBK cells were stained by crystal violet. As a control, a laboratory standard of Sendai virus-induced porcine leukocyte IFN- α (corre-

sponding to 2.3 Units porcine IFN ml⁻¹) was titrated on each assay plate. One unit per millilitre of this standard corresponded to the dilution that, in a series of initial assays, protected a mean of 50% of MDBK cells in a culture. All sample IFN- α titres were expressed in terms of such units.

Statistical analysis

An analysis of variance was performed using a model including the effects of sire, dam within sire, and set of data. The dam term contains the additive genetic contribution of the dam plus environmental and dominance genetic effects common to litter-mates. The analysis was carried out with the Statistical Analysis System program package (SAS Institute, 1985) using the GLM procedure. Quantitative genetic variation was analyzed using an individual animal model with a relationship matrix including sires, dams and grandparents. A common environmental effect, i.e. litter, was included as a third random effect. A REML (restricted maximum likelihood) program package using a derivate-free algorithm was used (Meyer, 1988). This program gives estimates of heritabilities (h^2) and the common litter effect (c^2), i.e. the proportion of the total variation attributed to additive genetic variation and litter variation, respectively. In addition, the influence of fixed effects is tested. As the model includes a relationship matrix, it was possible to link the data from an earlier generation on Con A induced proliferation and IL-2 production (Edfors-Lilja et al., 1991), with the present data by adding an additional earlier generation to the relationship matrix. When data from several sets were analyzed simultaneously (total and differential WBC counts, Con A induced proliferation and IL-2 activity, pseudorabies virus induced IFN- α activity and serum levels of IFN- α and Ig), the model included set as a fixed effect. The effects of sex and selection line were tested, but not included in the model as they turned out to be non-significant for all the traits.

The proliferative response was analyzed as total cpm values in mitogen stimulated cultures, as net cpm values (spontaneous proliferation subtracted from the mitogen induced proliferation) and as a stimulation index (SI), i.e. mitogen induced proliferation divided by spontaneous proliferation. The IL-2 activity was similarly analyzed as total and net cpm values and expressed as percent of the laboratory standard (see earlier).

In order to achieve a normal distribution of data, the in vitro induced IFN- α activity as well as the SI values, were transformed using square root transformation. The phagocytic capacity was analyzed as peak CL values per 10⁶ PMNL.

Results

Serological status

Absence of subclinical viral infections in the animals was indicated by the low serum levels of IFN- α (1.5 U IFN ml⁻¹ serum, SD=1.3, $n=121$). The

total level of Ig was 16.6 mg per millilitre serum ($SD=8.0$, $n=124$) with a slight difference ($P \leq 0.10$) between Sets 1 and 2. At this age (8 weeks), a large litter effect ($c^2=0.50$) was estimated for the levels of immunoglobulin in serum, while the heritability was 0.00. Serum antibody titres to PPV were determined in Set 1. Immediately before weaning, the antibody titres to PPV were less than 1:10 (two litters), 1:10 (five litters), 1:50 (two litters) and 1:250 (one litter). Two weeks later, the titers to PPV remained unaltered or were, for 12 out of 40 piglets, decreased.

Total and differential WBC counts

As shown in Table 1, the total number of WBC and differential cell counts were within the normal range, although a rather large individual variation was found. Also between paternal offspring groups, a variation was found in the number of WBC and PMNL (Fig. 1). When analyzed for genetic variation (Table 2), a high heritability ($h^2=0.87 \pm 0.44$) was estimated for the numbers of PMNL, while no detectable heritability ($h^2=0.00$) was found for numbers of lymphocytes. A medium high heritability was estimated ($h^2=0.44 \pm 0.29$) for the total WBC count. The litter influence was pronounced for the number of lymphocytes ($c^2=0.41$), while this effect was small for the total number of WBC ($c^2=0.07$) and PMNL ($c^2=0.00$). It is notable from Table 2 that the estimates based on the absolute numbers of PMNL and lymphocytes diverge from those based on the proportions of PMNL and lymphocytes.

Table 1

Overall mean values (with one standard deviation) for the various immune parameters measured

| Trait | Mean | SD | n ¹ |
|--|--------|-------|----------------|
| WBC ($\times 10^9 l^{-1}$) | 20.6 | 4.4 | 124 |
| PMNL (%) | 42.3 | 10.7 | 124 |
| ($\times 10^9 l^{-1}$) | 8.7 | 2.9 | 124 |
| Lymphocytes (%) | 55.9 | 10.0 | 124 |
| ($\times 10^9 l^{-1}$) | 11.5 | 3.3 | 124 |
| Phagocytic capacity peak CL-value (mV) | 6.3 | 3.4 | 72 |
| IFN- α production (U ml ⁻¹) | 122.8 | 212.9 | 124 |
| <i>Proliferation (cpm)</i> | | | |
| spontaneous | 5502 | 6419 | 122 |
| 10 μ g Con A ml ⁻¹ | 140458 | 95625 | 119 |
| 2.5 μ g Con A ml ⁻¹ | 161134 | 71469 | 82 |
| <i>IL-2 activity (%)</i> | | | |
| spontaneous | 1.9 | 9.7 | 99 |
| 10 μ g Con A ml ⁻¹ | 71.3 | 61.1 | 99 |
| 2.5 μ g Con A ml ⁻¹ | 71.0 | 59.1 | 59 |

¹Set 1 included 84 piglets and Set 2 40 piglets. Owing to technical errors 25, 12 and four values are missing for the IL-2 activity, phagocytic activity and the proliferative capacity, respectively.

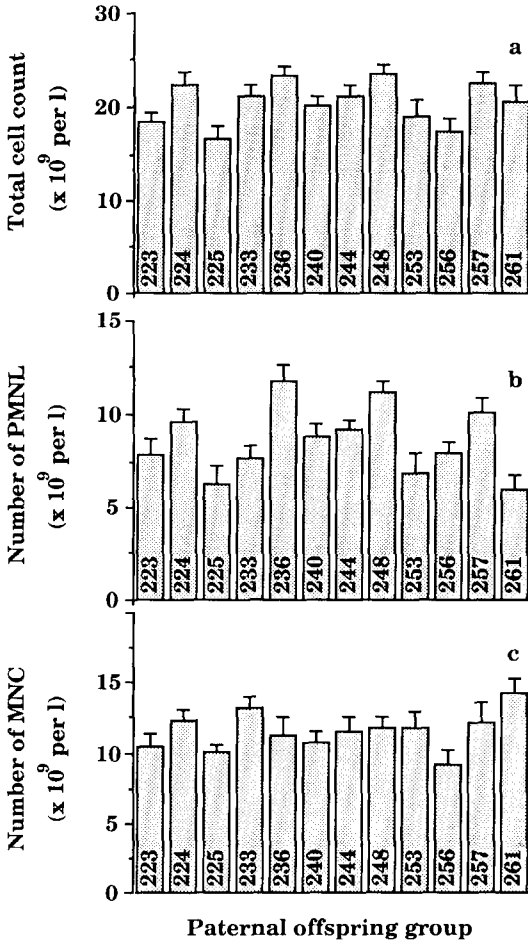


Fig. 1. The total number (mean values with one standard error) of (a) leukocytes, (b) polymorphonuclear leukocytes and (c) lymphocytes in porcine blood. The blood was collected from 124 piglets which were the offspring of 12 sires and 31 dams.

Phagocytic capacity of PMNL

The phagocytic capacity of PMNL was determined as the peak value of luminol enhanced chemiluminescence (CL) after uptake of zymosan particles. As seen in Table 1, the peak CL value was in average 6.3 mV with a large individual variation, i.e. the coefficient of variation (SD per mean) was more than 50%. One of the paternal offspring groups (sire No. 253) had almost twice as high peak CL value per 10^6 PMNL as the other groups (Fig. 2(a)). A medium high heritability ($h^2 = 0.32 \pm 0.32$), but no detectable litter effect ($c^2 = 0.00$) was found for the peak CL value per 10^6 PMNL (Table 2).

Table 2
Estimates of heritabilities (h^2) and common litter effects (c^2) for the various immune parameters

| Trait | $h^2 \pm SE^1$ | c^2 |
|---------------------------------------|-----------------|-------|
| <i>Number of:</i> | | |
| WBC | 0.44 ± 0.29 | 0.07 |
| PMNL | 0.87 ± 0.41 | 0.00 |
| lymphocytes | 0.00 ± 0.00 | 0.41 |
| <i>Percentage of:</i> | | |
| PMNL | 0.39 ± 0.28 | 0.31 |
| lymphocytes | 0.24 ± 0.21 | 0.39 |
| Peak CL/PMNL | 0.32 ± 0.32 | 0.00 |
| IFN- α production ² | 0.08 ± 0.12 | 0.39 |
| <i>Con A induced³:</i> | | |
| proliferation (SI) ² | 0.38 ± 0.21 | 0.26 |
| IL-2 production (%) | 0.44 ± 0.23 | 0.52 |
| Serum level of Ig | 0.00 ± 0.00 | 0.50 |

¹Calculated according to Falconer (1981): $SE(h^2) = \sqrt{1/a \times 8 \times h^2/T}$, where a = the additive relationship among animals and T = number of animals.

²Square root transformed.

³Including earlier results (Edfors-Lilja et al., 1991).

Con A induced proliferation and IL-2 production by MNC

In Set 1, two concentrations of Con A, 2.5 and 10 $\mu\text{g ml}^{-1}$, were used. The proliferation and IL-2 production induced by the lower mitogen concentration was not significantly different from that induced by 10 $\mu\text{g Con A ml}^{-1}$ which was used in both Sets 1 and 2 (Table 1). Therefore, only data from cultures stimulated with 10 $\mu\text{g Con A per ml}$ are presented in the following results.

As shown in Fig. 3, mitogen induced proliferation and IL-2 production varied between, as well as within, offspring groups. To reduce the influence of day-to-day variations in the assays, the analysis for genetical influence on proliferation and IL-2 production were performed on SI values and net IL-2 values, respectively. When tested for significance, the effect of dam, which includes both genetic and common environmental effects, was highly significant ($P \leq 0.001$). However, the effect of sire was, owing to the large dam effect, not significant.

To enlarge the amount of data for estimating the genetic (h^2) and litter (c^2) proportion of the total variation in Con A induced proliferation and IL-2 production, results from an earlier generation of pigs (Edfors-Lilja et al., 1991) were included. From these combined data, linked via the relationship

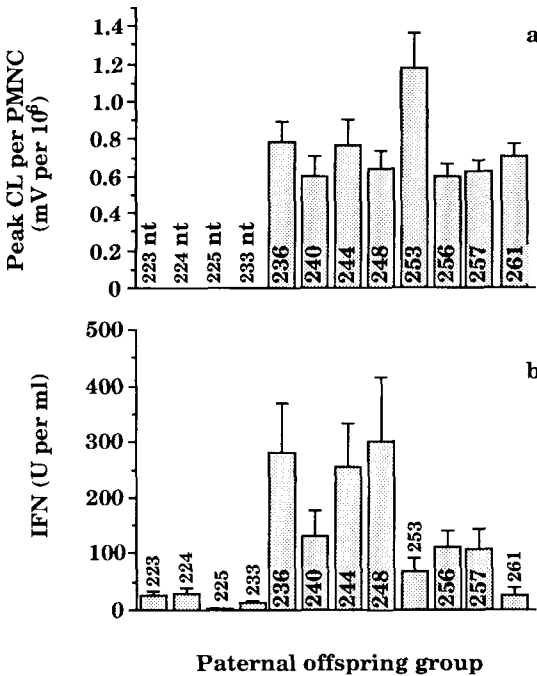


Fig. 2(a) The phagocytic capacity of polymorphonuclear cells and (b) the production of IFN- α in vitro of mononuclear cells (mean values with one standard error). The blood was collected from offspring of eight and 12 sires, respectively.

matrix, the heritability was estimated as medium high for both proliferation ($h^2 = 0.38 \pm 0.21$) and IL-2 production ($h^2 = 0.44 \pm 0.23$). A high litter effect was estimated for the IL-2 production ($c^2 = 0.51$) while a lower estimate ($c^2 = 0.26$) was obtained for the proliferation (Table 2).

Production of IFN- α by MNC in vitro

The capacity of MNC to produce IFN- α showed a large individual variation (Table 1). As shown in Fig. 2(b), the mean IFN- α producing capacity of MNC obtained from pigs in the different offspring groups varied from 3 to 302 U ml⁻¹ supernatant. The heritability estimate was low ($h^2 = 0.08 \pm 0.12$), while the litter effect was medium high ($c^2 = 0.39$), Table 2.

Correlations between immune parameters

The number of PMNL was correlated with the peak CL values ($r = 0.40$, $P < 0.001$) and with the IFN- α production ($r = 0.31$, $P < 0.001$). Accordingly, MNC from offspring of sire Nos. 236, 244 and 248, which in average pro-

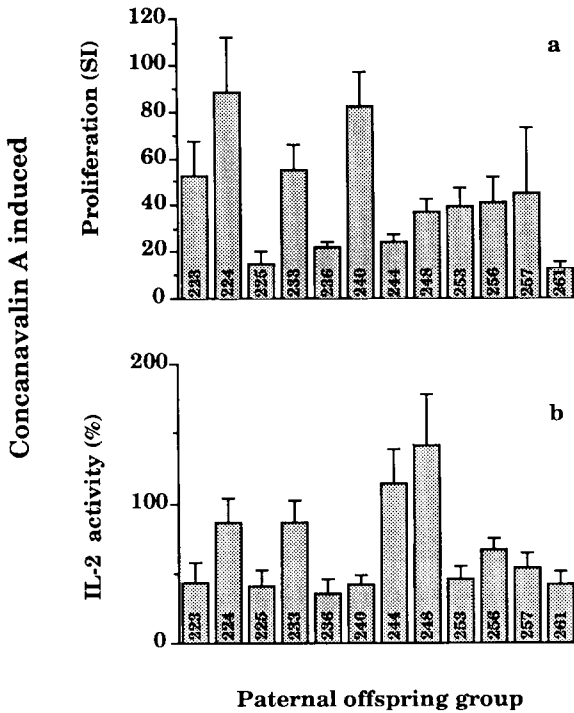


Fig. 3(a). Concanavalin A induced proliferation and (b) interleukin-2 production of MNC isolated from piglets in 12 paternal offspring groups. The proliferation is expressed as SI values and the IL-2 activity as net values (see Materials and methods). All values are mean values with one standard error.

duced more IFN- α than offspring of the other sires also had a high number of PMNL per litre blood. A medium high correlation ($r=0.48, P<0.001$) was found between Con A induced proliferation and IL-2 production which in turn both were correlated ($r=0.44$ and 0.37 respectively, $P<0.001$) to the pseudorabies virus induced IFN- α production. Lower correlations ($r=0.22$ to $0.27, P\leq 0.01$) were found between serum levels of Ig and total numbers of white cells, numbers of lymphocytes, Con A induced proliferation and IFN- α production.

Discussion

The present study demonstrates a genetic variation in some parameters reflecting immune competence of swine. Most striking was the high heritability ($h^2=0.87\pm 0.41$) found for the number of PMNL, suggesting a single gene effect. The genetic influence on the number of PMNL could be exerted during the hematopoiesis, e.g. via production of granulocyte colony stimulating fac-

tor(s). Alternatively, a large genetic variation in the response to external stimuli, such as infections or stress, could cause the high heritability estimate obtained for the number of PMNL. In the latter case, 'stress-hormones' such as glucocorticosteroids are known to rapidly influence the number of PMNL circulating in the blood (for review see Quinn, 1990). If PMNL are to be used as a marker, further studies are therefore needed to elucidate the regulatory mechanism(s) behind their maturation and distribution. Compared with PMNL, the differentiation and maturation of lymphoid subpopulations is influenced by a broader range of cytokines and thus regulated by a number of genes. This polygenic background could explain why such a low heritability ($h^2=0.00$) was found when the total number of blood lymphocytes was analyzed. Consequently, the medium high heritability estimate for the number of WBC ($h^2=0.44 \pm 0.29$) is most likely owing to the genetic variation in the number of PMNL.

In addition to the high genetic influence on the total number of PMNL, a medium high heritability ($h^2=0.32 \pm 0.32$) was found for their phagocytic capacity. These findings are in accordance with the large sire effect demonstrated both on number and phagocytic capacity of PMNL in cattle (Kehrli et al., 1991). Hence, the high heritability estimates for number and function of PMNL, together with the important role of PMNL in the hosts early defence against bacterial infections, make these two parameters potential markers of disease resistance. This proposal may become even more relevant since recent results suggest that PMNL also release immunoregulatory cytokines (reviewed by Loyd and Oppenheim, 1992) that can influence the ensuing immune response.

The cell-mediated immunity is commonly measured as mitogen induced proliferation which so far is the most extensively studied immune response trait. We have previously found half-sib differences in the time course, but not in the magnitude, of lymphocyte proliferation induced by Con A (Edfors-Lilja et al., 1991). Including these earlier data for the magnitude of Con A induced proliferation, a medium high heritability ($h^2=0.38 \pm 0.21$) was found. This estimate is in accordance with those previously reported for Con A induced proliferation ($h^2=0.225$) by Mallard et al. (1992), as well as for proliferation induced by phytohemagglutinin ($h^2=0.20$; Jensen and Christensen, 1980) and for keyhole limpet hemocyanin (h^2 =approximately 0.30; Joling et al., 1991).

Another measure of cell-mediated immunity is the ability to produce IL-2. As IL-2 production is essential not only for the cell-mediated, but also for the antibody-mediated immunity, this is a putative better marker of immune responsiveness than the mitogen induced proliferation. Studies examining genetic differences in IL-2 production are scarce in swine, while differences between inbred lines have been described in rats (Lukic et al., 1987) and in chickens (Knudtson and Lamont, 1989; Knudtson et al., 1990). We have pre-

viously reported a difference in the IL-2 production between paternal halfsib piglets (Edfors-Lilja et al., 1991). Including these earlier data, a medium high heritability ($h^2 = 0.44 \pm 0.23$) was found for the Con A induced IL-2 production with pronounced differences between the paternal offspring groups (Fig. 3(b)). The Con A induced IL-2 production was positively correlated with the magnitude of the proliferation ($r = 0.48$) which is in accordance with earlier results ($r = 0.35$; Edfors-Lilja et al., 1991). Even with this earlier data included, the data set was not large enough to obtain any reliable estimates of genetic correlations. In chickens however, results indicate that levels of IL-2 activity are usually associated with the magnitude of proliferation induced by Con A, but that the genetic control of the IL-2 activity is distinct from that of the proliferative response (Knudtson et al., 1990).

As a measurement of the innate immunity to virus, the ability to produce IFN α in vitro in response to a viral stimuli was chosen. In pigs (Charley and Lavenant, 1990), as in humans (Rönblom et al., 1983; Sandberg et al., 1990) an efficient, but infrequent, natural interferon producing (NIP) cell has been described. Swine breed differences in the frequency of NIP cells and the IFN yield per cell, were recently suggested (Nowacki et al., 1992). We found a low heritability ($h^2 = 0.08 \pm 0.12$) for the pseudorabies virus induced IFN- α production. The difference in IFN- α production between litters ($c^2 = 0.39$) as well as between individual animals was large (Table 1). The latter finding is in accordance with observations in mice and humans, for which a 100-fold individual variation in virus induced IFN- α production has been described (reviewed by De Maeyer and De Maeyer-Guignard, 1988). A number of murine non-structural genes e.g. the *If-1* locus, that influence the amount of IFN produced per cell have been identified. If a porcine *If-1* locus exists, very large individual differences in level of IFN- α could be expected as a result of the segregation of the *If-1^h* allele. Interestingly, it has been suggested that the *If-1* locus not only affects the amount of IFN produced per cell, but also the early stages of a signal transduction pathway common to virus induction of IFN genes, as well as TNF- α and IL-6 genes (Ray et al., 1992).

The serum level of Ig showed in the present study a very low heritability ($h^2 = 0.00$), but a large litter effect ($c^2 = 0.50$). The latter value indicates that maternal antibodies still were present at the time for blood sampling (8 weeks of age). In support of this, serum antibody titres to PPV, presumably of maternal origin, were present both immediately before weaning and 2 weeks later, but then at lower titres. It is not possible to decide whether a high Ig level in plasma of conventionally reared pigs reflects a good ability to mount protective antibody responses or/and a high incidence of infections. Therefore, the level of Ig, which earlier has been proposed as a potential marker of immune responsiveness (Almlid, 1981; Buschmann et al., 1985; Burton et al., 1989; Mallard et al., 1989b), must be interpreted with caution.

Positive correlations were recorded between some of the immune response

traits even if no paternal sire group was uniformly superior across all traits. In the present study, no correlations between immune response and production traits were estimated. However, the selection for lean tissue growth rate on different diets (Stern et al., 1993), did not cause any differences between the lines in the immune parameters measured. This is in accordance with findings in dairy cattle where no differences in a broad range of tests for non-specific immunity were seen between lines selected for genetic differences in milk production (Kehrli et al., 1991). Whether selection for improved meat production affects the immune competence will be examined using a reference pedigree for gene mapping consisting of crosses between Swedish Yorkshire and European wild pigs (Johansson et al., 1992). In addition to this basic research, both heritabilities and genetic correlations need to be estimated for the actual breeding populations before immune response traits are to be included in breeding programmes.

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References

- Almlid, T., 1981. Indirect selection of bulls for improved resistance to diseases in dairy cattle. *Livest. Prod. Sci.*, 8: 321–329.
- Appleyard, G., Wilkie, B.N., Kennedy, B.W. and Mallard, B.A., 1992. Antibody avidity in Yorkshire pigs of high and low immune response groups. *Vet. Immunol. Immunopathol.*, 31: 229–240.
- Artursson, K., Wallgren, P. and Alm, G.V., 1989. Appearance of interferon- α in serum and signs of reduced immune functions in pigs after transport and installation in a fattening farm. *Vet. Immunol. Immunopathol.*, 23: 345–353.
- Biozzi, G., Mouton, D., Stiffel, C. and Bouthillier, Y., 1984. A major role of the macrophage in quantitative genetics regulation of immune responsiveness and anti-infectious immunity. *Adv. Immunol.*, 36: 189–234.
- Burton, J.L., Kennedy, B.W., Burnside, E.B., Wilkie, B.N. and Burton, J.H., 1989. Variation in serum concentrations of immunoglobulins G, A, and M in Canadian Holstein-Friesian calves. *J. Dairy Sci.*, 72: 135–149.
- Buschmann, H., Junge, V., Kräusslich, H. and Radzikowski, A., 1974. A study of the immune response to sheep erythrocytes in several breeds of swine. *Med. Microbiol. Immunol.*, 159: 179–191.
- Buschmann, H., Kräusslich, H., Herrmann, H., Meyer, J. and Kleinschmidt, A., 1985. Quanti-

- tative immunological parameters in pigs – experiences with the evaluation of an immunocompetence profile. *Z. Tierz. Züchtungsbiol.*, 102: 189–199.
- Charley, B. and Lavenant, L., 1990. Characterization of blood mononuclear cells producing IFN- α following induction by coronavirus-infected cells (Porcine Transmissible Gastroenteritis Virus). *Res. Immunol.*, 141: 141–151.
- De Maeyer, E. and De Maeyer-Guignard, J. (Editors), 1988. Interferon and other related cytokines. J. Wiley, New York, pp. 364–379.
- Edfors-Lilja, I. and Fossum, C., 1991. Variations in number and functional capacity of circulating leukocytes between half sib pigs. *Anim. Genet.*, 22 Suppl., 1: 116.
- Edfors-Lilja, I., Gahne, B. and Petersson, H., 1985. Genetic influence on antibody response to two *Escherichia coli* antigens in pigs. II. Difference in response between paternal half-sibs. *Z. Tierz. Züchtungsbiol.*, 102: 308–317.
- 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.
- Falconer, D.F. 1981. Introduction to quantitative genetics. Longman Group Limited, London.
- Huang, J., 1977. Quantitative inheritance of immunological response in swine. Dissertation, University of Hawaii. University Microfilms International, Ann Arbor, MI, 92 pp.
- Jensen, P.T. and Christensen, K., 1980. Genetic studies on the in vitro PHA transformation of porcine blood lymphocytes. *Vet. Immunol. Immunopathol.*, 2: 133–143.
- Johansson, M., Ellegren, H., Marklund, L., Gustafsson, U., Ringmar-Cederberg, E., Andersson, K., Edfors-Lilja, I. and Andersson, L., 1992. The gene for dominant white color in the pigs is closely linked to ALB and PDGFRA on chromosome 8. *Genomics*, 14: 965–969.
- Joling, P., Wever, P.J.M., Mok, K.S., De Vries Reilingh, G. and Oskam, J.P.H., 1991. Estimation of porcine immune competence with help of keyhole limpet haemocyanin (KLH). *Anim. Genet.*, 22 Suppl., 1: 110–111.
- Jordan, G.W., 1972. Basis for the probit analysis of an interferon plaque reduction assay. *J. Gen. Virol.*, 14: 49–61.
- Kehrli, M.E., Weigel, K.A., Freeman, A.E., Thurston, J.R. and Kelley, D.H., 1991. Bovine sire effects on daughters' in vitro blood neutrophil function, lymphocyte blastogenesis, serum complement and conglutinin levels. *Vet. Immunol. Immunopathol.*, 27: 303–319.
- Knudtson, K.L. and Lamont, S.J., 1989. Association of genetics and sampling time with levels of interleukin-2 activity. *Vet. Immunol. Immunopathol.*, 22: 333–343.
- Knudtson, K.L., Kaiser, M.G. and Lamont, S.J., 1990. Genetic control of interleukin-2-like activity is distinct from that of mitogen response in chickens. *Poult. Sci.*, 69: 65–71.
- Kräusslich, H., Buschmann, H., Meyer, J. and Kleinschmidt, A., 1983. Ein Selektionsversuch auf Antikörperbildungsvermögen beim Schwein. *Z. Tierz. Züchtungsbiol.*, 100: 101–108.
- Lloyd, A.R. and Oppenheim, J.J., 1992. Poly's lament: the neglected role of the polymorphonuclear neutrophil in the afferent limb of the immune response. *Immunol. Today*, 13: 169–171.
- Lukic, M.L., Stojkovic, M.M., Kostic, M., Tucic, N. and Vukmanovic, S., 1987. Cellular and genetic basis of strain differences in IL-2 production in rats. *Transpl. Proc.*, 19: 3137–3139.
- Mallard, B.A., Wilkie, B.N. and Kennedy, B.W., 1989a. Genetic selection for improved immune response in Yorkshire pigs. Abstract, 2nd Int. Vet. Immunol. Symp. 24–29 July, Hannover, Immunobiology, Suppl., 4: 104–105.
- Mallard, B.A., Wilkie, B.N. and Kennedy, B.W., 1989b. The influence of the swine major histocompatibility genes (SLA) on variation in serum immunoglobulin (Ig) concentration. *Vet. Immunol. Immunopathol.*, 21: 139–151.
- Mallard, B.A., Wilkie, B.N., Kennedy, B.W. and Quinton, M., 1992. Use of estimated breeding values in a selection index to breed yorkshire pigs for high and low immune and innate resistance factors. *Anim. Biotech.*, 3: 257–280.

- Magnusson, U. and Einarsson, S., 1990. Effects of exogenous oestradiol on the number and functional capacity of circulating mononuclear and polymorphonuclear leukocytes in the sow. *Vet. Immunol. Immunopathol.*, 25: 235–247.
- Magnusson, U. and Fossum, C., 1992. Effect of estradiol 17 β treatment of gilts on blood mononuclear cell functions in vitro. *Am. J. Vet. Res.*, 8: 1427–1430.
- Meyer, K., 1988. Programs to estimate variance components for individual animal models by restricted maximum likelihood (REML). User Notes, Institute of Animal Genetics, Edinburgh University, West Mains Road, Edinburgh, UK.
- Nowacki, W., Cederblad, B., Renard, C., La Bonnardiére, 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.
- Quinn, P.J., 1990. Mechanisms of action of some immunomodulators used in veterinary medicine. In: Blecha, F. and Charley, B (Editors), *Immunomodulators in Domestic Food Animals Adv. in Vet. Sci. and Comp. Med.* Academic Press, San Diego. Vol. 35, pp. 43–99.
- Ray, N.B.K., Cheung, S.C., Rosztochy, I. and Pitha, P.M., 1992. Mouse genotype affects inducible expression of cytokine genes. *J. Immunol.*, 148: 1934–1940.
- Rothschild, M.F., Chen, H.L., Christian, L.L., Lie, W.R., Venier, L., Cooper, M., Briggs, C. and Warner, C.M., 1984a. SLA complex and immune response. Breed and swine lymphocyte antigen haplotype differences in agglutination titers following vaccination with *B. bronchiseptica*. *J. Anim. Sci.*, 59: 643–649.
- Rothschild, M.F., Hill, H.T., Christian, L.L. and Warner, C.M., 1984b. Genetic differences in serum-neutralization titres of pigs after vaccination with pseudorabies modified live-virus vaccine. *Am. J. Vet. Res.*, 45: 1216–1218.
- Rönblom, L., Ramstedt, U. and Alm, G.V., 1983. Properties of human natural interferon-producing cells stimulated by tumor cell lines. *Eur. J. Immunol.*, 13: 471–476.
- Sandberg, K., Matsson, P. and Alm, G.V., 1990. A distinct population of nonphagocytic and low level CD4+ null lymphocytes produce IFN- α after stimulation by Herpes Simplex Virus-infected cells. *J. Immunol.*, 145: 1015–1020.
- SAS Institute, 1985. *SAS User's guide*. Cary NR. SAS Institute.
- Stern, S., Johansson, K., Rydhmer, L., Andersson, K., 1993. Performance testing of pigs for lean tissue growth rate in a selection experiment with low and high protein diets. I. Experimental design and efficiency of selection. *Acta Agric. Scand.*, 43.