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Pig peripheral blood mononuclear leucocyte subsets are heritable and genetically correlated with performance

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Indicator traits used to select pigs for increased resistance to infection or improved health must be heritable and, preferably, be associated with improved performance. We estimated the heritability of a range of immune traits and their genetic and phenotypic correlations with growth performance. We measured immune traits on 589 pigs and performance on 1941 pigs from six farms, three of which were classified as 'high health status' (i.e. specific pathogen-free) and three were of lower health status. All pigs were apparently healthy. Immune traits were total white blood cells (WBC), and peripheral blood mononuclear leucocyte (PBML) subsets positive for CD4, CD8α, gamma delta ($\gamma\delta$) T cell receptor, CD11R1 (natural killer cell marker), B cell and monocyte markers at the start and the end of standard growth performance tests. At both time points, all immune traits were moderately to highly heritable except for CD8α^+ cells. At end of test, heritability estimates (h^2) ($\pm$s.e.) were 0.18 ($\pm$0.11) for total WBC count. For PBML subset proportions, the heritabilities were 0.52 ($\pm$0.14) for $\gamma\delta$ TCR$^+$ cells, 0.62 ($\pm$0.14) for CD4$^+$ cells, 0.44 ($\pm$0.14) for CD11R1$^+$ cells, 0.58 ($\pm$0.14) for B cells and 0.59 ($\pm$0.14) for monocytes. Farm health status affected the heritabilities for WBC, being substantially higher on lower health status farms, but did not have consistent effects on heritabilities for the PBML subsets. There were significant negative genetic correlations between numbers and proportions of various PBML subsets and performance, at both start and end of test. In particular, the proportion of PBML cells that were CD11R1$^+$ cells, at end of test, was strongly correlated with daily gain ($r_g = -0.72$; $P < 0.01$). There were also weaker but significant negative phenotypic correlations between PBML subsets measured at end of test and performance, for $\gamma\delta^+$ T cells, CD8α^+, CD11R1$^+$ cells, B cells or monocytes. Phenotypic correlations with daily gain were generally lower at the start of test than at the end of test. These results show that most of the major pig PBML subsets are heritable, and that systemic levels of several of these PBML subsets are genetically negatively correlated with performance. This approach provides a basis for using immune trait markers when selecting boars that can produce higher-performing progeny.

Keywords: pigs, innate immunity, performance, heritability, NK cells

Introduction

In the pig-breeding industry, infection is a major cause of loss of productivity and can only be partially controlled by use of antibiotics and biosecurity measures. This problem has been further complicated by recent European legislation, which prohibits the use of anti-microbial growth promoters. Hence, it would be desirable to develop a method for selecting animals with increased resistance towards a wide range of infectious diseases. However, this has proved problematic because disease resistance, especially towards a wide range of infections, is difficult to monitor as it is often not feasible, or even possible, to detect every pathogen that has significant impact within a herd. In particular, sub-clinical infections cannot always be detected on the basis of clinical signs or serology tests (Spurlock, 1997; Loeffen *et al.*, 1999).

In order to select animals for improved health or increased resistance towards infectious disease, an alternative approach would be to utilise measures of immune responses as a method of measuring responses towards a wide range of infectious diseases. This method is sometimes referred to as 'generalised immunity'. For measures of the immune response or immune traits to be useful as markers for resistance towards infectious disease, these markers should be heritable and, ideally, correlate with parameters related to disease resistance, such as performance or health, etc. They should also be easy to measure and reproducible without requiring any challenge of the animal; these criteria are fulfilled by peripheral blood mononuclear leucocyte (PBML) subsets. Studies by Edfors-Lilja *et al.* (1994) and Henryon *et al.* (2006a)

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have already reported moderate heritabilities for total and differential numbers of peripheral blood leucocytes, with respect to traits such as numbers of neutrophils, eosinophils, monocytes and lymphocytes. We have a particular interest in PBML subsets as potential markers for resistance towards infectious disease, which include $CD4^+$ cells, $CD8\alpha^+$ cells, $\gamma\delta^+$ T cells, B cells, monocytes and natural killer (NK) cells, which in pigs express the cell marker $CD11R1^+$ (Haverson *et al.*, 2001). This is because our past work has demonstrated that amongst apparently healthy pigs from a farm of intermediate health status, the proportions of specific PBML subsets, B cells, monocytes and $CD11R1^+$ cells were negatively correlated with performance (Clapperton *et al.*, 2005a). Also from the same pigs, we compared two divergent lines selected for lean growth under restricted feeding (LGR) and observed that high LGR line pigs had higher numbers of total white blood cells (WBC), $CD8\alpha^+$ cells and $CD11R1^+$ cells than low LGR line pigs (Clapperton *et al.*, 2006). Overall, these findings would suggest that specific PBML subsets correlate with performance, and can be influenced by genotype.

There are no reported heritability estimates for these particular PBML subsets in pigs, although these traits have been shown to be heritable in humans (Evans *et al.*, 1999; Hall *et al.*, 2000; Ahmadi *et al.*, 2001) and in other species (Wonigeit *et al.*, 1998; Chen and Harrison, 2002; Cheeseman *et al.*, 2004).

In the pig-breeding industry, boars are generally selected for performance under a high health status environment whilst the subsequent progeny are often reared under lower health status conditions. One major challenge is to be able to select AI boars that produce progeny that can perform equally well within either a high or a lower health status environment. Therefore, if immune traits are to be used as selection markers for 'generalised immunity', then it is desirable that these markers are both heritable and correlate with health parameters, e.g. performance, under both high and lower health status environments. In our previous studies, we only measured immune traits in pigs from a single farm, and thus could not ascertain whether changes in health status would affect the results. Furthermore, our data were insufficient to estimate heritabilities or genetic correlations between traits, and thus explore opportunities for genetically improving or altering these immune measures. In this paper, our aims are to determine whether pig PBML subsets are both heritable and genetically correlated with performance. Also, we wish to use our data to determine whether there is any trend in our results associated with broadly defined farm health status. The PBML subsets tested were $CD4^+$ cells, $CD8\alpha^+$ cells, $\gamma\delta^+$ T cells, B cells, $CD11R1^+$ cells and monocytes.

Material and methods

Populations studied and performance trait measurements

All measurements were performed on Large White pigs sampled from six farms labelled A to F. Three of these farms (A to C) were classed as high health status, i.e. free of all major swine pathogens and classified as specific pathogen-free (SPF), and the remaining three farms (D to F) were not SPF and hence classed as lower health status. Farms D to F were free of all major swine pathogens, except for the following organisms: Farm D was positive for enzootic pneumonia, Farm E was positive for *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Leptospira bratislava*, and also, *Salmonella typhimurium* phage type 104 was detected in faecal samples. Farm F was positive for PMWS (porcine multi-wasting syndrome). These six farms were grouped into four sources, where a source represented either the Roslin Institute farm (E) or one of the three pig-breeding companies (cited in the Acknowledgements), which contributed animals to the study.

Details of numbers of pigs tested per farm along with the number of sires and full-sibs families (i.e. litters) are shown in Table 1. Data from the commercial sources were split into two generations, G1 and G2. From G1, a small number of sires were selected by the companies on performance attributes and used to produce progeny (G2). For G1 and G2, immune traits were measured in a selection of animals chosen at random, whilst performance was measured in all G2 offspring from the selected G1 boars. The project had

Table 1 *Numbers of pigs tested, sires, lines and families per farm*

Source	1		2			3		4	Total
Generation	G1	G2	G1	G2	G2	G1	G2	G1	
Farm	A	A	C	C	D	B	F	E	
Health status	High	High	High	High	Lower	High	Lower	Lower	
No. of pigs tested for immune traits	99	0	92	68	59	96	0	175	589
At start-test	99	0	0	0	0	96	0	112	307
At end-test	47	0	92	68	59	96	0	175	537
No. of pigs tested for performance	99	684	92	259	138	96	398	175	1941
No. of genetic lines	8	4	1	1	1	1	1	8	
No. of sires	34	4†	23	5†‡	5†‡	10	5†	64	
No. of full-sib families	65	155	53	53	35	19	53	78	

For each source, G2 progeny were derived from G1 boars. Performance was measured in all pigs tested for immune traits.
†All sires were measured for immune traits when they were growing pigs.
‡These were the same sires.

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initially been designed to enable us to contrast immune and performance traits between farms of high and lower health status; however, it can be seen in Table 1 that genetic links between high and lower health status farms were achieved only in sources 2 and 3.

The average age of animals at the start of test and end of test was 89 and 148 days, respectively. Animals were blood sampled, a 12 ml sample collected via the external jugular vein into a tube containing EDTA anti-coagulant, at the start and the end of test, and sampling was staggered so that pigs were tested in weekly groups of 20 to 30 pigs. With the exception of farm E, sampling for each farm at each time point took place over a period of 4 to 6 weeks. Daily weight gain (g/day) was measured from the start to the end of test and was available for blood-sampled animals as well as for their non-sampled littermates.

Immune measurements

Total and differential WBC were performed immediately after blood collection, as described by Clapperton *et al.* (2005a). Using previously described methods (Clapperton *et al.*, 2005a), peripheral blood mononuclear leucocytes were isolated from whole blood by density centrifugation and the numbers of cell subpopulations were enumerated using flow cytometry. Briefly, single- and dual-colour analyses of cell subpopulations were determined using primary monoclonal antibodies (mABs) recognising the cell surface markers CD4 (MIL-17), CD8α (MIL-12) (Saalmuller *et al.*, 2001), gamma delta ($\gamma\delta$) T cell receptor (TCR) (PPT16) (Davis *et al.*, 2001), the immunoglobulin light chain on B cells (K139 E1) (Denham *et al.*, 1998), CD11R1$^+$ on NK cells (Haverson *et al.*, 2001) (MIL-4) and SIRPα on monocytes (74-22-15) (Thacker *et al.*, 2001), together with secondary antibodies goat anti-mouse (GAM) IgG1-fluorescein isothiocyanate (FITC), GAM IgG2a-phycoerythrin (PE) and GAM IgG2b-fluoresecein isothiocyanate. Data acquisition and analysis were carried out using a FACScan and CELLQuest software (Becton Dickinson, Oxford, UK). The PPT16 cell line supernatant was provided by Huaizhi Yang, Institute for Animal Health, Pirbright, Surrey, UK. The other antibodies were provided by the following companies: Serotec, Oxford, UK, for MIL-4, MIL-12, MIL-17 and K139 E1; Southern Biotechnology Associates Inc., Birmingham, AL, USA, for 74-22-15 and GAM IgG2b; Oxford Biotechnology Ltd., Kidlington, Oxfordshire, UK, for GAM IgG1 and IgG2a.

Data analysis

Preliminary analyses of the data were performed using the GENSTAT package (Lawes Agricultural Trust, 1983) to determine significant fixed effects and to test the distributional assumptions for the traits. A single performance trait was available and consistent across all farms, this being daily live weight gain. The immune traits were total WBC count, and the PBML subsets categorised into CD4$^+$ cells, CD8α^+ cells, $\gamma\delta^+$ T cells, CD11R1$^+$ cells (which were further subdivided into CD11R1$^+$ CD8α^+ and CD11R1$^+$ CD8α^- cells), B cells and monocytes. The WBC PBML subsets were enumerated at both the start and the end of test, although not all animals were measured at both time points. The PBML subsets were expressed both as a proportion of total PBML and as total estimated numbers of each subset.

Significant fixed effects for most traits included farm, weekly batch of pigs nested within farm, line nested within farm, sex and age at measurement (fitted as a covariate). Pigs from sources 1 and 4 were subdivided into lines, and line effects from source 4 (i.e. Roslin Institute farm E) were previously presented by Clapperton *et al.* (2006). Many of the traits were right skewed and required log-transformation prior to analysis. These included all end-test measures except for $\gamma\delta$ cells, CD8α^+ and B cells at start test, and all the PBML subset numbers.

Genetic parameters, and their standard errors, were estimated using the ASREML package (Gilmour *et al.*, 2004), fitting an animal model including all known pedigree relationships. In total, 1941 animals were included in the dataset and the pedigree; however, many more animals had performance than immune trait data (see Table 1). Fixed effects described above were fitted to each trait in the ASREML analysis. Random effects fitted were the residual term plus the direct genetic effect. Litter was fitted as an additional random effect; however, as assessed by the likelihood ratio test, it was never significant and was subsequently dropped from all analyses. Univariate analyses were performed for each trait described above using all available data for each trait (see Table 1). Furthermore, the dataset was subdivided into high and lower health status farms and the univariate analyses repeated for the immune measures. Using the whole dataset, bivariate analyses were performed to estimate correlations between performance and immune traits, and between the immune traits at the start and the end of test.

Results

Summary of data

Mean values for immune and performance traits at the end of test for each farm are shown in Table 2. Similar values were also obtained at the start of test (data not shown). Although there was considerable variability between farms in the immune traits, there was no obvious relationship between farm health status and immune traits, in terms of either mean values or variability. Performance tended to be lower on the lower health status farms; however, the effects of health status, farm husbandry system and animal genotype are confounded.

Heritability estimates

Estimated heritabilities for WBC and proportions of PBML subsets at the start- and end-tests are shown in Table 3. All PBML subsets tested were moderately to highly heritable at both time points, except for CD8α^+ cells. There was also a large disparity in heritability estimates for this PBML subset between the two time points. For WBC, heritability estimates were generally somewhat lower than those for the PBML subset proportions. The heritability for daily gain was 0.47 (s.e. 0.07).

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Table 2 *Summary of immune and performance traits at end-test for each farm*

Farm Health status	A High	B High	C (G1) High	C (G2) High	D Lower	E Lower	F Lower
Measurement (units)							
White blood cells (10^6 cells/ml)	21.9 (5.6)	29.2 (9.1)	26.7 (10.3)	29.2 (13.1)	21.0 (13.1)	32.6 (9.5)	N/A
$CD4^+$ cells (%)	20.2 (4.1)	15.4 (3.6)	18.0 (5.9)	18.8 (3.6)	21.2 (3.2)	13.9 (4.1)	N/A
$CD8\alpha^+$ cells (%)	28.3 (5.6)	27.1 (5.8)	27.6 (7.5)	24.3 (5.0)	29.9 (6.7)	28.7 (6.1)	N/A
$\gamma\delta^+$ T cells (%)	27.3 (7.9)	30.3 (8.4)	34.6 (9.2)	32.9 (8.2)	25.0 (5.6)	49.1 (9.8)	N/A
$CD11R1^+$ total (%)	9.1 (3.3)	14.7 (4.3)	13.5 (5.6)	14.4 (4.4)	15.9 (3.8)	10.5 (5.1)	N/A
$CD11R1^+$ $CD8\alpha^+$ (%)	2.5 (1.7)	5.2 (2.0)	5.5 (2.8)	3.6 (1.9)	3.9 (2.3)	4.8 (2.3)	N/A
$CD11R1^+$ $CD8\alpha^-$ (%)	6.6 (2.5)	9.2 (2.3)	8.6 (3.7)	10.8 (3.4)	12.0 (3.7)	6.6 (3.7)	N/A
B cells (%)	10.7 (2.4)	11.8 (3.8)	6.4 (3.2)	14.8 (4.3)	14.7 (4.6)	12.5 (7.0)	N/A
Monocytes (%)	13.5 (5.8)	14.7 (6.9)	13.0 (4.7)	11.0 (3.3)	13.0 (4.1)	9.5 (3.5)	N/A
Daily gain (g/day)	724 (169)	992 (191)	980 (160)	972 (136)	675 (105)	830 (110)	927 (203)
Age, at start-test (days)	69 (4.6)	90 (4.4)	96 (5.5)	95 (6.9)	90 (6.1)	86 (10.5)	98 (9.4)
Age, at end-test (days)	146 (5.2)	140 (4.2)	147 (5.7)	142 (7.5)	163 (7.4)	157 (12.9)	141 (40.0)

Data are expressed as mean (s.d.).

Table 3 *Heritability (h^2) estimates of total white blood cell numbers (WBC) ($\times 10^6$ cells/ml) and the proportions of peripheral blood mononuclear leucocyte subsets (%) of ca. 500 Large White pigs from three high health status and two lower health status farms at start- and end-test (live weights ~30 and ~90 kg, respectively)*

	Start-test		End-test	
Immune trait	h^2	s.e.	h^2	s.e.
WBC	0.24	0.15	0.18	0.11
$\gamma\delta^+$T cells	0.62	0.18	0.52	0.14
$CD4^+$ cells	0.36	0.18	0.62	0.14
$CD8\alpha^+$ cells	0.60	0.18	0.18	0.13
$CD11R1^+$ cells total	0.58	0.18	0.44	0.14
$CD11R1^+$ $CD8^+$ cells	0.71	0.20	0.34	0.15
$CD11R1^+$ $CD8^-$ cells	0.31	0.18	0.48	0.13
B cells	0.59	0.16	0.58	0.14
Monocytes	0.52	0.17	0.59	0.14

Table 4 *Heritability (h^2) estimates of total white blood cell numbers (WBC) ($\times 10^6$ cells/ml) and proportions of peripheral blood mononuclear leucocyte subsets (%) of ca. 500 Large White pigs from three high health status and two lower health status farms at end-test (live weight ~90 kg), divided according to farm health status*

	High health status			Lower health status		
Immune trait†	σ^2_p	h^2	s.e.	σ^2_p	h^2	s.e.
WBC	0.09	0.06	0.11	0.06	0.37	0.16
$\gamma\delta^+$ T cells	70.0	0.55	0.18	57.7	0.39	0.21
$CD4^+$ cells	0.06	0.82	0.16	0.06	0.34	0.20
$CD8\alpha^+$ cells	0.05	0.03	0.13	0.04	0.54	0.22
$CD11R1^+$ cells	0.11	0.56	0.18	0.15	0.32	0.20
B cells	12.1	0.31	0.17	23.1	0.69	0.21
Monocytes	0.15	0.58	0.18	0.10	0.58	0.18

†All traits log-transformed prior to analysis except $\gamma\delta^+$ T cells and B cells.

Results for heritability estimates for WBC and proportions of PBML subsets at end-test, separated according to farm health status, are shown in Table 4, although these results must be interpreted with caution as pig genotype and farm health status were confounded. Heritability estimates for individual measurements appeared to vary according to farm health status, especially for PBML subsets, $CD4^+$ cells, $CD8\alpha^+$ cells and B cells; however, the standard errors of the estimates were always large. The average heritability calculated from all PBML subsets was 0.475 and 0.477 for high and lower health status environments, respectively. For WBC, the heritability estimate was higher for lower health status farms than for higher health status farms ($0.05 < P < 0.1$).

Correlations between the start- and end-tests for WBC numbers or PBML proportions are shown in Table 5. The phenotypic correlations between the two time points, which under the assumption of equal variances across time may be interpreted as the across-time repeatabilities, were generally lower than the genetic correlations, but were positive and significantly greater than zero for all cell types tested. Genetic correlations were positive albeit with large standard errors.

Genetic and phenotypic correlations with daily gain

Genetic, phenotypic and environmental correlations of daily gain with WBC or PBML subset numbers measured at the end test are shown in Table 6a. In this table and in subsequent analyses presented, the genetic correlations were nearly always stronger than the phenotypic correlations. WBC numbers were uncorrelated with daily gain; however, significant correlations were observed between PBML numbers and daily gain. Most of these correlations were negative, indicating that improved performance is associated with decreased values for these traits. In particular, there were strong and significant negative genetic correlations between the numbers of B cells or $CD11R1^+$ cells and daily gain. $CD11R1^+$ cells comprise $CD8\alpha^+$ and $CD8\alpha^-$ subsets, and these subsets were negatively correlated, both genetically and phenotypically, with daily gain.

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Table 5 *Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between start-test and end-test for total white blood cell numbers (WBC) ($\times 10^6$ cells/ml) or proportions of peripheral blood mononuclear leucocyte subsets*

Immune trait	r_g	s.e.	r_p	s.e.	r_e	s.e.
WBC	0.85	0.62	0.23**	0.07	0.05	0.14
$\gamma\delta^+$ T cells	0.81**	0.16	0.69**	0.04	0.60**	0.13
$CD4^+$ cells	0.87**	0.17	0.46**	0.06	0.02	0.24
$CD8\alpha^+$ cells	0.19	0.42	0.36**	0.07	0.49**	0.17
$CD11R1^+$ cells	0.41	0.28	0.24**	0.07	0.06	0.24
$CD11R1^+$ $CD8\alpha^+$ cells	0.65**	0.26	0.43**	0.07	0.21	0.33
$CD11R1^+$ $CD8\alpha^-$ cells	0.30	0.36	0.28**	0.08	0.27	0.20
B cells	0.88**	0.21	0.34**	0.08	−0.33	0.33
Monocytes	0.15	0.28	0.16*	0.08	0.17	0.26

*$P < 0.05$; **$P < 0.01$.
Data represent 1941 pigs from three high health status and three lower health status farms.

Table 6a *Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between total white blood cell numbers (WBC) ($\times 10^6$ cells/ml), or numbers of peripheral blood mononuclear leucocyte subsets measured at end-test and daily gain (g/day)*

Immune trait	r_g	s.e.	r_p	s.e.	r_e	s.e.
WBC	−0.16	0.31	−0.02	0.05	0.05	0.12
$\gamma\delta^+$ T cells	0.04	0.29	0.15	0.06	0.23	0.13
$CD4^+$ cells	0.14	0.31	−0.08	0.06	−0.19	0.13
$CD8\alpha^+$ cells	−0.15	0.45	−0.13	0.06	−0.14	0.12
$CD11R1^+$ cells	−0.50	0.27	−0.20**	0.06	−0.04	0.14
$CD11R1^+$ $CD8\alpha^+$ cells	−0.52**	0.18	−0.27**	0.05	0.05	0.16
$CD11R1^+$ $CD8\alpha^-$ cells	−0.63**	0.18	−0.27**	0.05	0.05	0.16
B cells	−0.63*	0.27	−0.17**	0.06	0.05	0.13
Monocytes	−0.45	0.23	−0.17**	0.06	0.02	0.14

*$P < 0.05$; **$P < 0.01$.
Data represent 1941 pigs from three high health status and three lower health status farms.

Table 6b *Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between proportions of peripheral blood mononuclear leucocyte subsets measured at end-test and daily gain (g/day)*

Immune trait	r_g	s.e.	r_p	s.e.	r_e	s.e.
$\gamma\delta^+$ T cells	0.35	0.35	0.31**	0.08	0.28	0.26
$CD4^+$ cells	0.11	0.20	−0.09	0.06	−0.30	0.16
$CD8\alpha^+$ cells	0.00	0.44	−0.19**	0.05	−0.27**	0.11
$CD11R1^+$ cells	−0.72**	0.17	−0.22**	0.06	−0.11	0.14
$CD11R1^+$ $CD8\alpha^+$ cells	−0.38	0.24	−0.27**	0.05	0.04	0.15
$CD11R1^+$ $CD8\alpha^-$ cells	−0.61**	0.18	−0.32**	0.05	0.03	0.15
B cells	−0.30	0.19	−0.17**	0.06	−0.03	0.18
Monocytes	−0.30	0.19	−0.20**	0.06	−0.08	0.17

*$P < 0.05$; **$P < 0.01$.
Data represent 1941 pigs from three high health status and three lower health status farms.

Genetic, phenotypic and environmental correlations between daily gain and the proportions of PBML subsets, as opposed to numbers, measured at the end-test are shown in Table 6b. There were significant negative genetic correlations between daily gain and the proportions of $CD11R1^+$ $CD8\alpha^-$ cells or total $CD11R1^+$ cells. There were also significant negative phenotypic correlations for daily gain with the proportions of $CD8\alpha^+$ cells, total $CD11R1^+$ cells, $CD11R1^+$ $CD8\alpha^+$ cells, $CD11R1^+$ $CD8\alpha^-$ cells, B cells or monocytes, and these correlations were similar to those seen for PBML subset numbers. The $\gamma\delta^+$ cell proportions were positively correlated with daily gain.

Genetic, phenotypic and environmental correlations of daily gain with WBC or PBML subset numbers measured at start-test are shown in Table 7a. Once again, WBC was uncorrelated with daily gain. Overall, genetic and phenotypic correlations between PBML subsets and daily gain tended to be weaker at start-test compared to end-test, and

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Table 7a *Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between total white blood cell numbers (WBC) ($\times 10^6$ cells/ml), or numbers of peripheral blood mononuclear leucocyte subsets measured at start-test and daily gain (g/day)*

Immune trait	r_g	s.e.	r_p	s.e.	r_e	s.e.
WBC	0.03	0.30	−0.04	0.07	−0.08	0.14
$\gamma\delta^+$ T cells	0.23	0.24	0.15	0.08	0.08	0.17
$CD4^+$ cells	−0.38	0.32	−0.06	0.07	0.10	0.15
$CD8\alpha^+$ cells	−0.58*	0.27	−0.10	0.07	0.22	0.16
$CD11R1^+$ cells	−0.41	0.23	−0.11	0.07	0.22	0.22
$CD11R1^+$ $CD8\alpha^+$ cells	−0.34	0.60	0.00	0.07	0.10	0.14
$CD11R1^+$ $CD8\alpha^-$ cells	−0.25	0.25	−0.07	0.07	0.08	0.18
B cells	−0.40	0.30	0.01	0.07	0.25	0.15
Monocytes	−0.02	0.37	−0.05	0.07	−0.06	0.14

*$P < 0.05$; **$P < 0.01$.
Data represent ca. 1941 pigs from three high health status and three lower health status farms.

Table 7b *Genetic (r_g), phenotypic (r_p) and environmental (r_e) correlations between proportions of peripheral blood mononuclear leucocyte subsets measured at start-test and daily gain (g/day)*

Immune trait	r_g	s.e.	r_p	s.e.	r_e	s.e.
$\gamma\delta^+$ T cells	0.32	0.23	0.12	0.07	−0.09	0.21
$CD4^+$ cells	−0.22	0.29	−0.03	0.07	0.09	0.17
$CD8\alpha^+$ cells	−0.62**	0.20	−0.12	0.07	0.38	0.20
$CD11R1^+$ cells	−0.29	0.23	−0.14*	0.07	0.33	0.27
$CD11R1^+$ $CD8\alpha^+$ cells	−0.50**	0.20	−0.04	0.07	−0.01	0.17
$CD11R1^+$ $CD8\alpha^-$ cells	−0.08	0.30	−0.12	0.07	0.06	0.21
B cells	−0.27	0.21	−0.05	0.07	0.22	0.21
Monocytes	0.01	0.24	−0.04	0.07	−0.09	0.19

*$P < 0.05$; **$P < 0.01$.
Data represent 1941 pigs from three high health status and three lower health status farms.

they are accompanied by large standard errors. However, there were strong negative genetic correlations between numbers of $CD8\alpha^+$ cells and daily gain.

Genetic, phenotypic and environmental correlations between daily gain and proportions of PBML subsets measured at start-test are shown in Table 7b. With the exception of the correlations with $CD8\alpha^+$ cells and $CD11R1^+$ $CD8\alpha^+$ cells, these correlations are weaker than those seen at end-test. Once again, however, significant correlations with daily gain were invariably negative.

Notably, the two PBML subsets for which low genetic correlations across time were seen, viz. $CD8\alpha^+$ cells and monocytes (Table 5), were those for which correlations with daily gain differed markedly with time. $CD8\alpha^+$ cells were only correlated with daily gain at start-test, whereas monocytes were correlated with daily gain at end-test.

Discussion

This paper has presented several results that are of potential importance for breeding pigs with improved performance or health. Firstly, all the immune traits investigated, with the possible exception of $CD8\alpha^+$ cells, were moderately to highly heritable. Secondly, for the PBML subsets tested, changes in farm health status did not have a consistent effect upon heritabilities, although the conclusion must be tempered by the large standard errors and the confounding of farm and genotype. Thirdly, for specific PBML subsets, particularly $CD11R1^+$ cells (which have NK cell characteristics (Haverson *et al.*, 2001)) and especially the $CD11R1^+$ $CD8\alpha^+$ subpopulation, there were strong negative genetic correlations with performance, accompanied by phenotypic correlations in the same direction.

These results, particularly the genetic correlations with growth rate, provide optimism that immune traits may be used to breed pigs for enhanced performance and health, under circumstances where performance is used as a proxy for health. Moderate to high heritabilities towards a wide range of immune measures have previously been reported in pigs (Mallard *et al.*, 1989 and 1998; Joling *et al.*, 1993; Edfors-Lilja *et al.*, 1994 and 1998; Henryon *et al.*, 2006a). However, showing relationships between immune traits and disease resistance has been more difficult. This is partly due to the fact that although genetic variation in disease resistance under intensive production conditions has been demonstrated (e.g. Lyons *et al.*, 1991; Henryon *et al.*, 2001; Snowder *et al.*, 2006), heritabilities under such conditions are often relatively low, particularly when the 'disease' represents a general category of infections or syndromes rather than a specific identifiable disease. In a comprehensive study, Henryon *et al.* (2006b) were unable to demonstrate consistent genetic correlations between various

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immune parameters and the incidence of a range of infectious diseases within a herd of Duroc and Landrace pigs. However, it is notable that the only overlap in immune traits between our study and that of Henryon *et al.* (2006b) was WBC count, and we did not find any relationships between WBC and performance. It was partly due to the difficulties of recording and demonstrating genetic variation in the incidence of sporadic disease under intensive conditions that we chose performance as a proxy for health.

The choice of immune traits in studies such as this is potentially contentious, and different studies have invariably focussed on different measures. Other workers (Eurell *et al.*, 1992; Grellner *et al.*, 2002; Galina-Pantoja *et al.*, 2006) have also reported negative phenotypic relationships between immune traits such as PBML subsets, acute phase proteins (APP) *v.* weight gain. However, an alternative approach by Mallard *et al.* (1998) used an immune index comprised of both antibody- and cell-mediated immune responses as a tool for selecting pigs for increased weight gain. In order to find markers that reflect resistance towards a wide range of infectious diseases, we chose the immune traits used in this study because they are repeatable, easy to measure compared to other labour-intensive assays for cell function and relatively stable across time (Clapperton *et al.*, 2005a). Also, since levels of these traits were shown to phenotypically negatively correlate with performance (Clapperton *et al.*, 2005a) and to be influenced by pig genotype (Clapperton *et al.*, 2006), this suggests that these markers may both be heritable and genetically correlate with animal health status. The focus on traits that are largely representative of the innate immune system was also deliberate, as this is more likely to confer appropriate responses to a wide variety of diseases than measures of acquired or adaptive immunity. However, whether our measures are indicative of baseline immune levels as argued by Henryon *et al.* (2006b) is debatable, particularly as the infection status of our pigs at the time of measurement is unknown.

This is the first report to measure the heritability of individual PBML subsets in pigs. Our results correspond with similar studies performed for human PBML subsets, which reported moderate to high heritabilities for B cells, NK cells, $CD4^+$ T cells and $CD8\alpha^+$ T cells (h^2 ranging from 0.46 to 0.7). For other species, e.g. mice, chicken and rats, there is also substantial evidence that PBML subset levels are under genetic control (Roberts *et al.*, 1997; Wonigeit *et al.*, 1998; Duarte and Penta-Goncalves, 2001; Chen and Harrison, 2002; de Haan *et al.*, 2002; Morrison *et al.*, 2002; Myrick *et al.*, 2002). Given the consistency of significant heritabilities for various PBML subsets across species, it would seem likely that such genetic variation would have biological consequences to the host, most likely in the balance between effectively responding to infectious agents and inducing serious pathological sequelae. Understanding the genetic factors underlying variation in PBML subsets could lead to new methods to both enhance disease resistance and reduce susceptibility to autoimmune or other undesirable reactions.

Whilst moderate to high heritabilities have been reported for human $CD8\alpha^+$ cells ($h^2 = 0.5$ to 0.7) (Hall *et al.*, 2000, 2002; Ahmadi *et al.*, 2001), the heritability for porcine $CD8\alpha^+$ cells in our study was variable, being high at start-test ($h^2 = 0.6$) but relatively low at end-test ($h^2 = 0.18$). One possible explanation for this disparity in the end-test result is that in humans $CD8\alpha^+$ cells mainly represent one major cell type, classical $CD8\alpha^+$ T cells, whilst in pigs $CD8\alpha^+$ cells comprise various T and non-T cell types, $CD8\alpha^+$ NK cells, $CD4^+$ $CD8\alpha^+$ cells and $CD8\alpha^+$ $\gamma\delta^+$ T cells (Yang and Parkhouse, 1996). Some of these cell types, e.g. $CD4^+$ $CD8\alpha^+$ cells, are only released in response to infection and therefore levels of these cell types may depend more on type of exposure to environmental pathogens than on genetic factors (Ober *et al.*, 1998; Zuckermann, 1999). Nonetheless, genetic variation in levels of double-positive T cells has been reported in both monkeys (Lee *et al.*, 2003) and chickens (Luhtala *et al.*, 1997).

For WBC, the heritability was 0.18 and 0.24 at start- and end-test, respectively. Whilst these values are lower than those reported by Edfors-Lilja *et al.* (1994) ($h^2 = 0.44$), they are similar to the estimate quoted by Henryon *et al.* (2006a) ($h^2 = 0.25$). The genetic correlation between start- and end-test was high, indicating that either measurement time would suffice if selection were to be performance on WBC, despite the low phenotypic correlations between these time points. In general, for the PBML subsets, genetic correlations between start- and end-test were high, with the exception of $CD8\alpha^+$ cells and monocytes. This low genetic correlation between time points is also manifested in inconsistent relationships between these subsets and growth rate, across time. $CD8\alpha^+$ cells showed significant genetic relationships at start-test, and monocytes at end-test.

For immune traits to be valuable as predictors of performance under various infectious challenges, understanding their behaviour in different health environments is also required. Our definition of health status is based on the presence of major swine diseases that consistently affect both animal welfare and performance (Poppe *et al.*, 1998; Pallarés *et al.*, 2000; Regula *et al.*, 2000; Segalés *et al.*, 2004), although the use of APP as potential indicators of health status is currently under investigation (Parra *et al.*, 2006; Tecles *et al.*, 2007). In general, the traits we measured were consistent between high and lower health status farms, with two notable exceptions, levels of WBC and $CD8\alpha^+$ cells. Under high health status conditions, these two traits were not heritable, but under lower health status there was a significant genetic component to the observed between-animal variation. Although we could not examine the infectious disease challenges between the high and lower health status farms, it seems likely that the pigs from the latter farms were subject to more sub-clinical infections than those from the high health status farms. If so, this would suggest that the genetic component to the levels of WBC and $CD8\alpha^+$ cells may more reflect the host's ability to mount a response rather than simply reflecting a baseline level.

Correlations between immune and performance traits are potentially valuable from a breeding perspective. Although

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more cell types were significantly correlated with performance at the phenotypic level, the genetic correlations were generally stronger than the phenotypic correlations, and significant correlations were mainly observed at the end-test. Additionally, in the main, similar patterns were seen for both numbers and proportions of the PBML subsets. Some patterns do stand out. Firstly, large or significant correlations between cell type and performance were almost always negative. Secondly, only some of the traits showed significant correlations with performance even though most of them were heritable and correlated across time. In particular, WBC, $\gamma\delta$ T cells and CD4 cells were notable in that none of them had any significant genetic, phenotypic or environmental relationship with performance. In contrast, $CD11R1^+$ cells were the subset most consistently associated with performance at both the genetic and phenotypic levels, the latter confirming our earlier results with fewer pigs (Clapperton *et al*., 2005a). Galina-Pantoja *et al*. (2006) also corroborated our findings as they also found that NK cell levels were negatively phenotypically related to future daily gain, using immune measures at birth, weaning and at 9 weeks age. These workers enumerated the NK cells by measuring the proportion of $CD2^+CD16^+$ subsets. Along with NK cells, PBML that expressed CD16 (monocyte and NK cell marker) and $CD8\alpha$ were also negatively correlated with future weight gain but only at certain time points. However, NK cells were consistently negatively correlated with future weight gain at each time point. All pigs used in the study of Galina-Pantoja *et al*. (2006) were derived from a single lower health status farm; similar outcomes were seen in their study and ours, despite differences in both health status and timing of the immune measurements between the two studies. Importantly, the heritability of levels of $CD11R1^+$ cells was consistent between start- and end-tests as well as between different health status farms. It should be emphasised that this trait can be measured without the need for a deliberate challenge and thus could represent an animal's potential to resist disease. Taken together, these characteristics suggest that this trait in particular would be a strong candidate for selective breeding of pigs for enhanced performance under commercial conditions.

$CD11R1^+$ mononuclear cells in pigs express markers and function consistent with NK activity (Haverson *et al*., 2001; Denyer *et al*., 2006). In addition, we have described a novel $CD11R1^+$ population that does not express $CD8\alpha$, which is larger and more granular than the $CD11R1^+$ cells positive for $CD8\alpha$ (Clapperton *et al*., 2005a). Although at the end-test both populations are negatively associated with performance, strikingly only the $CD8\alpha^+$ population is correlated genetically at the start-test, suggesting that these two populations may also differ functionally.

Although our results suggest that selecting animals for lower proportions or numbers of $CD11R1^+$ cells would lead to higher-performing progeny, it would be advisable to proceed with such selection with some care, as other potential correlated responses are unknown. Health monitoring would be advisable, as there is a risk that using an immune trait as a selection marker for improved performance could lead to adverse correlated changes in other immune responses, which may be detrimental to the host. For example, there was an increased incidence of arthritis in response to *Mycoplasma hyorhinis* infection in Yorkshire pigs selected for both 'high' immune response accompanied by increased weight gain compared to contemporaneous pigs selected for both 'low' immune response accompanied by decreased weight gain (Jayagopala Reddy *et al*., 2000). In another study, selecting mice for a high antibody response was associated with either an enhanced or reduced ability to respond to an infection depending on the type of pathogen involved (Gheorgiou *et al*., 1985; Pedrini *et al*., 2005). Selection responses should be monitored to ensure that such effects do not arise.

NK cells are part of the innate immune system and function as both immune surveillance monitors and as effector cells against invading pathogens and developing tumours (Lodoen and Lanier, 2006). NK cells are activated by engaging highly polymorphic sets of receptors and carry out their effector functions by producing cytokines, particularly interferon-γ, and by lysing cells infected with pathogens. NK cells can also help to regulate the adaptive immune responses by producing interferon gamma, which can promote the maturation of dendritic cells and generate a T helper type 1 (Th1) response. There is also evidence that NK cells can kill dendritic cells that fail to become fully activated (O'Connor *et al*., 2005). The strong negative association of NK cell levels with performance might indicate that they are the major players in the initial stages of response to a broad range of pathogens, and the genes underlying variation in their numbers in peripheral blood are strong candidates for selecting animals with highly efficient generalised immunity. The relationship between NK cells and performance could be causal, with higher NK cell numbers leading to the production of more cachexic cytokines, for example. Alternatively, the presence of elevated NK cell levels may indicate a greater propensity to sub-clinical infections, which would lead to reduced performance. If so, other factors may lead to animals differing in their susceptibility or response to infection, and the NK cell levels are diagnosing the outcomes. The latter hypothesis would explain why the NK cell levels are particularly strongly associated with performance at end-test compared to start-test. As previously discussed, all pigs in this study were apparently healthy, but it is certainly possible that sub-clinical infections, which were, by their nature, undetectable, could account for this relationship. An alternative way of diagnosing infection status is to measure APP that are released in response to infection, and elevated APP levels have been associated with both clinical infections and, in apparently healthy pigs, sub-clinical infections (Petersen *et al*., 2002). We have previously demonstrated negative phenotypic correlations between APP and performance (Clapperton *et al*., 2005b). Therefore, we are currently generating data to investigate genetic relationships

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of APP levels with performance, in an attempt to further address this issue.

To conclude, we have shown that various PBML subsets ($CD4^+$ cells, $CD11R1^+$ cells, $\gamma\delta^+$ T cells, B cells, monocytes) are moderately to highly heritable. In addition, several of the PBML subsets are negatively genetically correlated with performance. These results potentially pave the way to use these immune traits to assist in the selection of pigs that are both healthier and more productive. Identifying the genes and mechanisms underlying our results is the subject of further research.

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