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Genetic Aspects of Biological Processes Underlying the Defense System in the Neonate

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Preparation for postnatal life of a neonate is determined by (1) adequate pre-natal development (2) development of innate immunity and (3) transfer of maternal antibodies. In this contribution the trait "piglet survival" will be explored using quantitative methods, searching for genetic variation and for biological explanations for possible genetic differences in piglet survival. Whereas for the chicken " innate immune system defined as natural antibodies (NAbs)" will be explored using a genomic approach searching for Quantitative Trait Loci (QTL) in an F2 cross of chicken lines selected for high and low antibody responses to SRBC (Sheep Red Blood Cells). By revealing variation which underlie biological processes of neonates it is shown that the defense system of the neonate is under genetic control. Genes of the neonate influence its innate defense system, taking care of general defense and of adaptation possibilities to novel antigens. The genes of the dam help, through maternal antibodies, to survive through the early days of life. We find that the innate defense system can be improved through direct selection against mortality and that the system of natural antibodies might have single gene variation, worthy of further investigation.

Key words: chicken, piglet, natural antibodies, mortality, Quantitative Trait Loci, quantitative approach.

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1. The story of life

The neonatal defense system starts its development already during prenatal life. Preparation for postnatal life of a neonate is determined by (1) adequate pre-natal development (2) development of innate immunity and (3) transfer of maternal antibodies.

Undoubtedly a genetic basis of the defense system exists. Two possible approaches may be taken for its understanding: (1) exploration of the process from a firm understanding of the biology and physiology of the animal (basics/phenotype) or (2) working the way back from apparently fit (in Darwinian terms) animals towards the basics of the physiology.

In this paper the current knowledge on quantitative and genomic approaches are explored in order to understand the start of the defense system using examples in two species: *Gallus gallus* (basics/phenotype) and *Sus scrofa* (phenotype/basics).

1.1 *Gallus gallus* – complexity of the defense system

After birth animals have to face the outside world. The need of a strong and properly developed defense system is extensive.

From hatching day onwards, individuals activate the innate immune system. Innate immunity is represented by natural barriers such as skin, physiological factors such as pH, temperature, oxygen tension, lysozyme-, and phagocytic- cells. Innate immunity also involves cells such as natural killer cells, mast cells, dendritic cells and phagocytes. Innate immunity itself usually terminates infections before the onset of disease. Maternal antibody transmission is defined as the transfer of antibodies by an immuno-competent adult to an immunologically naive neonate through the placenta or by the colostrum or yolk. Maternally derived antibodies provide the humoral immune defense for offspring early in their life. In the chicken, maternal antibodies are transferred into the egg and subsequently transported into the de-

veloping embryo. Maternally transmitted IgG is catabolised by offspring for 14 days after hatching. IgG is the primary immunoglobulin isotype of the egg yolk, while IgM and IgA are mainly found in the albumen. However, considerable amounts of IgM and IgA of unknown origin are found one day prior to hatching in the yolk sac. These antibodies are not synthesized de novo by the embryo proper, thus pointing to a transfer from the albumen into the egg yolk during development (KASPERS *et al*. 1996).

An important part of the innate immune system is formed by natural antibodies (NAbs). They are present in non-immunised individuals. NAbs have low binding affinity and a broad specificity repertoire. In mammals, NAbs are mainly of the IgM isotype, however, IgA and IgG have also been reported. It is very likely that NAbs are present in chickens.

1.2 *Sus scrofa*: peri-natal survival

In Sus scrofa, the domestic pig, embryonic development results in an elongation phase around day 12. After elongation placental attachment takes place. Size of individual placenta is directly related to later birth weight (VAN RENS *et al*. 2005), making uniformity in placental development important for uniformity in birth weight at parturition.

In the domestic pig the transition from intra- to extra uterine life is crucial; between 5 and 10% of piglets die during parturition and between 10 and 15% die during the suckling phase. At the end of the expulsion phase oxygen intake (breathing) has to start, until that moment the umbilical cord provides oxygen. The umbilical cord has to have sufficient length and strength and at the same time be weak enough to break when the neonate is born and works its way to the udder to collect colostrum, the source of maternal antibodies.

Concerning the immune system, pre- and neonatal vertebrates have limited ability to synthesize antibodies (GRINSTAFF *et al*. 2003). Therefore maternal antibodies provide a primary form of humoral – immune defense for the early weeks of life. In Sus scrofa passive immunity, comprised of maternally transferred antibodies, lasts for about 4-8 weeks. It takes an individual about 6 wks to develop its own active immunity.

Uniformity in development is important to have (1) a similar stage of maturity at parturition, (2) adequate body size and body reserves to start extra-uterine life and at the same time small enough not to obstruct the cervical passage and (3) equal opportunity of suckling after parturition Postnatal survival has equally to do with vitality of the neonate and mothering ability of the adult animal (LE DIVIDICH 1999).

2. Genetic approaches : quantitative vs. QTL approach

A genetic understanding of the biological process could start at an end result (*Sus scrofa*) or start from knowledge of physiology (*Gallus gallus*). Geneticists nowadays have two powerful tools available to investigate the genome; one is the quantitative and the other the genomic toolkit. In this contribution the trait "piglet survival" will be explored using quantitative methods, searching for genetic variation and for biological explanations for possible genetic differences in piglet survival. Whereas for the chicken "innate immune system defined as natural antibodies (NAbs)" will be explored using a genomic approach searching for Quantitative Trait Loci (QTL) in an F2 cross of chicken lines selected for high and low antibody responses to SRBC (Sheep Red Blood Cells).

2.1 Quantitative approach

The model underlying the quantitative approach is the infinitesimal model; variation in a biological trait, e.g. number of ovulations, is caused by an infinite number of genes. This assumption allows the use of theory developed for normal distributions, among others the estimation of components of variance. The trait 'peri-natal survival of piglets' was analyzed in order to estimate the magnitude of the genetic control and to assess its genetic correlations with biologically associated traits like body reserves, birth weight and litter size. The quantitative approach estimates similarities between groups of animals and compares this similarity with the average similarity of random animals in the dataset. For perinatal survival, similarities can be found between full and half sibs (additive or direct heritability), between animals who shared the same uterus (common environment, partially genetic, that is maternal effect) and between animals who shared the same nurse sow (mothering ability). Based on this genetic analysis, two groups of 25 sows each were selected with very low and very high estimated breeding values (EBV) for survival, respectively, mated them to low and high EBV males and performed Caesarian sections on them 1 day prior to expected parturition. Piglets were euthanised, weighed and dissected, and their placentas weighed.

2.2 QTL or single gene approach

Quantitative traits are phenotypes that exhibit quantitative variation. Therefore, studying quantitative traits depends on measuring rather than counting. Genes or loci with an effect on a quantitative trait are called QTL. One or more QTL may influence a trait. In principle, QTL mapping is based on

strains within species which differ in: 1) alleles affecting the trait of interest and 2) polymorphic molecular DNA markers. Such strains are reproduced to create a F1 cross and followed up by either backcross or an F2 generation, which can be used as a mapping population. QTL mapping involves analysing the genome with one DNA marker at a time. All individuals are divided into marker genotype classes and a statistical test is performed to determine if there is a significant difference in phenotype between the marker genotype classes. In case of such a difference, one assumes that the QTL is linked to the DNA marker. In this contribution a case study of the F2 generation of chicken will be used, assume differences in alleles affecting traits of interest in founder lines (high vs low line), and explore a QTL study for a non specific immune responses (NAbs) (SIWEK 2005).

Results

3. Natural antibodies in the chicken (Nabs)

3.1 Choice for Nabs

Natural antibodies were chosen because they are probably involved in early recognition and clearance of foreign material. They enhance processes of antigen uptake and antigen presentation via dendrites or B cells. Specific immunity and protection may also be enhanced by NAb and idiotype – anti-idiotype networks. In other words, NAbs are the key factors during very early development which influence future immune system function.

3.2 Genetic differences in Nabs

Nabs were defined as an antibody response to LPS and LTA. Lipoteichoic acid (LTA) and lipopolysaccharide (LPS) act as homotopes. Homotopes are microbial stimulators of the innate immune system. LTA is shared by gram positive bacteria, whereas LPS is shared by gram negative bacteria. Specific cellular and humoral immune responses depend on the previous activation of the innate immune system. The crucial role of homotopes is polarization of the specific immune system.

The estimated heritabilities varied from 0.03 (LTA at 5 wks of age) to 0.17 (LPS at 5 wks of age), which indicates that genetic factors might be involved in the traits. QTL were detected for LTA and LPS at both ages. Detected QTL were mapped on different chromosomes: GGA3 at 66cM (LTA 5 wks of age), GGA3 at 106 cM (LPS 18 wks of age), GGA8 at 38 cM (LPS 5 wks of age), GGA10 at 56 cM (LTA 18 wks of age) (SIWEK 2005).

3.3 Survival in young pigs

3.3.1 Genetic background

The genetic analysis of piglet mortality, during and direct after parturition, revealed significant genetic differences between and within different commercial lines of pigs (KNOL *et al*. 2002a, 200b) to a magnitude that within a few generations survival of piglets could be markedly increased (KNOL 2001). This result was consistent with that of others (GRANDINSON *et al*. 2003; HERMESCH *et al*. 2002). The genetic correlation of survival with birth weight was almost zero, in contrast to the expected high and positive correlation. Genetic correlations with especially appetite and the accretion of body fat were clearly positive, indicating that the current practice of selection in the domestic pig towards decreased fatness might be detrimental to peri-natal survival.

3.3.2 Underlying biological processes

In the high/low sampling experiment, the high group showed on average slightly lower birth weights, slightly higher uniformity and slightly higher efficiency of the individual placentas, all significant at $P<0.05$. No differences were found in oxygen concentration in the blood, nor in haematocrite levels. Piglet dissection resulted in heavier small intestines, heavier stomachs, heavier livers and higher glycogen levels in the body and especially in the liver of the piglets of the high EBV group.

The most striking difference by far was the cortisol level. The high EBV animals exceeded average cortisol levels of the low EBV animals by a factor of 50% ($P<0.0001$).

Discussion

4. Adequate pre-natal development

For the domestic pig, a polytocous species, the placental development is important for later perinatal survival. This placental development influences uniformity and with that it influences the onset of parturition and the uniformity of the litter at birth. The analogy with the chicken is difficult, since a chicken could be seen as monotocous species in the sense that individuals of the same female do not influence one another. Or the chicken could be seen as a polytocous species, because time of hatching is highly uniform and neonates are raised together in a natural environment.

The result from the high low sampling showed that clear and consistent differences between genetically high and low EBV animals exist in a number of traits important for early survival. The key factor appears to be cortisol. Cortisol helps the in maturation of lungs and synthesis of glycogen (LEENHOUVERS 2001). Quick uptake of oxygen after parturition and rapid access to body energy reserves as glycogen are vital for peri-natal survival. The pre-natal role of cortisol in preparation for a life transition after hatching is unknown as far as the authors are aware.

4.1 Consequences of innate immune responses

It has been proven that maternal antibody transmission is partially genetically based (GRINDSTAFF *et al*. 2003). In this paper we move one step further to innate immune response. Initially, the immune response towards any antigen is in the hands of the innate immune system. This is a subject of intensive research in man and mouse after being discarded for a couple of decades. Several complementary mechanisms are at play in innate immune responses, although the finesses (rules) are not fully understood at present. Nevertheless, it is clear that several cell types, e.g. phagocytes or dendritic cells and natural killer cells, but also acute phase proteins, complement, and the recently acknowledged natural antibodies play a role in the innate immune response. Upon exposure to pathogens, one of several paths can follow: infection might lead to death or chronic disease, might be terminated by innate immunity itself or might be taken over by adaptive immunity. The role of innate immunity is emphatic. Invertebrates survive infections with an innate immune system only. Natural Antibodies (Nabs) are probably the first reactive molecules in the whole cascade of immune responses to encounter foreign antigens. The low genetic correlations between the Nabs level to LTA and LPS indicate that the genetic regulation of innate humoral immune responses is different at different ages (5wks and 18 wks) and different for both investigated homotopes (LTA and LPS). This is supported by the lack of QTL that influence both traits. The next step in this approach, after detecting the QTL regions is narrowing down the genomic region of interest (QTL fine-mapping) and looking for functional and positional candidate genes mapped in this area.

4.2 Survival and (maternally transferred) immune response

Any genetically based variation among mothers in the developmental environment which they provide for offspring in terms of transmission of antibodies is described as indirect or maternal genetic effect. Differential survival of offspring as a result of the maternal antibodies received will alter the distribution of maternal antibody transmission in the offspring generation. The concentration and diversity of the IgG antibody population transferred are correlated between mother and offspring (HELLER *et al*. 1990; LEITNER *et al*. 1990). In chickens exposed to Escherichia coli or infectious bursal disease, post hatching survival is positively correlated with the hen's titer (HELLER *et al*. 1990; GODDARD *et al*. 1994). In dairy cattle mortality is much lower in calves with high level of maternally transmitted antibodies. Maternal antibodies guide the diversity of immune repertoire in offspring, as well as improve strength of offspring immune responses. In chickens derived from bursectomized mothers, a lack of maternal IgG causes lower frequency of MHC class II cells in the spleen (YASUDA *et al*. 1998). An absence of maternal antibodies depress the immune responsiveness of offspring which may lower their survival.

4.3 Beyond quantitative approaches

The holy grail of many types of genetic or genomic studies seems to be to find the single gene which explains the majority of the variation in a specific trait of interest. WEST *et al*. (2004), found a chicken yolk sac IgY receptor. IgY receptor is a functional equivalent of mammalian MHC related Fc receptor. Maternal IgY is an avian counterpart of mammalian IgG. In mammals, IgG is transferred from mother to offspring by MHC related receptor FcRn which binds IgG in acidic endosmoses and releases it at basic pH into blood. MHC, which stands for Major Histocompatibility Complex, is the key player in immune responses. Histocompatibility molecules are glycoproteins expressed at the surface of almost all vertebrate cells. Their role is to display antigens in such a way that they can be recognized by T lymphocytes. The importance of MHC molecules is emphatic. The *Sus scrofa* case study pointed towards cortisol as a candidate gene which play an important role in early survival. The *Gallus gallus* study pointed towards genetic regions where candidate genes might be located, without knowing their function.

To be able to verify the gene function in the biological process, an expression study should be performed. Genomics combines sequence polymorphism with variation in expression level. Therefore it is important to establish how robust RNA measurement is against sequence variation, e.g. Single Nucleotide Polymorphism in the transcript. (DE KONING 2005). To be able to understand the function of SLA and the surrounding region an ex-

pression array was approach has been developed. (GAILLARD *et al*. 2005). The region of swine major histocompatibility complex (SLA) seems to be involved with many physiological traits including immune responsiveness to a variety of parasites and male and female reproduction performances, which makes this region an excellent candidate for Marker Assisted Selection (MAS).

Conclusion

The defense system of the neonate is under genetic control. Genes of the neonate influence its innate defense system, taking care of general defense and of adaptation possibilities to novel antigens. The genes of the female help, through maternal antibodies, survive through the early days of life. The innate defense system can be improved through direct selection against mortality and the system of natural antibodies might have single gene variation, worthy of further investigation.

Relatively novel research tools such as gene expression areas might speed up this process.

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