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Genetic aspects of auto-immune profiles of healthy chickens

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ABSTRACT

Auto-antibody profiles binding liver antigens differed between chicken lines divergently selected for specific antibody responses to SRBC, and were affected by ageing suggesting both genetic and environmental effects. Presence and levels of IgM and IgG antibodies binding chicken liver cell lysate (CLL) fragments in plasma at 5 weeks of age from 10 individual full sibs and their parents from 5 H_{srbc} and 5 L_{srbc} line families was studied to reveal genetic relations. Non-genetic maternal effects were studied by comparing auto-antibody profiles of 36 weeks old hens from 2 other unrelated lines with the profiles from their chicks at hatch.

IgM and IgG antibodies from parents and progeny from both H_{srbc} and L_{srbc} lines bound CLL fragments. Significant line and generation differences and their interactions were found for both isotypes. Higher staining of CLL fragments was usually found for H_{srbc} line birds. Lines were clustered by auto-antibody profiles, but staining by birds of both lines in both generations was very individual for IgG and IgM. The current data with full sibs therefore not supported a genetic basis for auto-antibody profiles. IgG but not IgM auto-antibody profiles of chicks correlated with maternal auto-antibody profiles.

The results suggest that the auto-antibody repertoire of healthy chickens is largely stochastically initiated and may be affected by environmental challenges during ageing, but genetic mechanisms may underlie staining intensity of individual bound CLL fragments. The present results suggest that identification of fragments or profiles to be used at early age for genetic selection for health traits is not feasible yet. Secondly, the IgM profile of neonatal chickens seems non-organised independent of the maternal profile, but the neonatal IgG profile is much more related with the maternal profile. Consequences of these findings for disease susceptibility or breeding for optimal health are discussed.

1. Introduction

Natural antibodies (NAb) are defined as antibodies present in normal healthy animals under the absence of (previous or concurrent) deliberate antigenic stimulation or infection (Avrameas, 1991; Baumgarth et al., 2005). In many mammals (Avrameas, 1991; Lutz et al., 2009; Nagele et al., 2013) and chickens (Parmentier et al., 2014) NAb were found to bind self-antigens. These so-called natural auto-antibodies (NAAb) may be involved in maintenance of homeostasis (Ochsenbein et al., 1999; Lutz et al., 2009) by inactivation of cytokines, masking auto-antigens, and clearance of obsolete or damaged cells and metabolic waste (neo-epitopes) as part of anti-tumour surveillance and prevention of inflammation. NAAb are always present in the body, but levels may increase with age. In man and mice, quantitative (Western) immunoblotting (Lacroix-Desmazes et al., 1995, 1999; Mouthon et al., 1996; Stahl et al., 2000), or proteomics (Nagele et al., 2013; Madi et al., 2009) were used to analyse the NAAb repertoire of individuals and inbred strains to various tissues like liver, kidney, brain and muscle, or auto-antigen chips. In general, the binding repertoire and levels of IgM binding auto-antigens increased during aging and remained stable later on, resulting in corresponding repertoires between individuals (Stahl et al., 2000). IgM patterns appeared to evolve without exogenous stimulation (Haury et al., 1997), suggesting that they are not formed randomly (Hooijkaas et al., 1984). Auto-IgG patterns contained the same bands as IgM with the addition of more specific bands. Since auto-IgG patterns

Abbreviations: CLL, chicken-liver-cell-lysate; NAAb, natural (auto-) antibodies.

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were not expanding with age and remained stable at a young age (Stahl et al., 2000) it was suggested that auto-IgG profiles could represent an 'antibody fingerprinting' of an individual (Francoeur, 1988). Different auto-antibody binding profiles in different murine inbred strains (Nobrega et al., 1993) indicated a genetic component underlying the NAAb repertoire. However, Francoeur (1988) reported after disappearance of maternal antibodies a large individual variability of NAAb in healthy man, independent of genetic background and environment suggesting a stochastic origin of NAAb. In addition, it was proposed that IgG autoantibodies function as an adaptive mechanism to clear cellular debris (Nagele et al., 2013).

IgM and IgG auto-reactivity to chicken liver lysate (CLL) were different in plasma samples obtained from chicken lines that were divergently selected for specific agglutination responses to sheep red blood cells (SRBC) suggesting a genetic component underlying the auto-antibody repertoire in poultry as well (De Jong et al., 2013). Also at 52 weeks of age different auto-reactivity to CLL was found than at 5 weeks of age in the same chickens (Parmentier et al., 2014). Using ELISA, heritabilities and significant non-genetic maternal effects were found for IgM autoantibodies in chickens, but no or very low for IgG (Bao et al., 2016). The purpose of the present study was threefold, first we evaluated staining of corresponding fragments of progeny with their parents (a generation effect after disappearance of maternal antibodies), and between full sibs to identify genetic relations, using plasma prior to immunization of chickens from two lines that were divergently selected for high (H_{srbc} line) or low (L_{srbc} line) specific antibody agglutination titres to SRBC at 5 days after subcutaneous immunization with SRBC at 5 weeks of age. Five families consisting of a cock and hen from the 30th generation and approximately 10 progeny (31st generation) from both lines were studied. Higher NAb and NAAb levels were found at all ages in the H_{srbc} line as compared to the non-selected control, and L_{srbc} lines (Parmentier et al., 2004). Second, line effects were tested per stained fragment in the SRBC selection lines. Third, we studied auto-antibody profiles of hens and their chicks at hatch to indicate non-genetic maternal effects, such as maternal antibody transfer, in two other selection lines. In man, neonatal auto-IgM profiles were found non-organized whereas the neonatal auto-IgG profile was very much alike the maternal auto-IgG profile (Madi et al., 2009). Merging of the auto-IgM and auto-IgG profiles in man during ageing was proposed as a marker for maturation of the immune system (Madi et al., 2011).

We correlated and grouped the staining patterns of IgM and IgG antibodies per line, generation and family using principal component analysis. Our results from the SRBC lines suggest first that both generations and lines can be characterized by auto-antibody profiles, and second that auto-immune profiles are individually restricted as was indicated for man previously (Francoeur, 1988). Comparison of the auto-reactivity of hens and their chicks at hatch suggested no relations between the neonatal and the maternal IgM profiles, whereas many correlations were found between the IgG profiles of hens and chicks. A stochastic origin of NAAb may hamper opportunities to relate auto-antibody profiles at a young age in healthy chickens with genotypes and phenotypic traits such as immune responses, disease resistance and (metabolic) disorders at a (later) given age. However, the individual IgM patterns at hatch and proposed merging of IgM and IgG profiles during ageing may provide tools to relate auto-responsiveness and health status.

2. Materials and methods

2.1. Plasma samples

Two experiments were conducted to measure genetic (Experiment 1) and non-genetic (maternal) effects (Experiment 2).

2.1.1. Experiment 1

Plasma was obtained at 5 weeks of age prior to immunization with SRBC, from 119 ISA-Brown medium heavy laying birds. The chicks were from two different lines, which were either divergently bred during 30–31 generations for high (H_{srbc} line) or low (L_{srbc} line) primary (agglutinating) antibody responses at day 5 after primary intramuscular immunization with SRBC at 37 days of age. From 5 H_{srbc} line and 5 L_{srbc} line cock-hen matings (families) from the 30th generation approximately 10 progeny, both hens and cocks of the 31st generation were sampled at 5 weeks of age one year later as well. The lines thus represented genotypic high (H_{srbc}), and genotypic low (L_{srbc}) hens and cocks from 2 generations in 10 families.

2.1.2. Experiment

Plasma was obtained at 36 weeks of age from 14 White Leghorn layer hens and 1–3 chicks per hen at hatch. Hens originated from lines divergently selected for high or low levels of natural antibodies (NAb) binding keyhole limpet haemocyanin (KLH) for 3 generations (Berghof et al., 2015). Eight hens were of the high NAb line: H_{klh}, 6 hens were of the low NAb line: L_{klh}.

2.2. Housing and management

For both experiments, chicks from both generations (Experiment 1) sampled at 5 weeks of age, or sampled at 22 d of age (Experiment 2), from one sex were kept at the moment of blood sampling in a density of 80 birds per 2×2 m^2 surface with wooden shaving. Chicks had free access to standard rearing diet 1 (152 g kg-1 crude protein, 2817 kcal kg-1 metabolizable energy) and water. All chicks from both experiments originated from solitary battery housed hens at 36 (Experiment 2) or 42 (Experiment 1) weeks of age, with normal access to standard layer diet (192 g kg-1, 2790 kcal kg-1). The hours of light per day were regulated with age, starting with 23 h of light per day in the first week, which reduced to 8 h in week 4 until week 15, and then increased again until 16 h from week 22 of age onwards. All chicks in both experiments were vaccinated for Marek's disease at hatch; infectious bronchitis at hatch (MA 5), days 70 (primer), and 112 (H52); infectious bursal disease at day 21, and New Castle Disease at days 10, 28, and 98 of age.

2.3. Reagents

One stored aliquot of earlier made chicken liver cell lysate (CLL) was used (Parmentier et al., 2014). CLL was made by freezing 0.5 g liver tissue from a five week old $Control_{srbc}$ line hen in liquid nitrogen. The liver was lysed with 5 ml lysis buffer (50 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.1% SDS, 2% Glycerol, pH 7.4) with addition of 2.5 ml protease inhibitor cocktail (Sigma-Aldrich, St. Louis, USA). The mixtures were centrifuged for 15 min (13,500 g) after which the supernatant containing CLL was kept in aliquots at −20 °C until use.

2.4. Natural auto-antibody profiles

Western blot analysis was used for the determination of natural (auto-) antibody binding profiles to CLL in plasma of 119 chickens obtained at 5 weeks of age, both parents and progeny (Experiment 1), and 14 hens at 36 weeks of age and 19 chicks of these hens at hatch (Experiment 2). First, CLL was diluted 1:30 based on preliminary optimization and incubated 1:1 v/v with sample buffer (1:20 β-mercaptoethanol and Laemmli sample buffer (BIORAD,