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Evaluation of the effects of dexamethasone-induced stress on levels of natural antibodies in immunized laying hens

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ABSTRACT

Natural antibodies (NAb) are an important humoral component of innate immunity, playing a pivotal role as first line of defence against pathogens even without prior antigen-specific activation or antigen-driven selection.

The levels of NAb in plasma of young laying hens were explored in more detail and identified 2,4,6-trinitrophenyl bovine serum albumin (TNP-BSA), as the non-self antigen showing the highest levels of IgϒY- and IgM-NAb. Subsequently, the relation between specific antibody (SpAb) levels and NAb levels, and the effect of dexamethasone (DEX)-induced stress on the acquired Ab response and on NAb levels were examined.

According to obtained results, the affinity of NAb and SpAb, measured using the thiocyanate elution method, resulted higher in SpAb than in NAb. After stress induction, IgM-NAb and SpAb levels showed a transient decrease, whereas the levels of IgϒY-NAb were not changed. Moreover, statistical analysis showed positive correlations between IgϒY- and IgM-NAb levels and between IgM-NAb and SpAb levels that are lost as stress has been induced, whereas no correlation was observed between IgϒY-NAb and SpAb levels, neither before nor after the DEX-administration. This indicates that IgM-NAb assessment could be a valid tool to estimate the potential of the acquired Ab response and that the dexamethasone-induced stress condition causes depression of IgM-NAb levels and the acquired Ab response, but it has no evaluable effects on IgϒY-NAb levels.

1. Introduction

Natural antibodies (NAb) are termed as antibodies (Ab) that circulate in normal individuals, able to bind to a particular antigen or pathogen even without prior antigen-specific activation or antigen-driven selection (Boes, 2000). This characteristic makes them of particularly interest in the immune status of non-immunized individuals, acting in the first line of defence against pathogens by directly neutralizing the pathogen and activating the complement system (Oschenbein and Zinkernagel, 2000). Thus, while it is customary to divide the immune system into non-specific and specific immune system NAb can be regarded as a direct linkage between the innate and the acquired immunity (Carroll and Prodeus, 1998), demonstrating that these are integrated systems. Even if NAb may be of IgM, IgG and IgA isotypes, IgM is the predominant one in human and mouse (Berland and Wortis, 2002; Dono et al., 2004). In mammals, NAb are mostly secreted by the long-lived, self-renewing B1 subset of B-cells, generated during foetal or neonatal development (Boes, 2000). As opposed to antigen-induced antibodies (SpAb), which are mono-reactive, most NAb are characterized by a broad specificity repertoire, including self and non-self phylogenetically conserved structures, such as carbohydrates, heat shock proteins, nucleic acids and phospholipids, with usually low binding affinity (Casali and Schettino, 1996; Boes, 2000).

NAb belong to two distinct types with different activities (Lutz et al., 2009). The first one is directed to non-self antigens, playing a role in infectious disease prevention. The second type, generally called cryptic or natural autoantibodies (N(A)Ab), is directed to self-antigens or to altered self-antigens, formed as a result of cell damage or inflammation, thereby performing homeostatic roles like regulation of cytokines and clearance of obsolete or damaged cells and metabolic waste, and in anti-tumour surveillance (Balsari and Caruso, 1997; Reid et al., 1997).

NAb have been found for a long time in several mammalian species (Karsenti et al., 1977; Guilbert et al., 1982), and also in fish (Gonzalez et al., 1988), in which NAb functions are considered more

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important than in higher vertebrates, because of the poor affinity maturation of SpAb responses and class switch incapability (Magor and Magor, 2001).

The presence of NAb reacting against self or non-self antigens has been previously shown in avian species as well (Neu et al., 1984; Barua and Yoshimura, 2001; Parmentier et al., 2004; Matson et al., 2005; Siwek et al., 2006; de Jong et al., 2013).

Although their function in avian species is not completely known, a relation between SpAb responsiveness and levels of NAb binding self or non-self antigens has been demonstrated in chickens (Parmentier et al., 2004; Siwek et al., 2006; de Jong et al., 2013). According to the authors, the comparative study of the NAb levels and the acquired immune response might provide innovative tools to breed chicken lines for health traits or to optimize hygiene and husbandry procedures.

Moreover, NAb levels increase during aging in chickens (Parmentier et al., 2004; Berghof et al., 2010), as also shown in dairy cows (Srinivasan et al., 1999), indicating that NAb may be the cumulative result of antigenic stimulation of the poly-specific receptors of B1 B-cells (Tomer and Shoenfeld, 1988). At the same time, Haghighi et al. (2006) showed a possible stimulation of NAb production in young chicks using a commercial probiotic administered orally on the day of hatch, as previously demonstrated in respect of the stimulation of the systemic Ab response (Haghighi et al., 2005). According to these authors, after two weeks treatment, both IgΥ- and IgM-Nab in serum and intestinal IgA-NAb levels increased in probiotic-treated birds, suggesting that the manipulation of the intestinal microflora by the use of probiotic positively interacts with the gut-associated lymphoid tissue (GALT) (Haghighi et al., 2006).

Recent papers have pointed out that levels of NAb binding the non-self antigen keyhole limpet hemocyanin (KLH) are heritable in poultry (Sun et al., 2013), and that the probability of hens to survive a laying period is positively related to the titres of NAb isotypes binding KLH (Sun et al., 2011). According to Berghof et al. (2015), selective breeding for NAb binding KLH can be done simultaneously on all NAb isotypes, in which the estimated heritability is between 0.07 (IgΥ) and 0.14 (IgM), thus depending on the different Ab isotype. Even if not only the genes of the mother but also a substantial non-genetic maternal environmental effect may influence the immune system of offspring, the selection for NAb may still be a promising strategy to enhance general disease resistance and health status of chicken (Berghof et al., 2015). Lastly, the selective breeding for enhanced NAb levels in chickens not only improve survival and disease resistance, but also may influence the performances of laying hens, suggesting genetic trade-offs between NAb and some production traits (van der Klein et al., 2015).

With the preceding as background, a closer look was made on the levels of NAb binding non-self antigens, with emphasis on the non-self antigen giving in the present study the highest IgΥ- and IgM-NAb levels in plasma of young laying hens. Subsequently, the relation between SpAb response and NAb levels, and the effects of dexamethasone-induced stress on the SpAb response and on NAb levels were examined. To induce a SpAb response, human-γ-globulins (HγG) were used as antigen, not only because this protein has a great immunogenic power (Cecchini and Saroglia, 2002), i.e. to induce a T-dependent Ab response, but also to avoid any possible interference with obligatory vaccinations of poultry.

2. Materials and methods

2.1. Experimental design, immunization and stress induction

The experiment was performed at a local farm and all the procedures were conducted in strict accordance with European legislation regarding the protection of animals used for scientific purposes (European Directive 2010/63), as recognized and adopted by the Italian law (DL 2014/26). No animals died during or as a consequence of the conducted experiment.

Thirty ISA Brown young hens of 16 weeks of age were maintained in floor pens in an environmentally controlled room (20 °C ± 1 °C), and tagged for identification. Animals were provided with free access to water and feed, using a commercial corn-soybean diet for layers. After 3 weeks of acclimatization, during which the light schedule was progressively modified at 15 h light and 9 h dark, hens were randomly divided into two groups of 15 hens pen^{-1} and submitted to the first blood sampling (day-1), that was repeated after 14, 28, 35, 42, 49 and 63 days from the beginning of the experiment. Blood samples were drawn from the brachial vein using a 2.5 ml syringe, and serum was collected after clotting by centrifugation (2500*g*, 15 min at 4 °C) and stored at −80 °C until analysed.

At day-28 and before blood sampling, all animals were immunized by intramuscular injection in the breast with 5 mg human-γ-globulins (HγG, Sigma-Aldrich, Italy) in 0.5 ml of saline solution. Further, after the immunization the specimens of the first group (stressed group) was submitted for 6 consecutively days to intramuscular injection of dexamethasone (DEX, Sigma-Aldrich, Italy), at the dosage of approximately 1.5 mg kg^{-1} body weight, as previously described (Huff et al., 1999), to induce a short/medium-term stress condition. A 200 mg ml^{-1} stock solution of DEX was previously prepared in absolute ethanol. The solution was suspended in sterile saline to provide an average volume of 0.5 ml for each animal. At the same time, hens of the second group (control group) received by injection 0.5 ml of saline solution for the 6 consecutively days. Fig. 1 summarizes the experimental protocol.

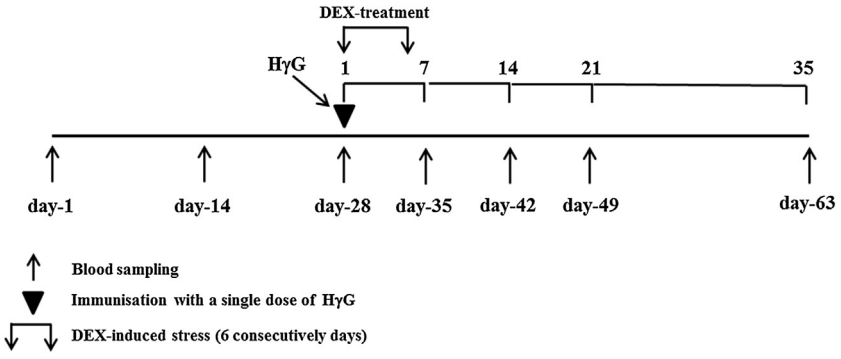

Fig. 1. Scheme of the experimental protocol.