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Variation in immunophenotype of lactating mice

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ABSTRACT

Immunological factors have been shown to play a crucial role in mammary remodelling in rodent models of lactation, particularly at the stage of mammary involution. However, the relationship between immunological factors and the ability of normal mammary gland to produce milk, as well as the genetic components contributing to lactation performance remain largely unknown. In this study, we assessed the lactation and immunological phenotypes of 11 inbred mouse strains, namely 129X1/SvJ (129), A/J, AKR, C3H/HeJ (C3H), CBA/CaH (CBA), C57BL/6J (C57), DBA/1J, DBA/2J, FVB/N (FVB), QSi5 and SJL/J (SJL) to identify potential links. Leukocyte analyses showed no direct link between the fraction of splenic leukocytes and lactation performance. However, significant strain differences were discovered in the fraction of CD8+ T lymphocytes ($P = 0.016$) and CD11b+Gr-1 mid-low monocytes ($P < 0.001$). Cytokine profiles in plasma were examined and a subset of plasma cytokines, namely CCL2, CCL3, CCL5, CSF2, CSF3, IL10, IL15, IL1B, IL4, IL5, IL7 and TNF, were fitted to a linear regression model for prediction of lactation performance (R-sq = 62%, S = 0.309). Significant strain differences in the plasma cytokine levels were also discovered amongst these inbred strains. Analysis of immunological phenotypes showed strong correlations between splenic immune cell subsets and their regulating cytokine levels in plasma. The results demonstrate the extent of genetic variability in the immunological phenotypes of lactating mice, and provide a basis for understanding the role of cytokines in milk production, and identifying potential biomarkers of lactation performance.

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1. Introduction

During mammogenesis and throughout the lactation cycle, the mammary gland undergoes a series of developmental changes that involve tissue remodelling, cell proliferation, apoptosis and differentiation. Interactions between the mammary gland and immunological factors have been described in great detail from an anatomical perspective over the past half century, however, investigation into the functional link between these seemingly dissimilar systems is a relatively new concept. It is now common practice to use microarray technology for the acquisition of high volumes of specific gene expression data and this has proved to be a highly effective tool for studying the behaviour of a number of genes simultaneously. This is especially useful for lactation studies where numerous complex biological processes modulate the mammary gland for lactogenesis.

The primary role of the mammary gland in mammals is to produce milk which supplies the nutritional

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needs of the juvenile in the form of milk proteins, sugars and lipids. Mammary gland secretions also provide the immunologically naive neonate with passive immunity from infection via consumption of immunoglobulin-rich colostrum produced at the onset of lactation (Reber et al., 2008a,b). Immunological factors have also been associated with the post-lactation remodelling of the mammary epithelium. For example, infiltration of leukocytes into the mammary gland occurs during the involution phase of the lactation cycle. Molecular characterisation of the factors that modulate the immune cascade during the involution phase has highlighted the importance of cytokines that activate signal transducers and activator of transcription 3 (STAT3) in the control of the inflammatory response during mammary tissue restructuring. STAT3 has been implicated in the regulation of apoptosis during the early phase of mammary gland involution by activating the transcription of pro-apoptotic genes. STAT3 may also activate anti-inflammatory genes during involution that suppress damaging inflammatory mediators, thereby preventing further tissue damage (Clarkson et al., 2004). Additionally, the key genes involved in the immune cascade have been described through an analysis of differential expression of genes during involution via the use of oligonucleotide microarrays. Gene expression profiles have identified a large number of candidate genes implicated in the activation of immunological activity, and have provided insight into the transcriptional changes that occur during involution in the mammary gland (Stein et al., 2004, 2007). Another study of expression of mammary transcriptome across lactation cycle also identified that regulation of cytokine biosynthesis and regulation of lymphocyte proliferation are amongst the most significant ontology terms of biological process (Lemay et al., 2007).

While a genomic approach to investigating the transcriptional activity of the mammary gland during the lactation cycle has had its immunological focus on the involution phase, other studies report immunological activity during mammogenesis. For example, leukocytes are not only involved in cell apoptosis during involution, but are also reportedly involved in mammary gland development (Gouon-Evans et al., 2000, 2002). The depletion of leukocytes has been shown to result in developmental failure of mammary gland terminal end buds (TEBs), since macrophages and eosinophils demonstrate crucial functions in TEB formation (Gouon-Evans et al., 2000). Although direct involvement of lymphocytes in mammogenesis has yet to be defined, the IL4/IL13/Stat6 signal pathway, which regulates the differentiation of naive CD4+ T helper (Th) cells into Th2 cells, has been reported to promote development of luminal mammary epithelial cells (Khaled et al., 2007).

Correlations between immunological factors and lactation performance have potential use as biomarkers of lactation performance, which may also be used across species. The mouse provides an ideal model organism for studying the genetic basis of reproductive traits as there are many inbred strains that possess phenotypic variation in these traits. The current study aimed to investigate the genetic component of lactation performance in order to build upon findings of previous studies. Correlations between immunological markers and lactation performance of 12 inbred strains of mice were investigated by using flow cytometric analysis of murine splenocytes for leukocyte subpopulations, and profiling of plasma cytokine levels.

2. Materials and methods

2.1. Animal housing and assessment of lactation performance

10 inbred strains of mice were sourced from the SPF certified Animal Resource Center, Perth, WA, Australia. The QSi5 mice were originally derived and bred within The Faculty of Veterinary Science, University of Sydney. All experimental mice were housed at an isolated room in the secured animal housing facility managed by the Faculty. Mice were kept in an environment of 12:12-h light:dark at an ambient temperature of 22 °C. A pellet diet (Rat and mouse cubes; Specialty Feeds, Glen Forrest, WA, Australia) and water were provided *ad libitum*. The mice used in the experiments were aged from 12 weeks to 16 weeks unless otherwise stated. Eight primiparous female mice from 11 inbred strains, namely 129X1/SvJ (129), A/J, AKR, C3H/HeJ (C3H), CBA/CaH(CBA), C57BL/6J (C57), DBA/1J, DBA/2J, FVB/N (FVB), QSi5 and SJL/J (SJL) were mated with males from each strain respectively. The date of parturition was recorded as day 1 of lactation (L1). The males were removed once pregnancy was confirmed. The litter size of each dam was standardised to eight pups for the QSi5 dams and six pups for the 11 other strains at L1. The females were euthanised at day 9 of lactation (L9), at which post-mortem tissue samples were collected. Lactation performance of the dams was assessed by measuring individual pup weight gain for the first eight days of lactation (Ramanathan et al., 2008). In brief, litter size was standardized to eight pups for the QSi5 strain and six pups for the rest of 10 strains. Lactation performance of each dam was measured by recording cumulative litter weights for the first eight consecutive days postpartum and dividing this value by the litter size. Animals which could not sustain the designated pup numbers for the entire 8 days of lactation period were removed from the experiment. A minimum of four animals from each strain were measured.

2.2. Fluorescence-activated cell sorting (FACS) analysis of splenocytes

Spleen collected from each dam was transferred into a 35 mm × 10 mm petri dish containing basal medium (DMEM or RPMI 1640). All plasticware used was pre-coated with PBS + 2% FCS (Sigma, NSW, Australia). Spleen tissue was disrupted mechanically and the cell suspension filtered through a 40 μm cell strainer (BD Biosciences, CA, USA). The filtrate was centrifuged and the splenocytes were collected and cryopreserved.

The fluorochrome-conjugated monoclonal antibodies used to identify T-lymphocyte subsets in splenocytes (labelled Tube 1) were anti-CD3e-biotin (145-2C11), anti-CD4-FITC (GK1.5), anti-CD8a-Alexa Fluor® 647 (53-6.7), anti-CD25-PE-Cy™7 (PC61), and anti-CD127-PE (SB/199).

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