1 Introduction

Animal health has become an increasingly important issue for livestock producers and consumers. Animal diseases, causing morbidity and mortality, significantly decrease the profitability of animal production. Antibiotics resistance of pathogenic organisms and newly emerged diseases in livestock production such as BSE have led for a call of genetic selection for disease resistance animals. Selective breeding of high disease resistance animals based on their phenotypic value and the presence or absence of some specific resistance genes in their genotypes are the important tools for animal breeders to improve genetics of disease resistance. In order to accomplish the breeding (improvement) goal, the relevant immune response traits from individual animals have to be defined. To date, numerous assays have been established and developed regarding immune response traits.

Molecular genetics approaches including the whole genome scan for quantitative loci (QTL) mapping and candidate gene study have been widely used to investigate genetic variation. Particularly, a combination of theses two approaches has been the most successful method of identifying “disease genes” to date. The QTL approach provides the ability to discover a number of genetic markers at the DNA level. The currents status in pig shows several QTL for various traits including meat production and quality, reproduction as well as disease resistance, that have been mapped on nearly all chromosomes in divergent breed crosses and commercial breeds (Bidanel and Rothschild 2002). The current status (February, 2007) of pig quantitative loci in the ‘PigQTLdb’ available at the website http://www.animalgenome.org indicating 1,657 QTL were reported from 110 publications represent 281 different pig traits. However, the QTL affecting immune response traits and disease resistance are still far less numerous (Rothschild 2003). The candidate gene approach is useful for quickly determining the association of a genetic variant with a disorder and for identifying genes of modest effect (Kwon and Goate 2000).

In this study, several experiments were conducted. Haemolytic complement activities were performed to determine the complement activities via classical and alternative pathways. Several enzyme-linked immunosorbent assays (ELISA) were performed to determine the antibody responses to different vaccination treatments in experimental

animals, including some important immunological parameters (e.g. haptoglobin, C3c). Moreover, molecular genetics methods regarding linkage mapping and candidate gene approaches were also employed in order to achieve the following aims:

1. Evaluation of the porcine immune competencies including total complement activities of classical and alternative pathways, complement component C3c, acute phase protein, and also antibodies of individual pigs in a backcross DUMI resource population.
2. Detection of quantitative trait loci (QTL) using genome scan over all autosomes in a backcross DUMI resource population regarding immunological traits.
3. Identification of single nucleotide polymorphism (SNP) in the porcine *MBL* candidate genes and their association on immunological traits in an F2 DUMI resource population.

2 Literature review

2.1 The immune system

The immune system is an organization of cells and molecules with special roles in defending against infection. It comprises of two functional types of responses, innate or natural and specific or adaptive responses (Figure 1). Its major functions are to differentiate self from non-self and to maintain host defences against foreign substances and pathogens. The innate responses use phagocytic cells including neutrophils, monocytes and macrophages, that release inflammatory mediators (basophils, mast cells and eosinophils) and natural killer cells. The molecular components of innate response include complement, acute-phase proteins and cytokines. The adaptive or specific immune response involves the proliferation of antigen specific B and T cells (Delves and Roitt 2000, Medzhitov and Janeway 1997). Its composition is a complex series of cells and molecular from various tissues that interact to protect the body against invading microorganisms (de Souza 2006). In multicellular animals, immune systems contain different kinds of cells such as tissues or organs and their molecular products that encompass and protect the whole organism against potentially harmful pathogens such as bacteria, viruses and parasites that inhibit the external environment (Cooper 2000). It is considered that the immune system must be capable of doing three actions including recognition of a diverse array of pathogens, killing these pathogens once they are recognized and sparing tissues of the host (Beutler 2004).

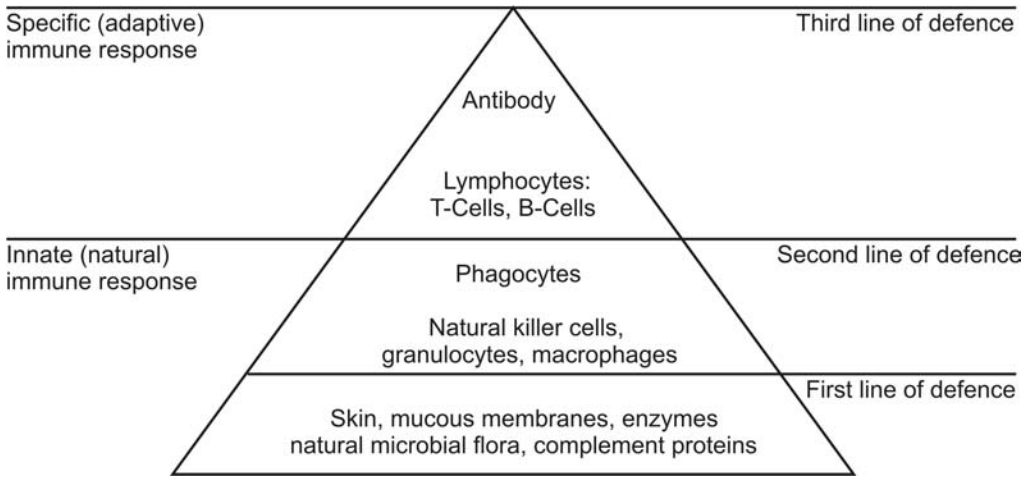

Figure 1: Scheme of the immune response

2.1.1 Innate immune response

The innate immune system can be expressed on the cell surface, in intracellular compartments or secreted into the bloodstream and tissue fluids using a variety of pattern recognition receptors (PRRs) (Medzhitov and Janeway 1997). PRRs functions include opsonization, activation of complement and coagulation cascades, phagocytosis, activation of proinflammatory signalling pathways and induction of apoptosis (Janeway and Medzhitov 2002). Two mechanisms are involved in the innate response in order to distinguish self cells from foreign organisms. The first mechanism involves an array of cell-bound and soluble molecules that have evolved to pathogen-associated molecular pattern (PAMPs) recognition, such as MBL. The second mechanism of self-nonself discrimination involves protecting self cells from the destructive effects of innate immunity such as the alternative pathway of complement activation on the surface of self cells (Parish 2005). The innate immune system evolved long before adaptive immune system in many respects (Beutler 2004).

2.1.2 Acquired immune response

The acquired immune response is a specific response against a particular pathogen or antigen, Lymphocytes are the primary effector cells, a memory response is generated and increases with each exposure to the antigen. The adaptive immune response is distinguished from innate immune mechanisms by a higher degree of specific reactivity for the including agent and recall memory (Doenhoff 2000). Its function is mediated by specific antibody or humoral immunity and a specific cellular immune response or cell-mediated immunity (CMI) (Corbeil 1991). These specificities are governed by antigen handling and recognition molecules on each of the three cell types such as, major histocompatibility antigens (MHC) on antigen-presenting cells (APC), T-cell receptors (TCR) on T-cell and immunoglobulin (Ig) molecules on B cells. One of the acquired immune system enhancements is also represented by successful vaccination against an infectious disease. Vaccines against bacterial and viral infections have employed attenuated live or inactivated whole organisms (Bahr 1999).

2.1.3 Complement system

The complement system is part of the innate immune system and plays an important role in both natural host defence against invading pathogens and the induction of acquired immunity (Roos et al. 2006). The complement system in mammals consists of a complex group of more than 35 soluble proteins and receptors that play an important role in innate and acquired host defence mechanisms against infection and participate in various immunoregulatory processes. The functions mediated by complement activation products include phagocytosis, cytolysis, inflammation, solubilization of immune complexes, apoptotic cells clearance and promotion of humoral immune responses. Activation of complement through any of the three pathways lead to activation of C3, the central protein of the complement system (Holland and Lambris 2005) and the formation of C5 convertase and enzyme complex that activates the terminal pathway and leads to the end product of complement activation, so called 'membrane attack complex' (MAC) (Sodetz and Plumb 2005). Most parts of the complement system are synthesized from liver and are taken into the circulation there (Carroll 2004).

2.1.3.1 Classical pathway

The classical pathway of complement is a major system of innate immunity and triggered through activation of several multimolecular proteases: C1, the MBL associated serine protease 2 (MBL-MASP-2) complex and the ficolin-MASP-2 complexes. These convert an initial recognition signal into proteolytic activity, thereby initiating the 'classical' and 'lectin' routes of complement activation. Both routes then lead to the formation of C3 convertase and the generation of protein fragments triggering diverse biological activities, such as opsonization, endocytosis and inflammation (Arlaud and Colomb 2005). The classical pathway was the first studied and found activated by either antibody released after humoral response or by natural antibody (Carroll 2004). It is triggered by activation of the C1-complex, either by C1q binding to antibodies complexes with antigens, or by binding C1q to the surface of the pathogen. The C1 complex is inhibited by C1-inhibitor. The C1-complex now binds to and splits C2 and C4 into C2a and C4b. C4b and C2a bind to form C3-convertase (C4b2a complex). The production of C3-convertase signals is the end of the classical

pathway, but cleavage of C3 by this enzyme brings us to the start of the alternative pathway.

2.1.3.2 Lectin pathway

The lectin pathway closely resembles the classical complement pathway. The major molecules of this pathway consist of mannose MBL, the homologous molecule to C1q and the MBL-associated serine proteases (MASPs) including MASP-1 and MASP-2, which are homologous to C1r and C1s (Nakao et al. 2001, Sato et al. 1994, Thiel et al. 1997) and form a complex molecule in the presence of the Ca^{2+} ions. This is similar to the C1q/C1r/C1s molecules of the classical pathway. The lectin pathway activation is initiated by binding of the complex between MBL and its associated serine protease MASP-1 and -2 to the mannose groups on bacterial cell surfaces, that leads to the activation of this protease (Fujita 2002, Walport 2001). By MASP2 activation, the complement component C4 and C2 are then cleaved to form the C3 convertase (C4aC2b), which is similar to the classical pathway activation (Fujita 2002). This C4aC2b molecule binds to C3b leading to the C5 convertase generation for the terminal pathway. Three members of this pathway have been identified including MBL, ficolin H and ficolin L (Carroll 2004).

2.1.3.3 Alternative pathway

The alternative pathway, the oldest and most important activation pathway of the complement system, assists in maintaining the integrity of an organism by inactivating invading organisms, pathogens and modified tissue cells (Zipfel 1999). It can be activated non-specifically without the necessity of antigen-antibody complexes (Corbeil 1991). The alternative pathway is activated by a variety of microorganisms including viruses, bacteria, fungi and protozoa. Although the initiation of the activation is essentially antibody-independent, aggregated antibodies have been shown to enhance the activation process. The alternative pathway is kept at a low level of steady-state activation as a result of the hydrolysis of the thioester group of native C3, which leads to the formation of hydrolysed C3 (Holland and Lambris 2005). This pathway is triggered on the surface of a pathogen. Then, C3 is split into C3a and C3b in the alternative pathway. Some of the C3b is bound to the pathogen where it will bind to

factor B; this complex will then be cleaved by factor D into Ba and the alternative pathway C3-convertase, Bb.

2.1.3.4 Terminal pathway

The terminal or lytic pathway of the complement system consists of C5-C9 components which are involved in the formation of the MAC. The MAC causes cell lysis in the pathogen (Holland and Lambris 2005). The activation of the terminal pathway begins with the activation of C5 convertase enzyme (C4b2a3b and C3b3bBb), then breakdown the C5 component to release C5a and C5b. The C5b molecule binds to C6, C7, C8 and C9 respectively, to form MAC that facilitates the killing of microorganisms by changing the permeability of their membranes causing the osmotic lysis of microorganism cells (Sodetz and Plumb 2005).

2.1.3.5 Complement activation

Once the complement system is activated, a chain of reactions involving proteolysis and assembly occurs, resulting in destruction of the pathogen membranes. Complement is activated by three different pathways, classical, alternative and lectin. All pathways share the common step of activating the central component C3, but their recognition mechanisms are different (Carroll 2004). Various pathogenic microorganisms, including bacteria and viruses, as well as many infected cells, efficiently activate the classical pathway after their recognition by antibodies. The activation is triggered upon interaction of the serum C1 complex with antigen-antibody complexes or immune aggregates containing immunoglobulin G (IgG) or IgM (Arlaud and Colomb 2005). The lectin pathway is triggered by C1-like complex proteases in which the recognition function is mediated by either MBL, a member of the collectin family (Turner 1996) or the L- and H-ficolins (Matsushita et al. 2002). The activation of the pathway starts in the fluid phase including plasma or serum by a change of C3 which leads to the enzyme complex formation that cleaves further C3 molecules and sets in motion an amplification reaction leading to complement activation and deposition of a large number of C3b molecules on the cell surface (Zipfel 1999).

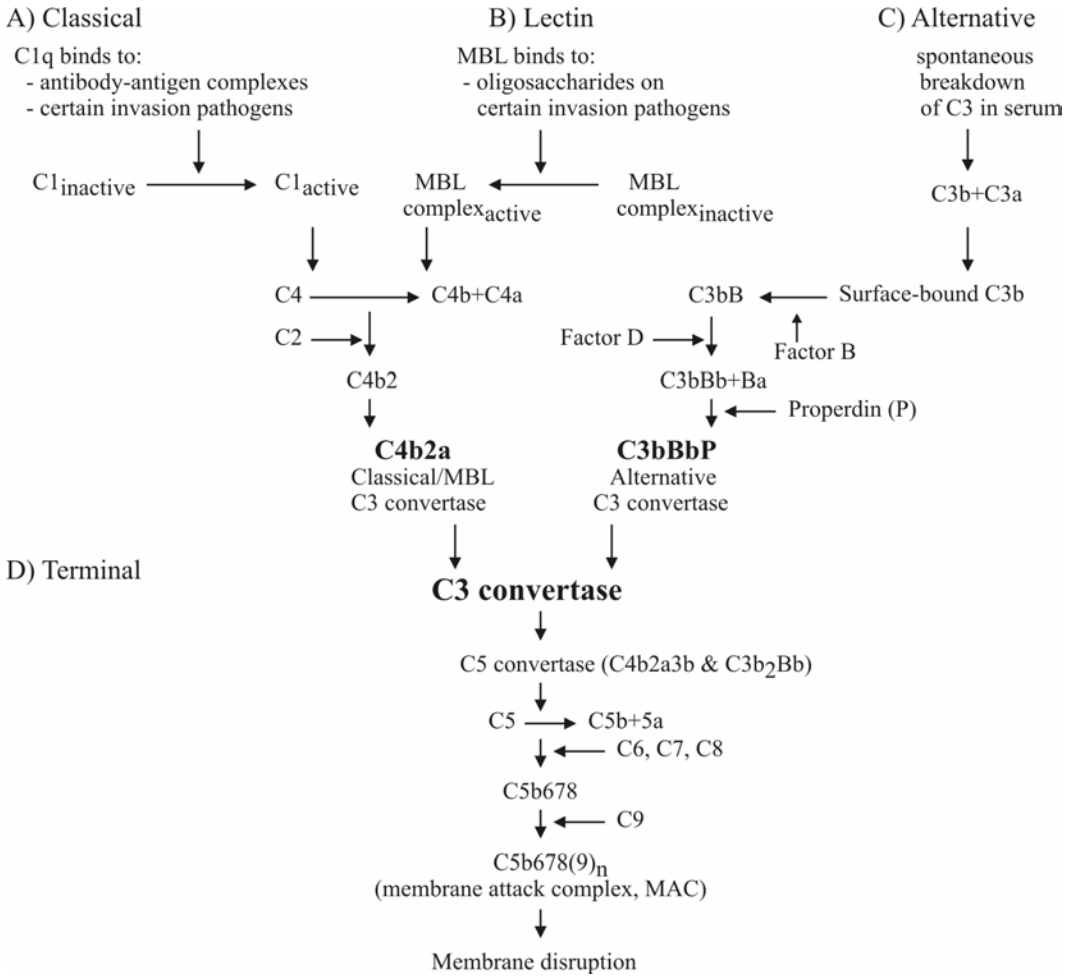

Figure 2: Activation of the complement system via A) classical, B) alternative and C) lectin pathways.

2.1.4 Antibody response

Antibodies are produced by B cells in response to antigens such as bacteria, viruses and protozoa. The development of the antibody response is dependent on the type of antigen and whether the immune system has previously encountered the antigen (Wingren 2001). Antibodies belong to glycoprotein families known as Ig. They circulate in the plasma and lymph, are present in mucosal and lymphoid tissues and can be found on the surfaces of B lymphocytes, where they function as receptors for antigens. From lymphocytes, antibodies are secreted in response to foreign antigenic stimulation, which consist of the principal component of the adaptive humoral immune response. Antibody molecules consist of four polypeptide chains including two identical light (L) chains and 2 identical heavy (H) chains as demonstrated in figure 3. The chains are linked by disulfide bonds and are arranged such that the H and L chains form pairs. The domain