

Genetic resistance to *Salmonella* infection in domestic animals

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Abstract

Genetic resistance to *Salmonella* infection in experimental animal models is well described. However, genetic resistance in domestic animals, which has potentially great value in terms of controlling *Salmonella* in the food chain, has been relatively poorly described. Recent advances in genetics and immunology have identified several factors that influence resistance in chickens and pigs in particular. Resistance to systemic salmonellosis in the chicken is encoded by a number of factors including Nramp1 (now termed Slc11a1) and a novel gene, *SAL1* that leads to increased macrophage activity against *Salmonella*. Studies in outbred, and in particular, inbred chickens have revealed considerable differences in levels of colonization of the gastrointestinal tract and responses to vaccination. Factors influencing this appear to include innate immune function, MHC and Nramp. In pigs several immune factors, including polymorphonuclear cell activity, have been shown to influence resistance.

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1. Introduction

The breeding of domestic animals with increased resistance to salmonellosis is an attractive option. Control methods currently or previously applied often have shortcomings. These include increased production costs associated with the use of vaccination, public health issues in the use of prophylactic antibiotics potentially leading to development of antibiotic resistant strains of pathogenic bacteria or only partially effective measures, such as the use of probiotic bacteria in competitive exclusion. Therefore, the breeding of resistant animals, in combination with good hygiene practices offers an easy, relatively low risk strategy to control both disease and colonization by *Salmonella*.

Studies of genetic resistance in domestic animals have focused on two broad aims. The first is to reduce the levels of disease and economic losses through high mortality rates of systemic salmonellosis caused by *Salmonella* serovars such as *Salmonella choleraesuis* in pigs (Van Diemen et al., 2002), or *Salmonella gallinarum* in poultry (Mariani et al., 2001; Wigley et al.,

2002). The second is to control colonization in domestic animals and subsequent transmission of 'food-poisoning' serovars such as *Salmonella typhimurium* or *Salmonella enteritidis* through consumption of meat or eggs (Lamont et al., 2002; Barrow et al., 2003). Advances in genetics and immunology in the study of domestic animals are now beginning to allow the identification of resistance genes and the determination of their underlying mechanisms.

2. Genetic resistance in laboratory animals

Genetic resistance to systemic disease caused by *S. typhimurium* in mice has been studied extensively and perhaps not surprisingly this has revealed that resistance or susceptibility is linked to many factors including the major histocompatibility complex (MHC) (Maskell and Hormaeche, 1986), Toll-like receptor 4 (Tlr4) (O'Brien et al., 1980; Poltorak et al., 1998) and the natural resistance associated macrophage protein (Nramp1) (reviewed by Blackwell et al., 2001). The MHC has been implicated in resistance and susceptibility to a number of both infectious and non-infectious diseases in mammals. Its role in resistance to salmonellosis is likely to reflect the ability of specific

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haplotypes to present specific antigens in the generation of an immune response. However, it should be noted that the only clear linkage of resistance to infectious agents with specific MHC haplotypes is in resistance to Marek's disease virus in chicken (Kaufmann et al., 1999). Several inbred mouse strains such as C3H/HeJ mice are highly susceptible to *S. typhimurium* and other Gram negative bacterial infections. A locus termed *Lps* was identified as being linked to this susceptibility and lack of response to lipopolysaccharide (LPS) (O'Brien et al., 1980). This locus has subsequently found to be Tlr4 (Poltorak et al., 1998), a transmembrane component of the LPS receptor complex that plays a key role in activation of immune responses to Gram negative bacteria. Perhaps the most studied of all these factors in mice is *Nrampl* (now termed *Slc11a1*), which has a range of effects on macrophage activation including MHC expression, production of pro-inflammatory cytokines and chemokines and the production of antimicrobial effectors such as nitric oxide and oxidative burst (reviewed by Blackwell et al., 2001). *Nrampl* is a divalent cation antiporter protein expressed on macrophage endosomes and lysosomes that is believed to play a role in divalent cation homeostasis and the generation of oxygen- and nitrogen-dependent antimicrobial activity. Mutations in the *NRAMP1* gene result in mice that are susceptible to a range of infections including *S. typhimurium*, *Leishmania* and *Mycobacteria* species, which can survive in macrophages. These susceptibilities gave rise to a number of former names for this locus including *Lsh*, *Bcg* and *Ity*. *NRAMP1* has been identified in a number of domestic species that will be discussed later.

3. Genetic resistance in poultry

The study of genetic resistance in the chicken has progressed fairly rapidly due to their small size, rapid growth, short generation time and the availability of a number of well-defined inbred chicken lines. Initial studies on genetic resistance centred largely on resistance of various chicken breeds to fowl typhoid (*S. gallinarum*) and pullorum disease (*Salmonella pullorum*) (Smith, 1956; Hutt and Crawford, 1960). More recent studies in inbred White Leghorn chickens revealed massive differences in the LD₅₀, pathogenesis and numbers of *Salmonella* in organs following oral or intravenous infection with *S. gallinarum* or *S. typhimurium* (Bumstead and Barrow, 1988, 1993). Subsequent experiments showed that lines W1, 6₁ and N were resistant to systemic disease, whereas lines C, 7₂ and 151 were highly susceptible. The greatest differences were found following *S. gallinarum* infection. Although chicks from these lines showed differences in susceptibility to *S. typhimurium* or *S. enteritidis* infection, these

became less pronounced in older birds which are innately resistant to systemic disease caused by these serovars. Subsequent studies in these lines showed linkage to both chicken *NRAMP1* and to the *Tnc* locus that is closely associated with *Lps*/Tlr4. These two loci account for up to 35% of the differences in susceptibility observed between lines W1 and C (Hu et al., 1997). However, in several lines, which showed great differences in susceptibility, there were no functional differences in the *NRAMP1* sequences. Further mapping has revealed a novel locus on chromosome 5 that accounts for a major part of the differences in susceptibility between these lines (Mariani et al., 2001). This novel gene has been termed *SALI* and subsequent studies have begun to elucidate the mechanisms of resistance of *S. gallinarum* infection (Wigley et al., 2002). Survival in macrophages is a vital stage of the pathogenesis of fowl typhoid (Barrow et al., 1994; Jones et al., 2001), therefore the ability to survive in and the antimicrobial activity of resistant- and susceptible-line macrophages was investigated. These studies showed that resistant-line macrophages clear *S. gallinarum* more efficiently through oxygen-dependent antimicrobial activity, thereby limiting progression of disease.

The role of *Nrampl* has been investigated in resistance to both experimental systemic infection, and gastrointestinal tract colonization by *S. enteritidis*. Studies with four outbred lines showed inheritance of a resistant trait to the amount and length of caecal colonization with *S. enteritidis* when orally infected (Duchet-Suchaux et al., 1997; Berthelot et al., 1998), to bacterial levels in the liver when infected intravenously (Girard-Santosusso et al., 1998) and to mortality in infected newly hatched chicks (Beaumont et al., 1999). Subsequent studies using one of these lines (L2) and commercial birds has shown a partial link of systemic resistance to *Nrampl* (Girard-Santosusso et al., 2002). Studies in a novel genetic cross termed the Iowa *Salmonella* response resource population (ISRRP) have been used to determine candidate genes for resistance to colonization by *S. enteritidis* previously observed in a number of lines (Kaiser and Lamont, 2001; Lamont et al., 2002). Polymorphisms in three candidate genes, *NRAMP1*, MHC Class 1 and *IAP1* (inhibitor of apoptosis 1) were found to be linked to the levels of *S. enteritidis* in the spleen of chicks infected at one-day of age, but appeared to play little role in gastrointestinal tract levels of *Salmonella*. The role of *Nrampl* having some effect in systemic disease, but little effect on intestinal colonization is perhaps logical, considering that its description as a resistance gene in mammals is generally in protection against systemic diseases where survival in macrophages is a key component in pathogenesis. Although survival in macrophages is undoubtedly essential in the development of systemic salmonellosis in the chicken (Jones et al., 2001; Wigley et al., 2001), survival in macrophages

does not appear to be a crucial component in intestinal colonization with *S. enteritidis* (Berchieri et al., 2001).

Studies of intestinal colonization by *S. typhimurium* or *S. enteritidis* in the inbred lines that were previously characterized for resistance to systemic disease (Wigley et al., 2002) have revealed large differences in levels and length of colonization between lines (Barrow et al., 2003). This is not related to MHC or to the *SALI* gene, or to development of adaptive immunity. The only observed differences between the most resistant (Line 6₁) and most susceptible lines (Line N) was a slight difference in the intestinal flow rate, and differences in the number of circulating heterophils (polymorphonuclear cells). Similar results are found following *Campylobacter jejuni* infection indicating this is not a species-specific effect. The ability of *Salmonella* or *Campylobacter* to colonize the gut may be limited by the innate immune system; both cells and secreted products such as defensins may play a crucial role in the genetic resistance to gut colonization. Heterophils are decisive components in the control of *S. enteritidis* invasion following oral infection of chickens (Kogut et al., 1994). Recent evidence indicates that there is a significant element of genetic control in the differential function of heterophils from various commercial broiler chickens (Swaggerty et al., 2003).

The role of MHC should not be overlooked in genetic resistance in chickens, especially considering its compact nature and lack of polymorphism in comparison to mammalian equivalents (Kaufmann et al., 1999). Studies in inbred lines of chickens indicate linkage of the ability to respond to *Salmonella* infection with particular MHC class I or class II haplotypes (Cotter et al., 1998; Liu et al., 2002). In particular the ability to generate a protective response following vaccination may be closely linked to MHC (Liu et al., 2002).

The role of Tlr4 in resistance has not been fully elucidated, though substantial differences in response to LPS have been found in macrophages from chickens with different genetic backgrounds (Dil and Qureshi, 2002). However, it has recently been shown that although polymorphisms are found in the chicken Tlr4 gene between a number of inbred chicken lines, these show little correlation to resistance to systemic salmonellosis (Leveque et al., 2003).

4. Resistance in pigs and other mammals

Although genetic-linked variance in immune responses is well known to occur in large domestic mammals such as pigs and cattle, resistance to specific pathogens is more poorly characterized (Van Diemen et al., 2002). *NRAMP1* has been identified and cloned in a number of domestic mammals including pigs (Sun

et al., 1998; Zhang et al., 2000), cattle (Ables et al., 2002) and sheep (Bussmann et al., 1998). In pigs *NRAMP1* is strongly expressed on macrophages and neutrophils following stimulation with LPS, but any role in *Salmonella* infection is yet to be described (Zhang et al., 2000). A reference population of pigs bred to study resistance to *S. choleraesuis* infection indicated that a number of inherited immunological traits influence resistance to disease (Van Diemen et al., 2002). The progeny of two boars showed increased resistance or susceptibility to infection. Neutrophils from the resistant animals showed increased phagocytic and antimicrobial activity and T lymphocytes increased mitogen-induced proliferation, though no genes associated with this resistance were described. Differences in susceptibility of cattle breeds to salmonellosis have been described, for example Friesian calves are more resistant to *S. typhimurium* infection than Jersey calves (Wray and Sojka, 1978), but no linkage to genes or mechanisms of resistance have been described.

5. Conclusions

There is undoubtedly a strong genetic association with resistance to salmonellosis in a number of economically important domestic species. However, as yet, selective breeding for resistance traits is not utilized in control of disease or the carriage of *Salmonella* in any of these species. The value of a particular resistance trait in reduction of disease must be balanced against other factors such as productivity of meat, eggs or milk. In addition there is no guarantee that increased resistance to one pathogen will not increase susceptibility to another. This may be particularly true in the case of MHC in chickens, where the number of MHC alleles expressed is limited. Nevertheless the low cost after initial breeding programmes and the high level of public safety afforded through genetic resistance makes it an attractive option. In addition it may be possible to breed animals that are more responsive to vaccination, particularly as our understanding of immunity in domestic animal species improves and new rational vaccines are produced. The control of *Salmonella* and *Campylobacter* colonization of the gastrointestinal tract of food animals, particularly poultry and pigs where intensive rearing, slaughter and processing are employed, would appear to be a particularly useful objective with enormous potential public health benefits. The reduction in numbers of infected animals and in the levels of bacteria colonizing infected food animals may be a significant factor in reducing the transmission of food-borne zoonotic infections. The breeding of genetically resistant animals, in combination with other control measures such as biosecurity and vaccination, may be a valuable tool in achieving this.

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