Identification Of A Porcine-specific Adhesin Of Salmonella Typhimurium And A Possible Mechanism Mediating Persistent Infections Of Swine

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Introduction

Salmonella enterica serotype Typhimurium (S. typhimurium) is one of the leading causes of food poisoning in man (4) and consumption of pork products is a major risk factor for disease (3, 5). A major outbreak of salmonellosis in Denmark caused by S. enterica and associated with the consumption of pork has stimulated renewed interest in Salmonella and food safety by pork producers, consumers, and the government (11). A combination of many factors has prompted the United States Department of Agriculture to place S. enterica at the top of its priority list of food borne pathogens (7). In Denmark, S. typhimurium is the most common serotype isolated from pigs (78.2 %) and is the second most common cause of food borne salmonellosis in humans (27.8 %) (1, 11). In the United States S. typhimurium is of similar significance.

The introduction of S. typhimurium into the food chain appears to be manifested by the early exposure of pigs to this organism which results in long term persistent, but subclinical infections. Infected pigs remain clinically healthy and frequently do not shed Salmonella in their feces. This makes identification of carrier animals difficult. However, these animals become reservoirs of Salmonella that can be spread to other animals or to contaminate food products at harvest. This is particularly true after shipment of pigs from the farm to the slaughter plant. The stress associated with shipment increases shedding of Salmonella in the feces of infected pigs (8).

Previously, two phenotypes of S. typhimurium strain 798, a strain known to persistently infect pigs, have been identified that maintain different in vivo and in vitro properties (9). The two phenotypes differ in their abilities to adhere to porcine enterocytes, to be phagocytized and persist in porcine white blood cells, in their sensitivity to serum complement, in the production of O-antigen, and in their colonial morphologies on Evans Blue-Uranine plates. Associated with cells of the adhesive phenotype are at least 10 unique envelope proteins, the production of fimbriae, and the presence of four unique non-fimbrial surface antigens detectable with specific antisera. All of these traits are coordinately regulated and are subject to phase variation. The rate of variation between the two phases is $10^3$ per generation when the transition is from non-adhesive to the adhesive phenotype and $10^{-3}$ per generation in the other direction. In this paper, we describe the production of a collection of mutations and the screening of the mutants for the loss of adhesiveness to enterocytes. Non-adhesive mutants were analyzed genetically to identify the adhesin. One non-adhesive mutant also was tested for its ability to colonize mouse and pig intestines and to cause systemic disease in mice.

Results

Transposon mutagenesis, using TnpA, was performed on cells in the adhesive phenotype and the mutants tested for loss of adhesiveness. Two non-adhesive mutants were identified. The TnpA and flanking DNA was cloned and subjected to DNA sequencing. In both cases, the transposon was located in fimA, the gene encoding the major subunit of type 1 fimbriae. When observed by electron microscopy, cells of the adhesive phenotype produced fimbriae that appeared morphologically like type 1 fimbriae, while non-adhesive cells (9) and the fimA mutants did not. To assess whether the mutation in fimA affected pathogenesis, one of the mutants was used in animal challenge studies using mice and pigs. There was a significant decrease in the ability of the mutant to colonize intestines, but the mutant retained the ability to invade and disseminate to peripheral tissues.

Discussion

Associated with the adhesive phenotype is the coordinate expression of several other traits associated with virulence including the expression of a long O-antigen, resistance to serum complement, enhanced ability to enter phagocytes, and the ability to resist intracellular killing by phagocytes. The mutation in fimA abolished the ability of the organism to attach to enterocytes, and this in turn reduced its ability to effectively colonize intestines but did not interfere with the ability of the organism to invade or to distribute systemically. Other than the loss of type 1 fimbrial production, the fimA mutant retained the other traits associated with the adhesive phenotype. The preferred route of
invasion leading to systemic spread of *S. typhimurium* is via M-cells (6, 10). *S. typhimurium* produce a unique adhesin, called long polar fimbriae (Lpf), that are specific for M-cells (2). Lpf do not appear to be regulated via the phase variation process described above and are distinct from type I fimbriae. Thus, if type I fimbriae are the enteroocyte-specific adhesin and Lpf are the M-cell adhesin, the *finA* mutant should continue to spread systemically yet have reduced ability to colonize intestines as was seen in the mouse and pigs models of infection.

We hypothesize that the mechanism regulating phase variation is responsible for the development of persistent infections with *S. typhimurium* and have developed the model shown in the figure to describe the role of phase variation in the establishment of persistent infections. Cells in the adhesive phenotype express an array of virulence related genes. These cells attach to enterocytes and M-cells and invade both types of cells. Once they invade M-cells, they encounter phagocytes in which they proliferate. We conclude that this is a virulent phenotype. On the other hand, cells in the non-adhesive phenotype should be avirulent. They are unable to colonize and invade through enterocytes because of the absence of type I fimbriae. They could still attach to M-cells using Lpf. However, those that did invade via M-cells would be killed when they encountered macrophages. Therefore, cells in the non-adhesive phase are either killed by the host immune system or cleared from the intestinal tract to the environment in feces. By regulating the proportion of cells in each phenotype through phase variation, a population containing a concentration of virulent cells below the threshold for disease could be maintained. In such a state, the population could be maintained at a level that would not result in disease but would be sufficient to maintain a sub-clinical infection.

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**References**


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**Acknowledgement**

This work was funded with a grant from the United States Department of Agriculture, National Research Initiative Competitive Grants Program (grant # 98-02811).