STUDIES ON SEROTYPE-SPECIFIC REGULATION OF ANTIGEN EXPRESSION IN *PASTEURELLA HAEMOLYTICA*

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Introduction

Bovine respiratory disease (BRD) remains a major economic problem for both the beef and dairy cattle industries in the U.S. (1). Recent estimates of the losses due to BRD exceed a billion dolars annually which is greater than all other diseases combined (2).

The most common sequel observed in BRD is a fulminating fibrinonecrotic pleuropneumonia called shipping fever or pneumonic pasteurellosis. Although BRD is a multifactorial disease involving interaction between a number of environmental factors, various viruses and bacteria, *Pasteurella haemolytica* biotype A serotype 1(A1) also called serotype 1 (ST1) has been established as the primary agent involved in the pathogenesis of the pneumonia (3). *P. haemolytica* ST1 can be isolated in pure culture from typical pneumonic lesions of the disease (4).

Pathogenesis

P. haemolytica ST1 is rarely found in the upper respiratory tract (URT) of healthy cattle (5). In contrast ST2 is easily isolated and comprises the major proportion of the normal *P. haemolytica* microfloral population in unstressed, healthy cattle (4)(6). Exposure of cattle to various environmental and management stress factors and/or viral infection leads to an explosive growth of *P. haemolytica* ST1 and its colonization of all areas of the URT of affected animals (4)(7). The greatly increased population of ST1 in the URT subsequently results in accumulation of critical numbers of the organism in the lungs where they initiate a series of pathophysiologic events culminating in the severe fibrinonecrotic pleuropneumonia characteristic of the disease (5)(8).

This phenomenon of commensal to pathogen conversion in the URT microfloral population, from predominance of ST2 to ST1 is consistently observed during the genesis of pneumonic pasteurellosis. Hence, colonization of the URT with *P. haemolytica* ST1 appears to be an initial event and a important prerequisite to the development of the disease. It is a logical hypothesis therefore that serotype-specific antigens unique to *P. haemolytica* ST1, such as surface structures acting as adherence factors possibly enable selective colonization of the bovine URT and could be the key to pathogenetic events that lead to pneumonic pasteurellosis. Disease

control could thereby be targeted towards intervention in this serotype-specific colonization process, in order to reduce the number of organisms reaching the lungs below the critical level needed for lesion formation. This could thereby facilitate bacterial clearance by the normal defense mechanisms present in the bovine lungs (9).

The Importance of ST1-Specific Antigens in Pneumonic Pasteurellosis

In the past, serotype-specificity of *P. haemolytica* strains were assumed to be based on antigenic differences of capsular polysaccharides alone (10). However, vaccination/challenge studies have shown that increased resistance to virulent bacterial challenge was correlated with high levels of antibody against a surface polysaccharide/protein complex rather than to purified capsular polysaccharide (11). Results of immunization studies using a cloned *P. haemolytica* leukotoxin in combination with other cell-free extracts indicated that higher levels of protection against challenge were obtained when antibodies to both leukotoxin and an ST1-specific agglutinogen were elicited, instead of anti-leukotoxin antibodies alone (12). Serotype-specific inhibition of nasal colonization has also been demonstrated following vaccination with an ST1-specific antigen extract (13).

These data provide evidence that the focus of future studies on pneumonic pasteurellosis need to be chanelled more towards serotype-specific antigens as critical factors in disease pathogenesis and possibly as immunogens to prevent clinical disease.

Molecular Studies of PHA1SSA (*P. haemolytica* A1 serotype-specific agglutinogen)

Gonzalez-Rayos et al (14) have previously isolated a genomic fragment from *P. haemolytica* ST1 which encoded an ST1-specific agglutinating antigen. Further studies revealed a proteinaceous nature of this antigen, based on its protease and heat susceptibility (15). It is possible that this *P. haemolytica* A1 (ST1) serotype-specific agglutinogen (PHA1SSA) functions as an adhesin to facilitate survival and proliferation of *P. haemolytica* ST1 in the URT of cattle under permissive conditions. Although expression of this antigen in *P. haemolytica* strains appears to be ST1-specific, the coding gene was found to have homologous sequences in serotypes other than ST1. Notably homology was detected in the *P. haemolytica* ST2 genome.

Considering the commensal to pathogen conversion from ST2 to ST1 which appears to be a prerequisite to clinical disease, it is possible that

the potential for phenotypic expression of this antigen is favored under certain stress-induced states in the host-tissue environment. Moreover, surface-expression of this antigen may confer a selective advantage to enable outgrowth of the ST1 phenotype over the other *P. haemolytica* serotypes in the bovine URT.

Studies on Serotype-Specific Regulation of PHA1SSA Expression in *P. Haemolytica*

The purpose of this present project is to attempt to elucidate the regulatory mechanism(s) involved in ST1-specific phenotypic expression of PHA1SSA.

Experiment 1: Serotype genomic distribution of PHA1SSA-coding gene.

This study was undertaken to determine whether gene deletions, inversions or presence of different expression loci are involved in regulation of PHA1SSA expression in *P. haemolytica*. Total genomic DNA extracted from *P. haemolytica* serotypes 1-12 were digested with different restriction enzymes and then hybridized with the [α^{32}P]dCTP-labelled PHA1SSA-coding DNA from ST1. Results showed identical restriction patterns in all serotypes which hybridized with the probe (ST1, ST2, ST5, ST6, ST7, ST9, ST12). Considering that serotyping test results showed ST1-specific agglutination reaction to this antigen, therefore there must a specific mechanism which regulates phenotypic expression of PHA1SSA in *P. haemolytica* ST1. The data obtained from this experiment so far has not identified obvious fragment deletions nor gene rearrangements similar to those commonly involved in regulating phase-variable expression of bacterial surface antigens (16).

Experiment 2: Cloning of a PHA1SSA-coding gene from *P. haemolytica* ST2.

A recombinant plasmid containing a 3.6 kbp *Hind* III DNA insert was obtained from a *P. haemolytica* ST2 genomic library, after screening with a 2.2 kbp *Hind* III/*Pst* I fragment subcloned from the ST1-derived PHA1SSA-coding gene. Serotyping tests on *E. coli* clones carrying this recombinant plasmid showed phenotypic expression of an ST1-specific agglutinating antigen. These results indicate that the homologous genome fragment in ST2 contains a functional gene which encodes a gene product indistinguishable from PHA1SSA in the serotyping tests.

Experiment 3: In vitro transcription/translation from ST1 and ST2-derived PHA1SSA-coding genes.

Recombinant plasmids containing ST1 or ST2-derived inserts were analyzed in an in vitro *E. coli* coupled transcription/translation system.

Autoradiography revealed identical insert-encoded polypeptide bands which were absent in the vector plasmid controls.

Immunoprecipitation was done to determine serotype-specificity of the PHA1SSA gene product, using polyclonal typing antisera raised against *P. haemolytica* ST1, ST2 or ST7. Results did not show ST1-specific recognition of the insert-encoded polypeptides. This indicates that *P. haemolytica* ST2 and ST7 could also be expressing these gene-encoded polypeptides in vivo. Therefore phenotypic specificity of the serotyping agglutination reaction could be due to mechanisms which affect stability, extracellular export or membrane anchorage or the gene product.

Conclusion

We conclude from the data obtained in these experiments that lack of phenotypic expression of the *P. haemolytica* ST1-specific antigen PHA1SSA in ST2 does not appear to occur through gene deletions, inversions nor gene transfer between different expression loci. Furthermore, it appears that synthesis of PHA1SSA-specific polypeptides may occur in other serotypes carrying genome sequences homologous to the coding gene. Further studies to identify other factors which could be responsible for regulating expression of the PHA1SSA phenotype will be undertaken.

References

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