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# Lactic acid is a potential virulence factor for group B *Streptococcus*

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ABSTRACT

Group B *Streptococcus* (GBS) is a Gram-positive bacterium that causes sepsis and meningitis in neonates and infants. Although several GBS-associated virulence factors have been described, the mechanisms of GBS invasive disease are not well understood. To characterize additional virulence factors, a novel *in vitro* infection assay was developed using rat fetal lung explants. However, application of GBS to the system induced rapid lung tissue destruction associated with increased media acidity. Since lactic acid produced by other streptococci is an important virulence factor, we hypothesized that lactic acid contributed to the virulence of GBS. Spent growth media and neutralized-spent media were applied to explants and results indicated that neutralization of the media completely protected the tissue from degradation. These results were verified using multiple viability assays and with transformed cell lines. Furthermore, comparable spent media from *Escherichia coli* did not induce tissue cytotoxicity, suggesting that GBS produces organic acids in excess of other potential bacterial pathogens. Analysis of the spent media indicated that L-lactate levels reached ~70 mM, indicating that lactic acid is a major constituent of the metabolic acid produced by GBS. Treatment of explants with lactic acid alone produced dose-dependent tissue degradation, indicating that lactic acid is independently sufficient to induce target-tissue cytotoxicity. Finally, both spent media and 23.6 mM lactic acid produced dramatic tissue autofluorescence; the basis for this is currently unknown. These studies demonstrate that GBS-produced lactic acid is a potential virulence factor and may contribute to GBS invasive disease.



## 1. Introduction

Group B *Streptococcus* (GBS) is a major cause of severe morbidity and mortality in neonates and young infants [1]. Despite the widespread usage of antibiotic prophylaxis in the United States, approximately 3600 neonates and infants contract invasive GBS disease annually, with considerable morbidity and mortality. Although antibiotic prophylaxis is effective, it does not prevent all early onset disease and has no influence on the incidence of late onset GBS disease. Multivalent vaccines are promising; however, they are not completely effective against all forms of the disease [1,2]. Therefore, additional strategies to combat GBS disease need to be developed.

The mechanisms of GBS pathogenesis are the subject of numerous investigations and are the subject of a recent comprehensive review [3]. Briefly, GBS appears to gain access to the neonate via the respiratory tract from aspiration of the organisms while traversing the birth canal. This is followed by penetration into the lung epithelia via processes including attachment and subsequent intracellular invasion. Attachment and invasion are mediated in part by the GBS-surface-associated proteins including the alpha C-proteins (ACPs) and Rib [4–8]. Other surface-associated proteins including fibrinogen-binding protein A (FbsA) and a C5a peptidase contribute to GBS virulence by facilitating binding to the extracellular matrix (ECM) [9–11]. Capsular polysaccharides allow GBS to evade the initial innate immune response [12]. These oligosaccharides, which often contain a terminal sialic acid residue, appear to protect the organism via molecular mimicry of host cell surface carbohydrates. Cytolytic killing of host cells is mediated by the β-H/C toxin which forms pores in host immune cells, including macrophages and neutrophils, and ultimately induces apoptosis [3,5,13–15]. Due to variability in the expression and structure of offensive and defensive virulence factors, invasive strains of GBS do not consistently express all of these factors [3,16–19]. Consequently, other components of the organism are likely to contribute to its pathogenicity.

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Interestingly, little attention has been paid to the metabolic products of GBS including lactic acid. Lactic acid is the end product of anaerobic fermentation of glucose as well as the most abundant product of GBS aerobic growth [20]. Lactic acid is recognized as an important virulence factor for multiple streptococcal species including *S. rattus* and *S. mutans* [21,22]. In humans, a replacement strategy to reduce dental caries caused by *S. mutans* involves inactivation of the gene encoding the lactate dehydrogenase gene *(ldh)* to reduce lactic acid output [22]. Thus, lactic acid is clearly a virulence factor in other species of streptococci that are evolutionarily similar to GBS. To the best of our knowledge, the role of lactic acid as a virulence factor in invasive GBS disease has not been explored. Thus, we investigated the role of GBS-produced lactic acid, including its potential role(s) in the development of GBS disease, using a novel *in vitro* infection model.

## 2. Results

### 2.1. Infection of GBS in fetal rat lung explants

To determine if GBS infects fetal rat lung tissue, explants from embryonic day 14 (E14) rats were exposed to GBS strain A909 [23]. This date was chosen because it represents the upper limit in development at which fetal lungs can be placed in culture as whole tissues and still appear as two-dimensional structures. Fig. 1A and B show that the lung tissue disintegrated during the incubation with GBS compared with the control (uninoculated). Fluorescent immuno-histochemistry was used to localize GBS within the lung post-infection. Antibodies against the N-terminus of the alpha C-protein (ACP) were used as a probe because of their ability to detect a surface-associated, immunologically important protein [24]. Fig. 1C shows that although these antibodies effectively detect GBS in the fixed tissue, few GBS appeared to be associated with the host cells. The reasons for observing relatively few GBS associated with host cells are not known, but it is possible that GBS do not have to be tightly associated with the tissue to induce necrosis.

To determine weather GBS need to physically associate with the host tissue to induce damage, rat fetal lungs were grown protected from the bacteria by a 0.4-μm membrane barrier. The rat fetal lung explants were incubated in Transwell inserts with either BGJb (media control) or BGJb plus GBS strain A909 placed in the well of the dish. Fig. 2 shows that the fetal lung tissue disintegrated even with separation of the organism from the tissue, suggesting the presence of soluble cytotoxic factor. Additionally, in the above-

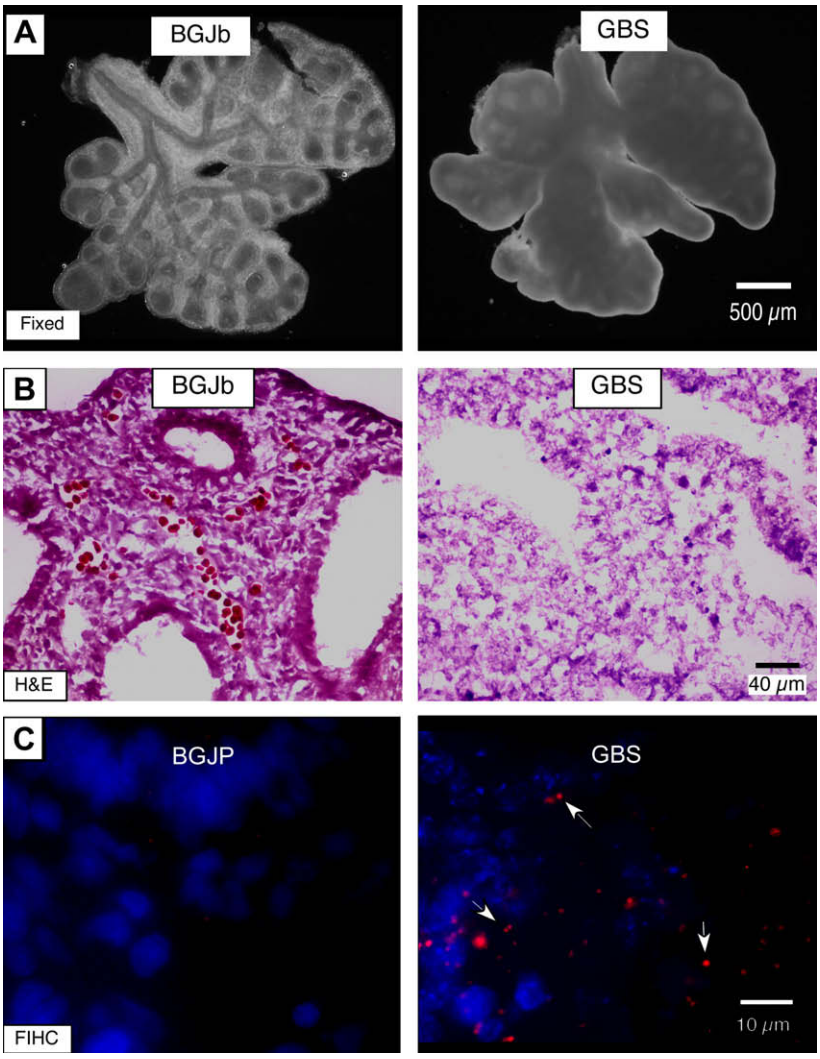


**Fig. 1.** Establishment of an *in vitro* infection model. GBS were inoculated into BGJb with freshly isolated rat fetal lung explants. (A) Fixed fetal lung tissues were photographed as shown. (B) Fetal lung tissues were sectioned and stained with hematoxylin and eosin (H&E). Arrows indicate erythroblasts. Note poor tissue integrity in tissue treated with GBS (strain A909). Insets represent high power images. (C) Fluorescent immuno-histochemistry using antibodies to the alpha C-protein (ACP) to detect GBS. Cell nuclei counterstained with Hoechst 33432 (blue). (For interpretation of the references to colour in the figure legend, the reader is referred to the web version of this article.)