An *in vitro* enterocyte cell-like model to study Group B streptococci internalization

Giuseppe Valerio De Gaetano,[1] Germana Lentini,1 Roberta Galbo,[2] Francesco Coppolino,[1] Agata Famà,[1] Giuseppe Teti,[3] Concetta Beninati[1,4]

[1]Department of Human Pathology, University of Messina, Messina; [2]Department of Chemical, Biological and Pharmaceutical Sciences, University of Messina, Messina; [3]Charybdis Vaccine Srl, Messina; [4]Scylla Biotech Srl, Messina, Italy

Abstract

We used a 2D in vitro growth model of Caco2 cells to study invasion of *Streptococcus agalactiae*. This model can be useful for evaluating the virulence of the most relevant enteric pathogens.

Introduction

Neonatal GBS linked-disease occuring in the first three months after birth is defined as late onset disease (LOD) and it is frequently caused by the "hypervirulent" BM110 strain belonging to CC17 clonal complex.[1] This strain often colonizes the infant gut but less is known about its ability to interact with the gut epithelium. The strict interactions between polarized enterocytes participate in maintaining the gut integrity, but, if lost, pathogens can be mostly advantaged to invade the host.[2] In this study, we aimed to investigate the GBS BM110 strain internalization processes into enterocytes by using, as *in vitro* model, the Caco-2 cell line which reproduces the main morphological properties of the colonic epithelium.[3]

Materials and Methods

To *in vitro* recapitulate the main features of the gut epithelium, the Caco-2 cell line was grown in RPMI-1640 medium supplemented with 10% (vol/vol) of fetal bovine seum (FBS) at 37°C in a humidified incubator. Cells were used at two stages of growth: islands of partially polarized and differentiated cells and fully polarized and differentiated monolayers, indicated as 5- and 21-days, respectively. Confluence and differentiation was constantly monitored by optical and immunofluorescent microscopy. To test the invasive properties of GBS, epithelial cells were infected with bacteria for 1h to allow the initial bacterial adhesion, to be later gently washed and further incubated for an additional 1h with a bactericidal amount of antibiotics. This method revealed to be useful to kill all extracellular adhered bacteria.[4] Then the number of intracellular bacteria was measured by plating the cell lysates on blood agar plates. To assess the possible route of invasion, Caco-2 cells were infected after treatment with media lacking bivalent cations and/or specific chelator as EGTA. To shed light on the mechanism of GBS internalization, infections were performed in presence of chemical inhibitors of chlatrin-, lipid-rafts- or caveolar-dependent endocytic pathways. Experiments were performed at least three times and data were statistically analyzed by the Mann-Whitney or the one-way ANOVA tests. A $p<0.005$ was used as a threshold for significance (Figure 1 and 2).

Results

Numerous Group B streptococci adhered to the lateral surfaces of Caco-2 islands, while a few number of them was confined to the apical section of fully polarized and differentiated monolayers. By opening the intercellular junctions, the abil-

Correspondence:Concetta Beninati, Department of Human Pathology, University of Messina, Messina; Scylla Biotech Srl, Messina, Italy.
E-mail: cbeninati@unime.it

Key words: Caco-2 cells; GBS; actin; endocytosis.

Acknowledgments: Work presented here was supported in part by the PRIN (Programma di Ricerca Scientifica di Rilevante Interesse Nazionale) 2017M8R5N9_002 grant from the Ministero dell'Università and Ricerca Scientifica (MIUR) of Italy.

Disclosures: All the authors declare no conflict of interest.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico, in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.
Accepted for publication: 7 September 2021.

Biomedical Science and Engineering 2021; 4(s1):167
doi:10.4081/bse.2021.167

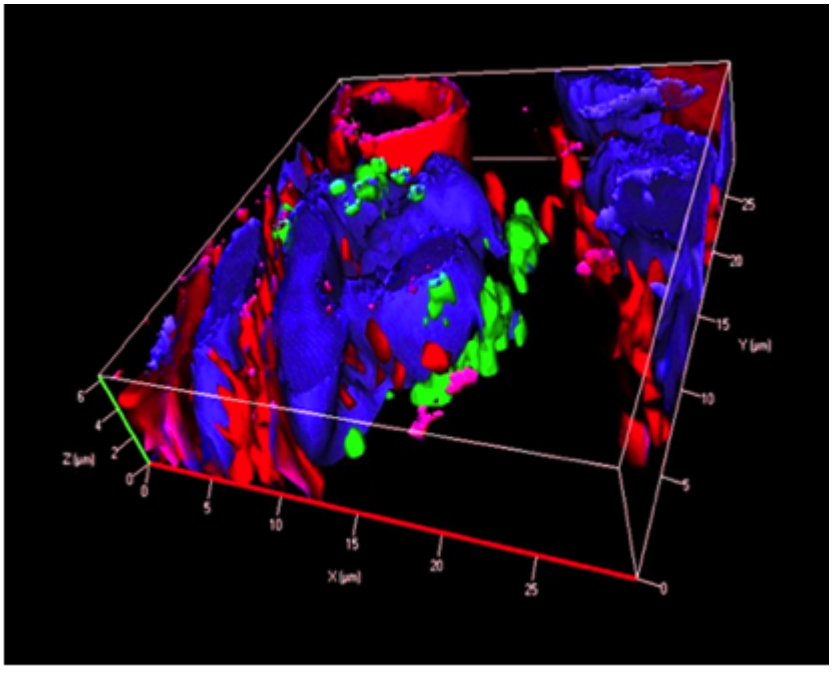

Figure 1. Three-dimensional reconstruction of invading streptococci (green) near cell nuclei (blue) after the disruption of the cytoskeletal architecture (red) of differentiated and polarized monolayers.

ity of GBS to adhere to or invade 5- and 21-day-old enterocytes was greatly enhanced. Immunofluorescent analysis of EGTA-treated cells revealed a huge number of invading streptococci in the parabasal and lateral sections of infected cells compared to their apical regions. Inhibitors of the clathrin endocytic way had no evident effect on GBS uptake, while methyl-β-cyclodextrin (MβDC) and genistein (Gen) treatment reduced the colony forming units (CFU) obtained from cell lysates. Intracellular actin inhibition by cythocalasin B (CytB) significantly decreased GBS invasion of enterocytes compared to microtubules inhibition. In the first minutes post-invasion, GBS was found enclosed in early endosomes, while, after 2h, inside acidified endosomes, as suggested by colocalizations with the marker EEA-1 and Lysotracker Red, respectively.

Discussion and Conclusions

In the present study, the absence of cell-to-cell interactions in differentiated monolayers facilitates GBS internalization suggesting the potential role played by specific cell receptors exposed mainly along the basolateral surface of enterocytes and absent in their apical side. This is particularly supported by the close GBS association with intercellular and/or the outer edges of intestinal islands. GBS adhesion to the lateral surfaces is favoured by the host actinic components and is followed by the involvement of lipidic and caveolar structures as suggested by the reduced number of viable intracellular bacteria in methyl-β-cyclodextrin- and genistein-treated cells. As confirmed by GBS colocalization with the endocytic marker EEA-1, bacteria firstly reside in the intracellular early endosomes, to be then trafficked into late endosomes remaining alive for a couple of hours, resembling the classical endocytic way used by other pathogens.[5] These results may be useful to develop new therapeutic strategies to reduce or prevent GBS infection of the infant gut. In light of this, the *in vitro* model used in the present work represents an interesting way to study more carefully each step of enteropathogens' invasion of epithelial cells and to better characterize the role played by their virulence factors in mediating it.

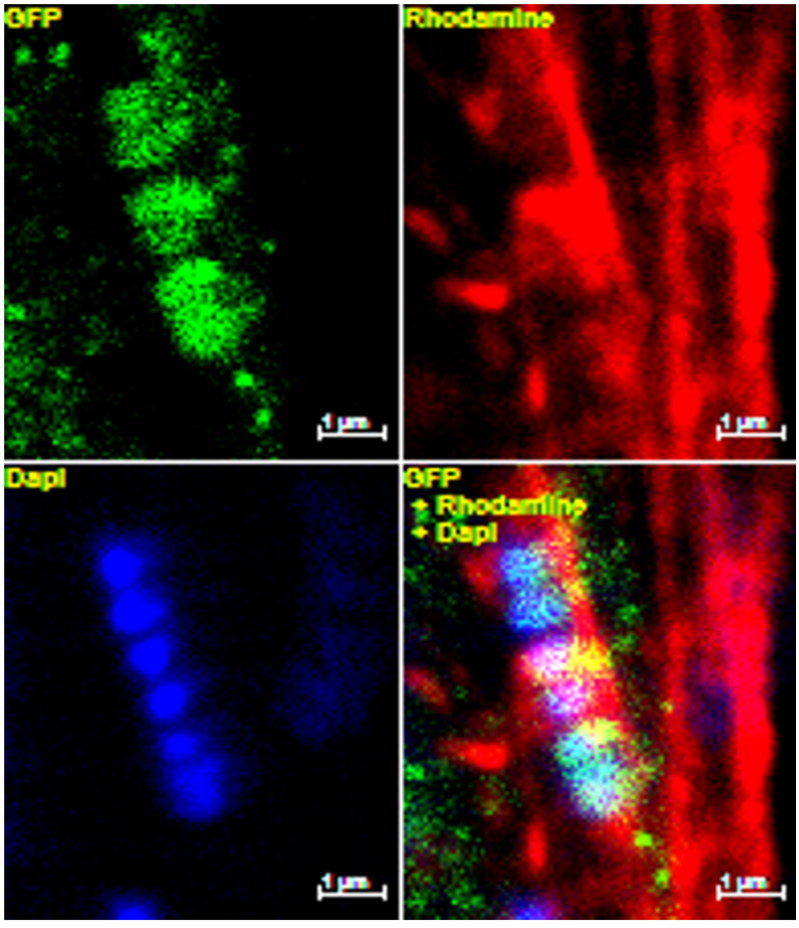

Figure 2. Co-localization of invading GBS (blue) with EEA-1 endocytic marker (green) and intracellular actin (red) in 5-days-old enterocytes.

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