

SIGA PROTECTIVE AND ANTI-INFLAMMATORY PROPERTIES IN HUMAN INTESTINAL EPITHELIAL CELLS

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INTRODUCTION

The intestinal immune system is the largest and most complex part of the immune system: it protects the organism against invasion and dissemination of pathogens and limits penetration of commensal microorganisms. **Intestinal epithelial cells** (IECs), the first component of mucosal surfaces, are in close contact with luminal contents and play a **crucial role** in signaling and mediating host innate and adaptive immune responses. The capacity of IECs to recognize factors including bacteria, viruses and antibodies is an integral aspect of first-line host responses. Cross-talk between cellular and molecular partners including **secretory immunoglobulin A** (SIgA) produced by plasma cells in the *lamina propria*, is an essential component of the complex function of the epithelium.

IEC lines have been frequently used to examine the consequences of the interaction with bacteria and proteins [1, 2, 3]. In such models, the **neutralizing capacity** of SIgA toward polarized epithelial cell monolayer is accompanied by the blocking of inflammatory signaling pathways and preservation of the epithelial integrity [4, 5]. We used these experimental settings to analyze the consequences of the interaction between IECs, microorganisms and SIgA based-immune complexes in models including **invasive bacterium** *Shigella flexneri* and **non invasive, commensal bacterium** *Lactobacillus paracasei*.

QUESTIONS ADDRESSED

- # How SIgA-based immune complexes are constituted?
- # How can bacteria or SIgA-based immune complexes modulate the inflammatory responses?
- # How commensal, non invasive bacteria can protect the epithelium integrity against invasive bacteria?
- # How IECs can discriminate between harmful and beneficial antigens?

MATERIALS AND METHODS

Materials

Cell line: Caco-2

Bacteria: *L. paracasei* ST11

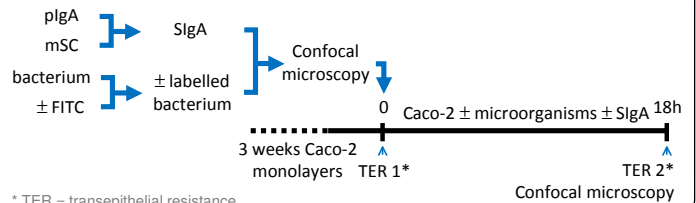
S. flexneri M90T

pIgA: anti-*S. flexneri* 5a LPS, C5

anti-*Salmonella* LPS, SAL4

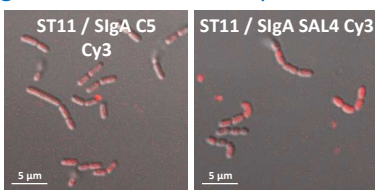
SC: mouse SC (clone 2H2)

Methods



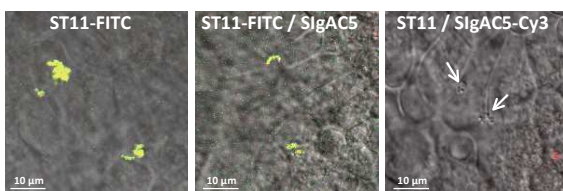
PRELIMINARY RESULTS (1)

SIgA-based immune complexes with commensal bacteria



Interaction between bacteria and SIgA is Fab-independent

The interaction between Caco-2 monolayers and commensal bacteria is affected by SIgA



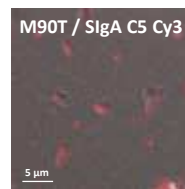
<i>L. paracasei</i>	ST11-FITC	ST11-FITC	ST11
SIgA	∅	C5	C5-Cy3
Distance from filter	39 µm	24.7 µm	10.4 µm

SIgA seems to impact on the distribution of commensals on the surface of epithelial cells

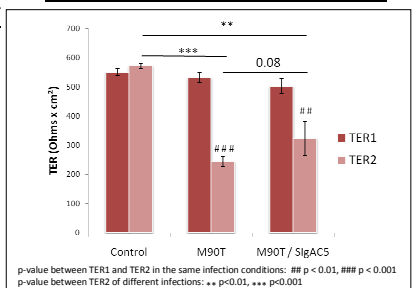
PRELIMINARY RESULTS (2)

The interaction between Caco-2 monolayers and *S. flexneri* is affected by SIgA

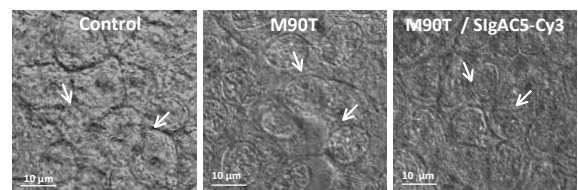
Interaction between M90T and its cognate SIgA



TER falls in presence of *S. flexneri*



Cellular morphology changes in presence of *S. flexneri*



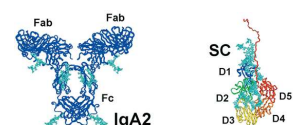
SIgA C5 seems to limit the infection by *S. flexneri* and reduce the alteration of the monolayer integrity

CONCLUSIONS/PERSPECTIVES

Our preliminary results first show that SIgA interacts with commensal bacteria in a **Fab independent way**. As SIgA is highly glycosylated, we plan to determine whether or not the interaction between SIgA and bacteria is **mediated through carbohydrate residues**. Deglycosylated SIgA will then be used to examine the possible entry of commensal / SIgA complexes within Caco-2 monolayers.

On the other hand, we have preliminary data demonstrating a **protective effect of SIgA** against an invasive bacterium. We will confirm this observation (TER measurements and confocal microscopy) and extend this analysis to proteins involved in **intercellular junctions** (eg, occludins). Another aspect, which needs to be taken in account, would be the protective effect of **commensals on the infection by *S. flexneri***. Finally, inflammatory signals emanating from Caco-2 cells, such as **cytokines /chemokines**, will be explored.

Structure and glycosylation (light blue) of IgA2 and SC [6]



REFERENCES

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