

# Anti-inflammatory properties of SIgA on intestinal epithelial cells during infection by *Shigella flexneri*

## INTRODUCTION



The intestinal immune system has the complex task to protect the sterile core of the organism against **invasion**. Most of invasive enterobacteria target **intestinal epithelial cells** (IEC) inducing major damages to the mucosa. *Shigella flexneri*, by invading IEC and inducing inflammatory responses of the colonic mucosa, causes bacillary dysentery, a bloody diarrhea that is endemic worldwide. The mechanism of entry of this bacterium is still **a matter of debate**. M cells participating in sampling antigens from the gut lumen through Peyer's patches are commonly considered as the primary site of entry of the bacteria. Once in the lamina propria, *Shigella* can invade IEC via their basolateral pole and spread from cell-to-cell leading to massive tissue destruction. More recently, data are accumulating demonstrating that bacteria can also enter the lamina propria directly via IEC, underscoring **IEC as another gate of entry**. In addition, the **protective role of secretory IgA** (SIgA) produced by plasmocytes of the lamina propria has been established in shigellosis context but few is known about its role in maintaining **IEC monolayer integrity**. Here, the impact of the bacterium was studied using polarized CaCo-2 cell monolayers apically infected with a virulent strain of *S. flexneri* either alone or complexed with its cognate anti-LPS SIgA.

## EXPERIMENTAL PROCEDURES

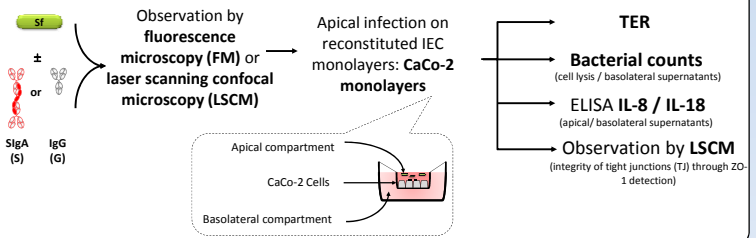
### Bacterial strain

*S. flexneri* M90TGFP (Sf): virulent strain which constitutively expresses GFP (2x10<sup>7</sup> bacteria/well)

### Hybridoma-isolated antibodies

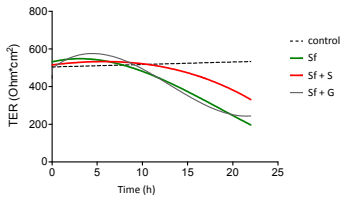
- pIgAC5 specific for *S. flexneri* serotype 5a LPS (10 µg/well) 
- > *In vitro* reassociation with SC
- IgGC20 specific for *S. flexneri* serotype 5a LPS (5 µg/well) 

### Apical infection of intestinal epithelial cell (IEC)



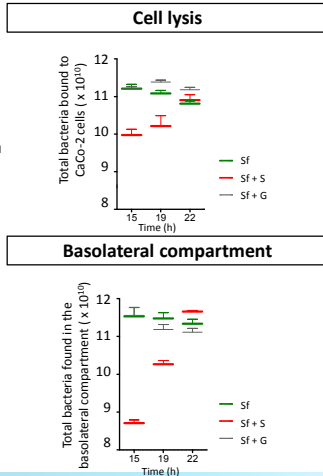
## RESULTS

### SIgA limits the drop of TER indicating the maintenance of the monolayer integrity

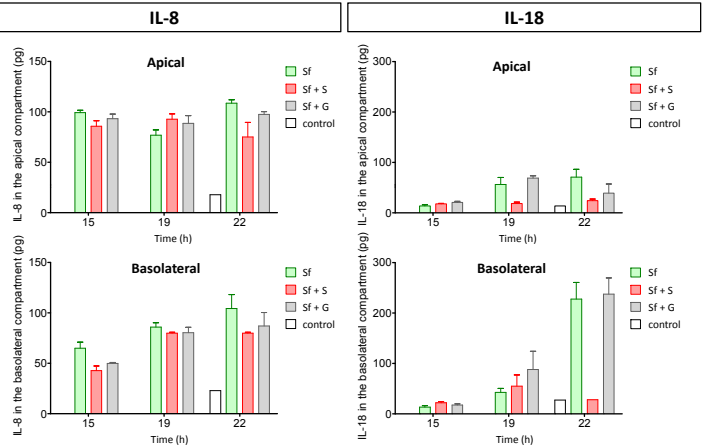


NB: experiments using a non-invasive strain results in absence of TER drop and bacterial growth in the basolateral compartment

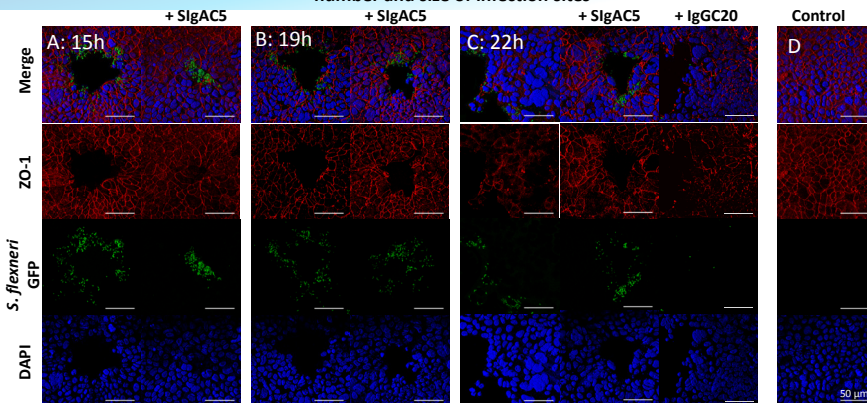
### SIgA delays the infection limiting both bacterial growth and proliferation in the basolateral compartment



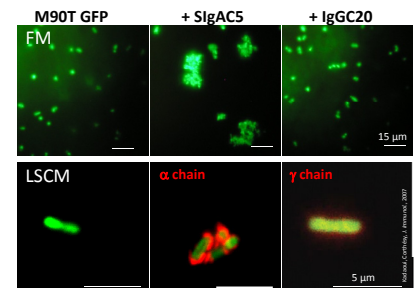
### Neutralization by SIgA results in various time-dependant cytokine release



### LSCM visualization demonstrates that SIgA is associated with ZO-1 network maintenance and with a reduction in the number and size of infection sites



### SIgA contrary to IgG induces the formation of large networks of coated bacteria with reduced viability (data not shown)



## CONCLUSIONS/OUTLOOKS

We demonstrate that bacteria are able to **infect IEC through their luminal-like pole**, inducing the **complete disruption of tight junctions (TJ)** and the destruction of the whole reconstituted CaCo-2 cell monolayer after one day post infection. Contrary to IgG, SIgA upon neutralization of bacteria led to the **maintenance of TJ** supporting IEC integrity, and the reduction of **cytokine release**. Together with anti-inflammatory properties of SIgA, the fact that apical bacteria can damage the IEC without the intervention of other cells such as M cells strongly suggests **new mechanisms** of invasion that can be involved in shigellosis.

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