ImmunoTools *FlowISiAM* Award 2024

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Uncovering the role of activated monocytes in *Clostridioides difficile* infection.

Background. *Clostridioides difficile*, a Gram-positive, spore-forming bacterium, is recognized as the primary cause of nosocomial antibiotic-associated diarrhoea and pseudomembranous colitis. In Europe, it ranks as the 6th most commonly detected microorganism among hospital infections, with an incidence of 3.48 cases per 10,000 patient days (Stockholm. ECDC; 2022). In the United States, it causes approximately 29,000 deaths annually (Lessa FC, *et al.* NEJM 2015). *C. difficile* is primarily transmitted from person to person through contaminated hands or environmental surfaces. Spores of *C. difficile* persist in the human gut for extended periods, but upon antibiotic exposure, the vegetative forms of the bacteria produce toxins, which are the principal virulence factors driving infection. **The toxins are immunogenic, recruit neutrophils and monocytes and promote inflammation**, tissue damage and diarrhoea (*Castagliuolo I, et al. Keio J Med 1999; Kordus SL, et al. Nature Rev Microbiol 2022*). Antibiotics successfully control initial infections. However, antibiotic administration is often associated with resistance and disease relapse, ranging from 15% to 25% post-initial treatment (*Song JH, et al. Gut 2019*). Subsequent recurrences further complicate the management of infections, with relapse rates increasing from 40% to 60%, highlighting the limitations of standard antibiotic regimens. Beyond antibiotics, alternative treatments such as toxin-neutralizing antibodies, molecular adsorbents, and faecal transplantation are potential options (*Bloom PP, et al. Expert Opin Biol Ther 2022*), although reliable markers for disease recurrence and treatment monitoring remain elusive.

Aims. This project has two main objectives: 1. Assess the expression of markers of tissue activated macrophages (CD14 and CD16, *Coy JF. Int J Mol Sci 2017*) in human primary macrophages exposed *in vitro* to *C. difficile* cultures. 2. Investigate the occurrence of tissue-activated macrophages carrying C. difficile antigens in the bloodstream.

Methodology: Human peripheral blood mononuclear cells obtained from healthy donors will be differentiated *in vitro* by incubating them with ImmunoTools GM-CSF, M-CSF. Subsequently, cells will be polarized using lipopolysaccharide and ImmunoTools IFN-γ, IL-4, or IL-10. Cells will be then exposed to toxigenic or non-toxigenic *C. difficile* standard strains or clinical isolates. We will use *FlowISiAM* to assess surface expression markers. Positive

cells will undergo sorting and intracellular bacterial determinants will be initially identified using molecular techniques. Collaborating with ImmunoTools and INVIGATE, we will then develop customized antibodies capable of detecting bacterial antigens and useful in *FlowISiAM* applications. Once set, the protocol will be translated *in vivo*. Upon the ethical commission approval, blood samples will be scrutinized using the *FlowISiAM* to detect *C. difficile* materials within activated cells. Results will be correlated with infection clinical scores or analyzed as potential predictive markers for disease reactivation.

Cooperation: Prof. Paola Brun's group works closely with the Microbiological Unit and the Gastroenterological Unit of the Padova University Hospital for biological sample recovery and will cooperate with ImmunoTools to establish *FlowISiAM* analysis. ImmunoTools will support the research by providing reagents such as recombinant growth factors for primary macrophages.

Prof. Dr. Paola Brun, alongside Dr. Sebastian Krause of INVIGATE, is committed to advancing their research on optimizing bacterial-specific monoclonal antibodies. Their collaborative efforts aim to generate compelling proof-of-principle outcomes essential for a forthcoming joint research grant application.

Impact: This project promises to deepen our understanding of processing antigens of *C. difficile* during infection and provides valuable insights for future non-invasive diagnostic advancements. As the findings of this study could be easily translated to other pathogens, we seek to develop and permanently improve *FlowISiAM*-based strategies for the early detection of infective agents.

ImmunoTools *FlowISiAM* AWARD for **Paola Brun** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.