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EDITORIAL

Single-cell analysis: Understanding infected cell heterogeneity

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Biological processes occasionally present a stochastic behavior. Variable performances can be observed for genetically identical organisms under the same environment conditions, which are invisible if phenomena are analyzed for the population as a whole, thus limiting a complete biological understanding. The technological developments in the field of fluorescence microscopy combined with increasingly automated analytical approaches, can now allow the study of extensive populations at the individual cell level. Over the last number of years, the number of publications reporting such an approach has increased with applications in a wide variety of fields including bacterial replication,[1,2] bacterial cell morphology,[3] and deformation of red blood cells.[4] In this issue of *Virulence* Ramos-Marquès et al.[5] combine up-to-date technologies and single cell analysis to obtain an insight of how bacterial pathogens interact with host cells.

They take advantage of fluorescent proteins (FP) expressed in bacteria and in eukaryotic cells to follow over time the host-bacteria/pathogen interaction.[6] FPs are usually not toxic and allow the long-term observation of organisms. Moreover, in most cases fluorescent tagging does not impact protein localization and function when compared to their native counterpart. These features, when applied in experiemental settings, therefore create great confidence regarding the meaningfulness of collected data. The team uses eukaryotic cells and *Salmonella* Typhimurium, expressing p65-GFP and DsRed respectively, to follow NF-κB activity during the infection process. From the initial stages in the gut through to chronic infection in the gallbladder, *Salmonella* modulates gene expression to colonize and survive in a large variety of cells and tissues. These bacteria initially activate the immune response to facilitate their spreading to different organs[7] but for a long-lasting chronic infection, they must hide from the immune system and dampen inflammation. NF-κB is a protein complex that controls the transcription of genes involved in inflammation and *Salmonella* targets the NF-κB signaling pathway in order to facilitate a more favorable adaptive reponse.[8]

A large number of studies have reported the modulation of the NF-κB pathway in response to a *Salmonella* infection.[8] However they generally use techniques that do not consider spatial and temporal parameters simultaneously. Microarrays, Northern- and Western-blotting are (so far) limited to the analysis of populations. By flow cytometry or PCR, it is not possible to track the same cells over time. Electron or classical fluorescence microscopy require the use of fixed samples that preclude an observation in a dynamic mode. This recent study[5] analyses at the single cell level the dynamics of the NF-κB pathway during an infection. Using a software that automatically defines cell segmentation based on the Hoechst nucleus staining while tracking the p65-GFP signal, they were able to calculate the nuclear to cytoplasmic ratio of the GFP intensity, which was then exploited as a read-out of NF-κB activation. Additionally DsRed in the cytoplasmic region was used to identify and compare responses in infected or uninfected fibroblasts.

Thanks to the implementation of this strategy, they found that when compared to naïve cells, infected and uninfected cells exhibit specific oscillatory NF-κB signaling dynamics and a variable capacity to respond to external signals, such as TNF-α challenge or secondary infection. These differences may be due to bacterial effector proteins that alter signaling in infected cells while these cells may keep the capacity to transmit a proinflammatory signal to adjacent cells, possibly via gap junctions as it was shown in HeLa cells.[9] These observation uncover the considerable benefit of performing a single cell study as compared to using average values from whole cell populations where such insights would be buried.

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While the ability to survive and replicate in macrophages is essential for virulence, *Salmonella* also targets other cell types. In the mouse model of infection, bacteria reside in various cells of the immune system[10] or epithelial cells of the intestinal tract and gallbladder.[11] Low numbers of *Salmonella* have also been found in non-phagocytic stromal cells of the intestinal lamina propria that are probably fibroblasts.[12] In this cell type *Salmonella* actively restrain their proliferation. Carrier state infections are commonly observed in human and domestic fowl following an acute phase. Fibroblasts infected at low level with dormant *Salmonella* may be a source of relapse and shedding. From this point of view and considering the different scenarios that can occur during a *Salmonella* infection, the development of this atypical fibroblast model is another valuable addition to the field.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

[1] McQuate SE, Young AM, Silva-Herzog E, Bunker E, Hernandez M, de Chaumont F, Liu X, Detweiler CS, Palmer AE. Long-term live-cell imaging reveals new roles for Salmonella effector proteins SseG and SteA. Cell Microbiol 2016; PMID:27376507

[2] O'Neill AM, Thurston TLM, Holden DW. Cytosolic Replication of Group A Streptococcus in Human Macrophages. MBio 2016; 7:e00020-16

[3] Shen S, Syal K, Tao N, Wang S. Note: An automated image analysis method for high-throughput classification of surface-bound bacterial cell motions. Rev Sci Instrum 2015; 86:126104; PMID:26724085; https://doi.org/10.1063/1.4937479

[4] Byeon H, Ha Y-R, Lee SJ. Holographic analysis on deformation and restoration of malaria-infected red blood cells by antimalarial drug. J Biomed Opt 2015; 20:115003-3; PMID:26544670; https://doi.org/10.1117/1.JBO.20.11.115003

[5] Ramos-Marquès E, Zambrano S, Tierrez A, Bianchi ME, Agresti A, García-Del Portillo F. Single-cell analyses reveal an attenuated NF-κB response in the Salmonella-infected fibroblast. Virulence 2017; 8(6): 719-740; https://doi.org/10.1080/21505594.2016.1229727

[6] Tsien RY. Nobel lecture: constructing and exploiting the fluorescent protein paintbox. Integr Biol (Camb) 2010; 2:77-93; PMID:20473386; https://doi.org/10.1039/b926500g

[7] Behnsen J, Pérez-López A, Nuccio S-P, Raffatellu M. Exploiting host immunity: the Salmonella paradigm. Trends in Immunology 2015; 36:112-20; PMID:25582038; https://doi.org/10.1016/j.it.2014.12.003

[8] Giogha C, Lung TWF, Pearson JS, Hartland EL. Inhibition of death receptor signaling by bacterial gut pathogens. Cytokine Growth Factor Rev 2014; 25:235-43; PMID:24440054; https://doi.org/10.1016/j.cytogfr.2013.12.012

[9] Kasper CA, Sorg I, Schmutz C, Tschon T, Wischnewski H, Kim ML, Arrieumerlou C. Cell-cell propagation of NF-κB transcription factor and MAP kinase activation amplifies innate immunity against bacterial infection. Immunity 2010; 33:804-16; PMID:21093316; https://doi.org/10.1016/j.immuni.2010.10.015

[10] Aussel L, Zhao W, Hébrard M, Guilhon A-A, Viala JPM, Henri S, Chasson L, Gorvel J-P, Barras F, Méresse S. Salmonella detoxifying enzymes are sufficient to cope with the host oxidative burst. Mol Microbiol 2011; 80:628-40; PMID:21362067; https://doi.org/10.1111/j.1365-2958.2011.07611.x

[11] Knodler LA, Vallance BA, Celli J, Winfree S, Hansen B, Montero M, Steele-Mortimer O. Dissemination of invasive Salmonella via bacterial-induced extrusion of mucosal epithelia. Proc Natl Acad Sci USA 2010; 107:17733-8; PMID:20876119; https://doi.org/10.1073/pnas.1006098107

[12] Núñez-Hernández C, Tierrez A, Ortega AD, Pucciarelli MG, Godoy M, Eisman B, Casadesús J, García-Del Portillo F. Genome expression analysis of nonproliferating intracellular Salmonella enterica serovar Typhimurium unravels an acid pH-dependent PhoP-PhoQ response essential for dormancy. Infect Immun 2013; 81:154-65; https://doi.org/10.1128/IAI.01080-12