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Highlight

Enemy at the gates☆

A major part of the permanent exhibition of the Mauer-museum at the Checkpoint Charlie, one of the crossing points between East and West Berlin during the Cold War, is dedicated to the countless infamous escapes, successful or not, through what was once supposed the world's most secure border system [2]: some fled the Eastern German regime with a hot-air balloon made of raincoats, others with an improvised zip line after shooting an arrow connected to a fishing line over the border, a man reached Denmark *via* the Baltic Sea dragged by a rudimentary submarine and numerous people managed to escape through the wastewater system thanks to an initial official authorization to inspect the tunnels for a school presentation [3,4].

Inevitably, the stroll through this collection of artifacts oozing with thrilling adventure raises an irrepressible, morbid desire in the carefree visitor, oblivious to the not so pleasant circumstances nearly half a century ago, to be part of *real* History and secretive meetings, nocturnal digging operations and encoded communications.

But just as much the human ingenuity calls forth admiration, the creativity deployed since millions of years of a co-evolutionary fight for the gates of the multicellular organism between hosts and pathogens definitively commands some respect, especially if we consider things for once from the microbial point of view, or as said one the French humorist Pierre Desproges – "The enemy is stupid. He thinks that we are the enemy while it's him!"

The various epithelia of the human body account for several hundreds of square meters of direct contact with the exterior world and its charming residents [5–7]. The cellular units of those border territories are tightly hold together and guarded by tight junctions as the first line of defense in the apical region, followed by the basolateral adherens junctions [8]. Tight junctions contain a variegated cocktail of proteins, including 24 types of claudins, occludins and junction adhesion molecules (JAMs) [8,9], whose precise composition varies according to the exact location and flux requirements in the organism [9]. In charge of the complex, hay paradoxical task of being both efficient barriers and channels, they monitor and modulate the paracellular flow of water, small solutes and macromolecules, bar the way to invaders but have to let rush through sometimes leukocytes in the middle of diapedesis [5,10,11]. Furthermore, at the cell level, junction proteins preserve cell surface polarity and the unique composition of the apical membrane - optimized for facing the harsh exterior conditions as well as for transport and signaling events - by impeding lipid diffusion [9,12]. And finally, the junction complexes represent the central information relay platform between the inside and outside worlds. Anyone used to tend to the various needs of cell cultures knows the importance of confluency and how easily cells feel lonely or crowded. Junction proteins transmit environmental clues to the cell interior so as to decide on cell proliferation, survival, differentiation and migration *via* a direct bidirectional connection with the cytoskeleton, adaptors and signaling proteins, and are in turn rearranged according to nuclear orders [10,12].

Defective composition or localization of junction complexes take some of the responsibility of diseases linked to fluid absorption and the resulting low grade inflammation, including inflammatory bowel disease (IBD), gallstones and Crohn's disease [9]. And of course, pathogens are having a field day with junction proteins. Not every microbe has time to spend with transmigration through an epithelial or endothelial cell, squeezing in between cells turns out to be a quick and discrete option to cross any border, including additional cellular blockades like the blood brain barrier or the placenta, while quickly pressing some buttons for internal cell signaling in passing [5,6,12]. Endowed with the blind resourcefulness of evolutionary tinkering, every pathogen developed its own favorite strategy, or rather strategies to trick or overpower the faithful guards of our innards [13]. While revisiting some stunning passages of recent History, here's an overview of the main roads to the human organism.

When confronted with a wall and when trying to climb in usually lead to being shot, the most nearby solution consists in a tunnel. When confronted with the intestinal epithelium and when trying to attach to it usually leads to get entangled in viscous mucus made of proteolytic enzymes and antimicrobial peptides, the only solution consists in getting across as fast and unnoticed as possible [14]. One of the most famous tunnels leading from East to West Berlin is the "Seniorentunnel", the tunnel of the elders, built by a group of 12 retirees in 1962 and

☆ Article highlight based on "Elucidating pathways of *Toxoplasma gondii* invasion in the gastrointestinal tract: Involvement of the tight junction protein occludin" by Caroline M. Weight et al. [1].

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of unusual height in order to reach freedom "comfortably and unbowed". Bacteria for their part use flagella and lipid charge neutralization, alter the mucosal pH, produce their own extracellular matrix or cleave mucins to reach the epithelial surface [14]. And then?

Brute force is of course an option. Several inhabitants of the GDR equipped vehicles with armor plates and bulletproof screens, or even stole a tank, and breached through the tollgates. In 1961, a young train engine driver crashed at full speed through the fortifications of the early wall with the "last train to freedom" [3]. Many pathogens also make their way without much subtlety by damaging tight junction components or entire epithelial cells [5,14]. Some secrete various toxins that dissociate junctional proteins, promote their internalization followed eventually by lysosomal degradation [9,12] or induce apoptosis and cell lysis [14]. Others wash their hands of responsibility and persuade the host cell's matrix metalloproteases (MMPS) to execute the cleavage [8] and some pro-inflammatory cytokines to increase the barrier permeability [5,13].

For those who don't necessarily want to cause a stir, hijacking the elements and attributes of the adversary might be a good alternative.

In order to fetch their third sibling from East Berlin, two brothers learned to fly, dressed in military uniforms and painted their planes with the Soviet red stars, allowing them to have an unmolested trip over the wall in early 1989 [4]. In a less exhausting fashion, it is told that a couple of border crossers just waved membership cards of the *Munich's Playboy Club*, coincidentally mimicking diplomatic passports, under the noses of the guards who let them pass [3].

All kinds of pathogens have figured out since a long time how to "legally" use host factors, including junction proteins, so as to attach and sneak either into the cell by provoking endocytosis or the intercellular space [6,7]. Taking advantage of the situation and given the eminent position of cell connectors in the cellular communication network, many pathogens target additionally components of the tight junctions to trigger signaling cascades that adjust host cellular behavior, such as the cell cycle stage or polarity, to their specific needs [8,12]. Reciprocally, pathogens have at their disposal many means to influence the host genetic expression and signaling networks, like the bacterial type III secretion system effectors, including those governing the expression levels of junction proteins, allowing them to reduce among others the number of fence-forming claudins and increase the channel-forming ones [5,8,12]. The masters of embezzlement are even slikked enough to reposition claudins and occludins into pores leading to uncontrolled calciulm influx and ultimately cell death [9].

One of the most ancient subterfuges though, according to mythology, is also quite popular among pathogens. Homer relates in his *Odyssey* how the Greeks built a huge and hollow horse under the guidance of Ulysses, hid inside and let the Trojans, convinced that the enemy had given in drag them into the impregnable city. The East German variant was the Trojan cow – a hollow exhibit piece, which could harbor one human [15]. Perpetuating the analogy with the Ancient Greeks, the role of the Achilles' heel of the intestinal epithelium is played by the unfortunate microfold cells, which are in charge of continuous luminal sampling of microbes in order to display them for the underlying antigen-presenting cells. Normally, this allows the immune system to tell apart commensal microorganisms from attackers and decide between tolerance and ringing the tocsin, but these M cells, devoid of mucus, are also a ticket to ride for pathogens to gain access to the lamina propria [7,8,14].

However, the bilateral continuous pressure kept evolution on its toes. Once word spread that one could escape the totalitarian system *via* the sewage tunnels, the system stroke back with more and more sophisticated railings and mechanic motion sensors [15]. The camouflage techniques allowing bacteria to dodge and counter the cationic antimicrobial peptides of the intestinal mucus layer caused the increase of the cationic properties as well as the appearance of numerous disulphide bonds and thus a more resistant conformation of the latter [14].

Frontiers are a sensitive issue, especially considering the current European politics and population dynamics, leaving both divided the public in terms of opinions and the specialists in terms of predicted outcome, although migration and border crossing have always been inherent motives of human History.

But also the precise events at our internal gates hold both many secrets and promises [13]. Numerous certainly very useful *ex vivo* epithelial cellular models in monolayers exist, however, as extensively discussed in a previous highlight, infection and invasion dynamics are only partially recapitulated in two dimensions [16]. Observations *in vivo* are hampered in turn by the rapidity of the processes – *Toxoplasma gondii* needs 40 s to infect small intestinal epithelial cells and reaches the lamina propria in 1 h while leucocytes squeeze between endothelial cells in one to 2 min [6,7,11]. Therapeutic hope comes from hepatitis C virus though, and its receptor claudin-1 (CLDN1) whose obstruction by antibodies efficiently inhibits HCV entry in mice [5].

At least judging by the ingenuity of our evolution-long enemies, according to Roosevelt[1], the epithelial guards deserve some praise.

1. Biosketch

Dr. Caroline Weight graduated from the University of York with a degree in Biology. She joined Prof. Cardings' Lab in 2006 working on the infection mechanisms of *T. gondii* in intestinal epithelial cells. In 2012 she started her postdoc with Prof. Parkos and Prof. Nusrat at Emory University in the USA where she investigated intracellular signaling pathways with a specific focus on the tight junction protein JAM-A. Caroline moved to Lund University in Sweden, in 2014, as a senior postdoc funded by a WennerGren Fellowship, and is currently working on negative feedback pathways in proximal T cell signaling in the Leukocyte Migration group.

[1] "I ask you to judge me by the enemies I made" (Franklin D Roosevelt).