Professor Dr Nicole Borel and her research group at the Center for Applied Biotechnology and Molecular Medicine illustrate new therapeutic strategies for treatment of chlamydial infections in humans and animals

More than meets the eye

Trachoma is the leading cause of infectious blindness in the world. This devastating disease is caused by the ocular serovars (A, B, Ba, and C) of the Gram-negative, obligate, intracellular bacterium *Chlamydia trachomatis,* which is hyperendemic in sub-Saharan Africa, the Middle East, as well as parts of Asia and central and South America. Globally, 84 million people, the majority of whom are children, suffer from active ocular *C. trachomatis* infection, and nearly eight million people are visually impaired as a result (Fig. 1).

Trachoma, a disease of poverty and poor hygiene, is categorised as a 'neglected tropical disease', and a number of global health organisations are working together to eliminate blinding trachoma by 2020; this includes the GET 2020 Alliance and the SAFE strategy of the World Health Organization.

The primary frontline antibiotics used to treat ocular chlamydial infection and prevent trachoma are oral azithromycin and topical tetracycline. However, there are drawbacks to antibiotic treatment of chlamydial infections, including unwanted and sometimes dangerous side effects, expenses (which may be prohibitive in poorer countries where trachoma is endemic), and the on-going and problematic emergence of antibiotic-resistant bacteria. Furthermore, incomplete or failed elimination of chlamydiae by antibiotic treatment may lead to deregulation of chlamydial development.

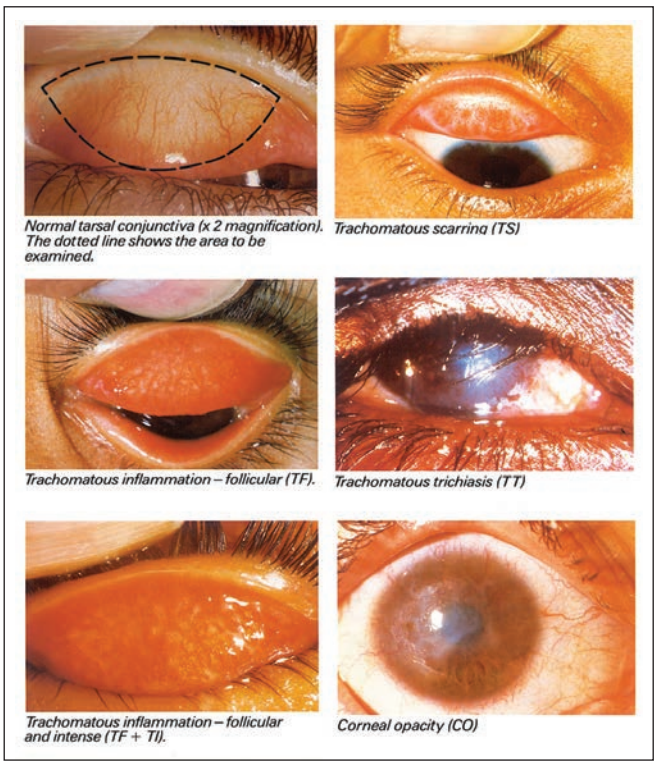

Fig. 1 Trachoma grading, adapted from the World Health Organization trachoma simplified grading card

Fig. 2 wIRA setting for *in vitro* experiments

Recent *in vitro* and *in vivo* studies indicate that a break in the normal chlamydial developmental cycle can result in chlamydial 'persistence' and long term infection that is refractory to antibiotic treatment. Such 'persistent' infections can cause a cascade of on-going inflammatory-induced sequelae, resulting in scarring of the conjunctiva and trichiasis (Fig. 1) which cannot be reversed by antibiotic treatment and can only be corrected by ocular surgery. Thus, novel but non-pharmacologic therapeutic strategies for trachoma are of high interest.

New therapeutic strategies

Water-filtered infrared A (wIRA) is short wavelength infrared radiation with a spectrum ranging from 780 to 1440nm. Light from a halogen bulb, passing through a water containing cuvette, emits wIRA and visible light (VIS) (Fig. 2). wIRA alone, or in combination with VIS (wIRA/VIS), has been used in various clinical settings, and its efficacy has been proven in acute and chronic wound-healing processes.

Due to its high tissue penetration and low thermal load on the skin surface, wIRA does not cause the skin irritation and overheating associated with unfiltered IRA irradiation. This makes wIRA an effective means to increase tissue temperature, oxygenation and perfusion, all important factors positively influencing wound healing. Consequently, wIRA has been shown to reduce the frequency of secondary wound infections.

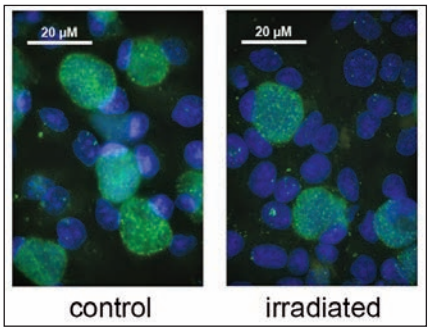

Fig. 3 Irradiation of chlamydial elementary bodies reduces their infectivity on host cells

Following abdominal surgery, a lower rate of wound infections was observed after post-operative wIRA/VIS irradiation compared to treatment with VIS alone. However, few preliminary data on the treatment of infectious conditions with wIRA irradiation have been reported so far. The direct effect of wIRA on pathogens *in vitro,* and in particular on obligate, intracellular agents such as chlamydiae, has not been shown before.

Acute chlamydial infection

Recent *in vitro* investigations by our group have revealed the exposure of chlamydiae prior to host cell infection. The exposure of Chlamydia-infected cells to wIRA/VIS irradiation reduces both the number of chlamydial inclusions that develop within host cells and the subsequent production of infectious chlamydiae, without any negative impacts on host cell viability. The efficacy of wIRA/VIS irradiation in reducing the infectious chlamydial forms (the elementary bodies (EB)) was demonstrated in animal-infecting as well as human-infecting chlamydial species.

Fig. 3 shows *C. trachomatis* EBs either irradiated with wIRA/ VIS (20 minutes, 3700W/m^2) or not irradiated (control), prior to infection of HeLa monolayers (human-derived cervical epithelial cell line). Cultures were incubated for 43 hours, fixed, and immune-labelled with anti-chlamydial LPS (green, chlamydial inclusions) and DAPI (blue, host cell nuclei). Frequency of inclusions per nucleus was calculated, and irradiation resulted in an approximately 50% reduction in the number of host cells infected (not shown; $p \leq 0.05$, $n = 3$, *t* test). Representative microscopic pictures at 1,000 times magnification are shown.

Furthermore, multiple-dose irradiation, as applied in clinical settings of wound healing and reduction of wound infection, resulted in an even more profound reduction of chlamydial burden *in vitro*. Importantly, we showed that wIRA/VIS does not induce cytotoxicity in two different permanent cell lines, one of human origin and one of non-human primate origin, even at high doses of wIRA/VIS or long term exposure.

Additionally, in a collaborative project with a group of the Darmstadt Technical University in Germany, wIRA treatment has been demonstrated to be undamaging to the pig eye. Incidentally, because wIRA/VIS reduces infectivity of directly irradiated extracellular chlamydial infectious EBs, potential applications for decontamination of healthcare and/or agriculture settings may be viable.

The benefits of wIRA treatment

Potential advantages of using wIRA to treat trachoma patients as an alternative or combination therapy in the future are manifold. The wIRA technology is already commercially available and has been shown to speed wound healing, reduce inflammation, and decrease secondary wound infections in clinical trials.

The wIRA device is applicable in the field, easy to use and does not require skilled medical personnel. The efficacy of a single dose of wIRA has been shown *in vitro* to reduce the chlamydial infectious burden by 50% without detriment to the host cells. Additionally, the anti-chlamydial effect of wIRA is most effective late in the chlamydial developmental cycle when anti-chlamydial drugs are less effective.

Inappropriate or inconsistent antibiotic therapy is a primary factor leading to the emergence of drug-resistant bacteria, which is particularly problematic in developing countries where antibiotics can be acquired only periodically and where use of expired or counterfeit medications is common. Because the use of wIRA may facilitate reduced dependence on antibiotic treatment, it has the potential to help reduce the incidence of bacterial antibiotic resistance. Because wIRA exposure reduces production of infectious chlamydiae, it may be expected to reduce/prevent *C. trachomatis* transmission as well as trachoma disease progression.

Current investigations

When it comes to treating trachoma patients, wIRA shows promise as a valuable therapeutic strategy. On-going work in our lab aims to demonstrate proof of concept for ocular wIRA treatment in an *in vitro* conjunctival cell culture model and an *ex vivo* animal model of ocular infection, respectively.

The transition to a more clinically relevant human conjunctival epithelial cell culture and ocular *C. trachomatis* strain wIRA exposure model, from our current successful and established non-conjunctival permanent epithelial cell and non-ocular *C. trachomatis* strain model, is now one of the main aims in the Borel research group at CABMM. It is expected that wIRA will reduce inclusion formation and production of infectious *C. trachomatis* EBs in conjunctival epithelial cells by 50% or more. Multiple or longer wIRA doses will likely further reduce inclusion formation and production of infectious EBs without deleterious effects on the host cells.

Our second aim will be to establish an *ex vivo* animal eye model (sheep) for infection with the animal chlamydial pathogen *C. pecorum* followed by exposure to wIRA. Natural infection with *C. pecorum* in sheep causes conjunctivitis. Preliminary *in vitro* data have already shown the effective reduction of wIRA/VIS in *C. pecorum*-infected cells. We expect that the *ex vivo* sheep eye infection model will show that wIRA exposure reduces chlamydial inclusion formation, shedding of infectious *C. pecorum,* and/or ocular inflammation.

The next step will be to establish an *in vivo* sheep model to evaluate the therapeutic effect of wIRA on conjunctivitis induced by *C. pecorum*. There are no existing animal models for *C. trachomatis* infections with the exception of a non-human primate model. The sheep model will be more technically and economically feasible than a non-human primate model. Shedding of infectious chlamydiae and subsequent inflammation will be scored and statistically evaluated so that both the anti-chlamydial and anti-inflammatory effects of wIRA *in vivo* can be determined.

In trachoma patients, the wIRA device could theoretically be used in conjunction with antibiotic therapy or as an alternative to antibiotic therapy, both to treat eye infections and to prevent disease transmission. The wIRA device has the potential to be utilised by technical personnel and/or physicians working to treat trachoma in a variety of settings.

Related projects

Chlamydiae not only induce trachoma but can also cause a wide range of acute and chronic diseases in animals and humans worldwide. They are responsible for economically important diseases such as mastitis, endometritis, conjunctivitis and pneumonia, and those which cause abortion in livestock. More recently, their role in asymptomatic gastrointestinal infections with recurrent chlamydial faecal shedding has been re-emphasised in veterinary medicine.

A recent investigation in our group demonstrated that more than 90% of the Swiss fattening pigs harbour *C. suis*, the pig-infecting chlamydial species, in their intestines. These chlamydiae, frequently resulting in entirely asymptomatic infection in swine, might be considered to be a part of the non-disease associated gut microbiome. However, on the other hand, *C. suis* is the sole chlamydial species demonstrated capable of acquiring stable antibiotic resistance (tetracycline resistance gene), presumably by lateral gene transfer. The gut may therefore represent an ideal niche and potential reservoir for dissemination of antibiotic-resistant chlamydiae.

The ability of chlamydial organisms to establish chronic, frequently subclinical infections has been hypothesised to correlate with chlamydial 'persistence' or 'the chlamydial stress response'. Fig. 4 shows that infectious EBs and replicative reticulate bodies (RB) comprise the characteristic biphasic chlamydial developmental cycle. Various stressors, including the host cytokine interferon-gamma and beta lactam antibiotics, such as penicillin, can cause the chlamydiae to enter persistence. Persistent chlamydiae are defined as viable but non-infectious and can resume production of infectious EBs upon removal of the stressor. *In vitro* and *in vivo* animal studies have shown persistent chlamydiae to be resistant to killing with azithromycin, a frontline anti-chlamydial drug.

This *in vitro* phenomenon is speculated to be associated with chronic clinical conditions in humans such as chronic bronchitis, asthma, atherosclerosis, reactive arthritis and genital infections in women leading to infertility. Notably, genital serovars of *C. trachomatis* are responsible for the most common bacterial cause of sexually transmitted diseases, and understanding the factors affecting the pathogenicity of chlamydial infection is therefore of broad human clinical and veterinary relevance.

Outlook

A current project in our lab investigates the mechanism leading to the 'persistence' chlamydial state in a co-infection model with chlamydiae and viruses naturally occurring in the porcine gut. Additionally, we have recently determined that damage/danger-associated molecules, typically released from mammalian cells/tissues in the course of pathogen infection and/or non-infectious trauma, inhibit both human-infecting and porcine-infecting chlamydial development *in vitro*. This suggests that these molecules might play a role in chlamydial development *in vivo*, both in humans and in animals of agricultural and economic importance, particularly in the context of poly-microbial infections.

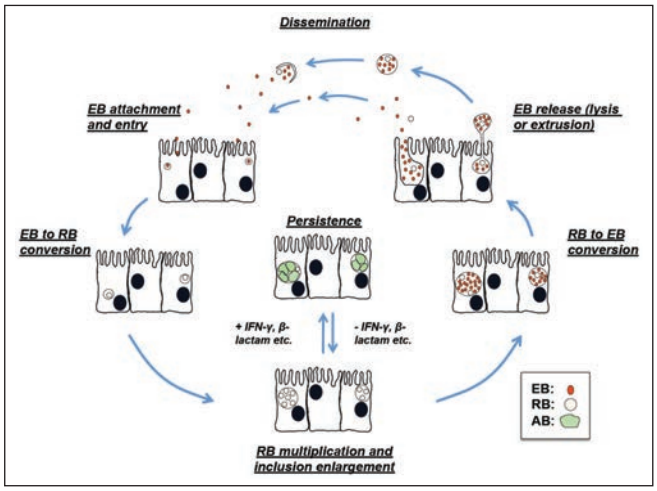

Fig. 4 Chlamydial developmental cycle, courtesy of R V Schoborg, *Microbes and Infection*, 2011

The inhibitory effect of wIRA/VIS on chlamydial infection has been proven *in vitro*. However, more preliminary experiments are necessary before the wIRA device can be implemented for the treatment of trachoma patients still suffering from the devastating leading cause of infectious blindness worldwide. Research in our lab is on-going in an effort to determine the mechanism(s) responsible for the anti-chlamydial effect of wIRA/VIS. Our current studies indicate that thermal as well as non-thermal effects contribute to the inhibitory impact of wIRA/VIS exposure on chlamydiae.

The long term goal of this project and the described related projects is to provide further insight into the host/pathogen interactions, pro-inflammatory mechanisms, and immune evasion strategies of this fascinating pathogen.

HORIZON 2020

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