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Commentary

DNABII targeting antibodies as vaccines against biofilm diseases

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Microbial biofilms, *i.e.* communities of microorganisms embedded in a complex matrix mainly composed by macromolecules of bacterial origin, play a significant role in several chronic and relapsing infections. A major hurdle in treating biofilm-mediated infections, including those formed on the surface of medical devices, is that bacterial cells are physiologically much more resistant to both antibacterials and host innate and adaptive immune mechanisms [1]. Astoundingly, the U.S. National Institutes of Health estimates biofilms account for 80% of human microbial infections [2]. This translates to roughly 2 million cases per year, with an estimated 268,000 deaths and a cost of $18 billion, which often requires extreme measures, such as the replacement or the removal of implanted devices or debridement of infected wounds [3,4]. Therefore, identification of novel strategies to treat or prevent biofilm-mediated infections is an urgent clinical need. In this optic, strategies that disperse bacteria from an established biofilm, or that prevent its formation by active host immunization (*e. g.* anti-biofilm vaccines), are now considered promising approaches.

In previous works, the authors demonstrated that antibodies targeting members of the DNABII family of bacterial DNA-binding proteins, integration host factor (IHF) and the histone-like protein, are able to sequester DNABII proteins from biofilms, resulting in the rapid collapse and subsequent detachment of bacteria from their protective biofilm matrix. This leads to the subsequent pathogen clearance by host immune effectors or antibiotics [5–8]. Importantly, this approach is species-independent and effective against *in vitro* biofilms of numerous bacterial species ($n = 22$), including members of the high priority *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp (ESKAPE) pathogens, and also in experimental biofilm models of chronic human diseases, including otitis media (OM) caused by non-typeable *Haemophilus influenzae* (NTHi) in chinchillas, lung infection by *Pseudomonas aeruginosa* in mice, and periodontal peri-implantitis by *Aggregatibacter actinomycetemcomitans* in rat.

In this issue of *EBioMedicine,* Novotny and colleagues report on significant progress towards the clinical application of this approach, by i) testing the ability of the Fab portion of a monoclonal antibody raised against a DNABII tip-chimeric peptide to resolve OM infection by NTHi, and ii) assessing the potential of this chimeric peptide to promote host's active immunization and thus preventing biofilm formation [9]. To this aim, authors firstly demonstrated that Fab fragments obtained from a murine monoclonal antibody raised against the DNA-binding tip region of the β-subunit of NTHi IHF ($NTHi_{IHF}$), termed β-tip Fabs, were able to significantly disrupt the biofilms formed by all tested bacterial species, including NTHi, *Moraxella catarrhalis*, *P. aeruginosa*, *Burkholderia cenocepacia* and *Staphylococcus aureus*. More significantly, in a chinchilla model of OM by NTHi, murine β-tip Fabs was capable in reducing the amount of established biofilm, resolving disease-associated inflammation, and eliciting the production of multiple host immune effectors to effectively eradicate biofilm-released bacteria. Subsequently, the authors generated a chimeric peptide composed of the DNA-binding tip domains of both the α-subunit and β-subunit of IHF_{NTHi} to obtain polyclonal rabbit anti-tip chimeric peptide Fabs. Two administrations of anti-tip Fabs were able to resolve NTHi biofilms in chinchillas, and significantly reduce the bacterial load, likely through stimulation of host immune effectors. Excitingly, the observed therapeutic effect was also durable. Furthermore, to assess the potential usage of these novel therapeutic agents in clinical trials, authors obtained a panel of humanised tip chimeric peptide-directed monoclonal antibodies (HuTipsMabs), and evaluated one of these HuTipMabs *in vitro*. This HuTipMab showed strong affinity to the target peptide, and significantly disrupt *in vitro* biofilms formed by NTHi, *P. aeruginosa* and *B. cenocepacia*. Significantly, this HuTipsMab was able to disrupt preformed NTHi biofilms in chinchillas in a lasting manner, indicating that the humanization process did not diminish its effectiveness. Additional advantage is that HuTipsMab did not induce overt inflammation incurred by Fabs generated from either murine or rabbit chimeric peptide sera. Lastly, the authors evaluated whether active preimmunisation of chinchillas with tip or tail chimeric peptide and adjuvant could prevent the induction of OM in chinchilla by superinfection of adenovirus and

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NTHi. The vaccine formulation significantly delays the onset of OM and showed an efficacy of 85% when compared to negative-control cohorts.

In conclusion, members of the DNABII family of bacterial DNA-binding proteins are critical components found in the biofilm produced by all bacterial species tested to date, and their high-degree of sequence conservation makes these proteins amendable for species-independent novel antibacterials targeting biofilm-mediated infections. The use of DNABII targeting Fabs in place of intact IgGs could prevent the formation of anti-antibodies in cases where repeated treatments are required, thus constituting a significant step forward toward clinical use for the treatment of biofilm-mediated diseases.

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Author contributions

MMD wrote the first draft of the manuscript. GWL and MMD co-edited the manuscript.

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