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The value of mucosal immunization against bacterial infections: will we conquer tuberculosis in the near future?

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INTRODUCTION

It is common knowledge that the barrier tissues of our body (skin, mucosae) are the major site of interaction with external microorganisms. Barrier tissues are stably associated with different kinds of microorganisms in a non-pathogenic and often beneficial cross-talk (such as in the case of commensals and symbionts). Over 90% of pathogens invade the body through mucosae in the upper respiratory tract, lung, gut and genito-urinary tract.[1] Focusing on bacteria, the major pathogens of concern in the respiratory tract are *Mycobacterium tuberculosis* (*Mtb*), *Streptococcus pneumoniae*, Group A streptococci and *Neisseria meningitidis*; in the gut *Shigella* ssp., *Salmonella* ssp., *Escherichia coli* and *Vibrio cholerae*; and in the genito-urinary tract *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and Group B streptococci. Each pathogen is well adapted to the specific mucosal environment that it infects, in terms of capacity to survive by eluding immune recognition and elimination. As an example, it is notable that a large part of the genome of *N. meningitidis* is dedicated to the complement neutralization and degradation of macrophage-derived oxidizing molecules,[2] underlining the complex co-evolution of bacteria and their host in reaching a co-survival equilibrium.

MUCOSAL VACCINES TARGETING DIFFERENT ANATOMICAL TRACTS

Since immunity at mucosal sites has peculiar characteristics and control mechanisms, mucosal vaccines need to be adapted to the pathogen's way of entry and interaction with the host mucosal tissues, in order to achieve the best local protective response against infection and invasion. A good knowledge of the natural infection and of its capacity to induce protective immune memory is required for the design of mucosal vaccines, since not in all cases mucosal vaccines would be the best solution. In the case of *N. gonorrhoeae*, the natural infection does not induce protection, thus a mucosal vaccine may not be able to overcome the immunosuppressive mechanisms triggered by the natural pathogen, while a parenteral immunization could work. In most cases, however, we expect the preferred immunization against respiratory pathogens be by inhalation or aerosol vaccines. Interestingly, there is evidence of tissue-specific immunity against mucosal pathogens, even in response to parenteral vaccination, and of cross-protection against pathogens affecting the same organ/tissue.[3] This opens the way to the design of mixed prime-boost vaccination strategies, with a first immunization by systemic route and a boost by mucosal administration, in order to achieve systemic immunity and memory, and efficient homing of recirculated antigen-specific memory cells to the mucosal sites.

THE CASE OF TUBERCULOSIS

Among bacterial pathogens, the *Mtb* complex causes tuberculosis (TB), a disease mostly affecting the lung, inducing a chronic state of latent infection (about 25-35% of the human population is currently infected with *Mtb*), which can be reactivated to an active life-threatening disease in about 10% of infected subjects. As for all human pathogens, it is important to know the history of the disease and to have knowledge of the protective mechanisms, in order to devise meaningful preventive and therapeutic strategies. The case of TB is particularly complex, because *Mtb* has lived with human beings at least for 70,000 years, and has evolved together with humankind.[4] Notably, *Mtb* is well adapted to low density host populations (long asymptomatic latency that facilitates reactivation, and slow disease progression) as well as to high density human populations (aerosol transmission and high virulence to ensure maximal infectivity), thereby allowing its widespread persistence in every human environment.

In the design of vaccines for preventing TB, ideal vaccines should be able to prevent infection in high density populations, and prevent reactivation in low density populations. For prevention of primary infection, although *Mtb* is an infection of the low respiratory tract (Figures 1A-B), it could be wise to increase anti-infective immunity at multiple levels. Building a multi-layered protective barrier, by enhancing nasal, pharyngeal and bronchial mucosal immunity, could effectively block the bacterial access to the lung. Ideally, both innate and adaptive immunity should be concomitantly activated at mucosal sites, to exploit the full breadth and duration of specific and non-specific protective mechanisms. Thus, vaccines that drive durable innate and adaptive memory in the airways are critical to prevent both primary infection and reactivation. A systemic priming followed by intranasal boosting may seed,

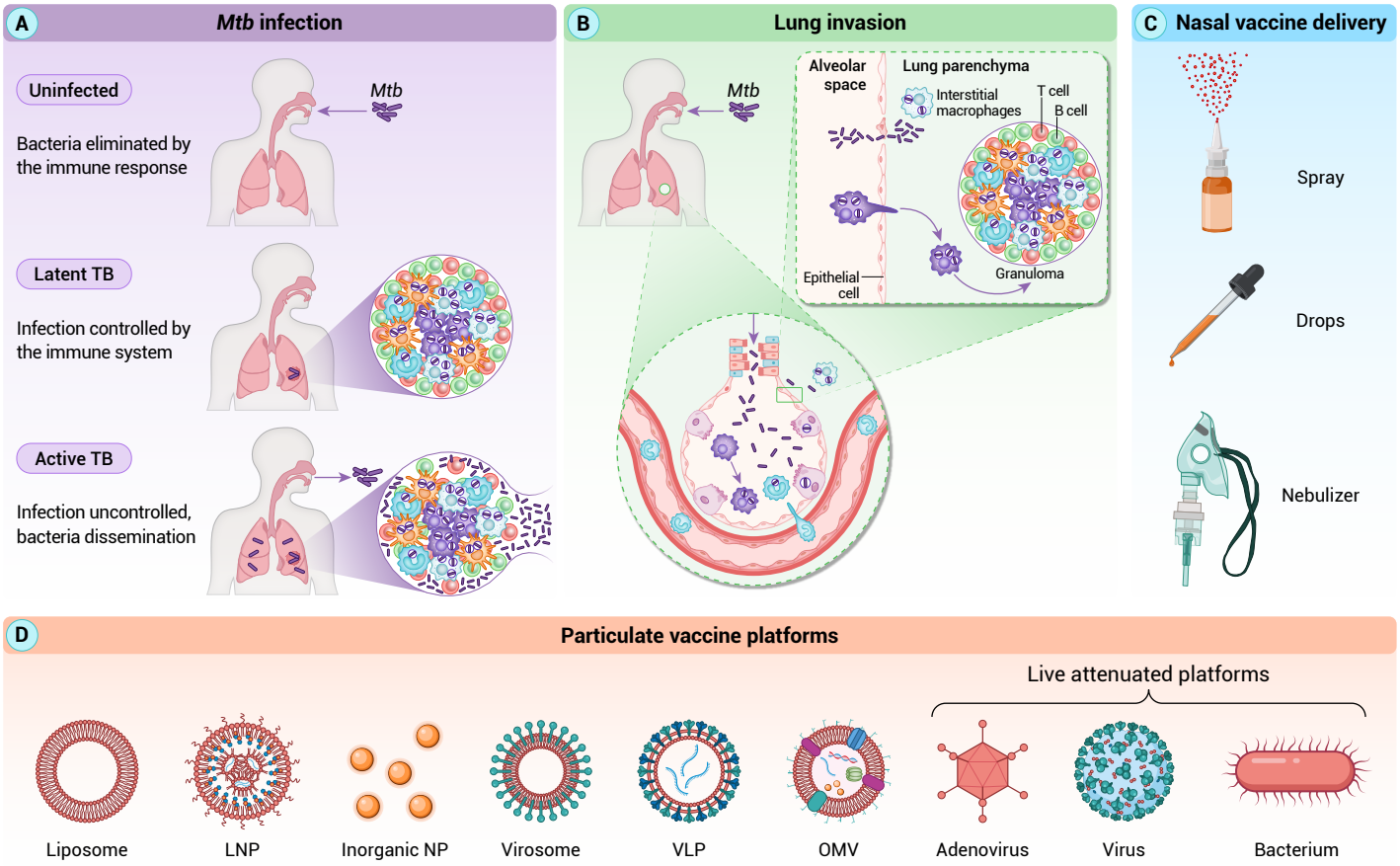

Figure 1. ***Mtb*** **infection and vaccine strategies against TB** (A) *Mtb* infection. Bacteria enter the body through the respiratory mucosa and, if the local immunity is effective, the infection cannot establish (upper panel). In latent infection, the bacteria have infected the lung, but the immune system controls and restricts the infection by forming granulomas, although allowing the bacterial survival (center panel). In active TB, immune restriction fails, granulomas become leaking, and bacteria can disseminate in the lung and outside the host (lower panel). (B) Lung invasion. Once entering the lung, *Mtb* mainly infects at the alveolar level. Both epithelial cells (in particular type II pneumocytes) and, mostly, alveolar macrophages can be infected. Monocytes egressing the bloodstream and entering the alveolar space (in the attempt to fight the infection) can also be infected. Magnification: bacteria can exit the alveolus upon death of infected pneumocytes, thereby infecting interstitial macrophages, and by egression of infected alveolar macrophages and monocytes. In the lung parenchyma, the infected cells are actively contained and secluded by the action of T and B lymphocytes that form a granuloma, but bacteria continue to survive within infected cells. (C) Nasal vaccine delivery. Mucosal immunization for respiratory infections such as TB should be ideally performed through the nasal route. Different delivery systems can be used, such as spray, drops and inhalation with nebulizers. These systems can deliver the vaccine to different mucosal tracts (nasal mucosa with drops or mild spray; pharynx and upper respiratory tract with spray and nebulizers; lower respiratory tract with strong nebulizers), thereby allowing to design a multilayered vaccine-induced local protection. (D) Particulate vaccine platforms. It is hypothesized that effective mucosal vaccines should mimic the characteristics of the infective agent, in order to trigger an appropriate immune response. Thus, it is advantageous, for vaccines against TB or other respiratory infections, to use particulate vaccines or vaccine carriers that reproduce the size/shape of the pathogen (such as liposomes, lipid nanoparticles -LNP-, inorganic NP, virosomes or virus-like particles -VLP-, or bacterial outer membrane vesicles -OMV-). An additional advantage for triggering immunity is the use of live attenuated vaccines, since the immune interaction with live agents is much stronger than with inert particles. Thus, adenoviral vectors (e.g., as carriers of mRNA vaccines), live attenuated viruses (engineered to express vaccine antigens) or live attenuated bacteria (naturally carrying relevant antigens, such as BCG, or engineered to express/display them) are the prioritized choice.

localize and sustain these responses in the airways and lung parenchyma, with emphasis on protection against active disease, to restrict transmission, when primary prevention is insufficient.

EXPLOITING KNOWLEDGE OF HOST-PATHOGEN CROSS-TALK IN MUCOSAL TISSUES

The cross-talk between host and pathogen is a key element for understanding the features of their cross-regulation and, consequently, to identify molecular targets that can tilt the balance in favor of the host. Such cross-talk can substantially vary depending on the pathogen, the target cells and the targeted mucosal tract. Depending on the location, different types of innate immune cells could be differentially involved (PMN, monocytes, macrophages, mast cells, ILCs, MAIT cells, DCs, etc.) and recruit specific immune cells (B and T lymphocytes) to induce different types of adaptive immune responses (persistent protective immunity, immunological memory) with a diverse specificity range and broadness. For instance, the production of secretory IgA at the mucosal surfaces is a very efficient adaptive immune mechanism with a recognition broadness that makes it effective across substantial pathogen variability.[5] Moreover, although innate immunity does not have classical antigen-specific memory, it can be "trained" to respond more efficiently upon re-exposure, i.e., it can develop an innate memory.

In addition to immune cells, several other cell types and mechanisms are active at mucosal sites. As an example, different types of epithelial cells in the mucosa can sense exogenous agents or tissue damage, and react by releasing cytokines and driving/regulating the development of inflammatory defensive reactions. Also, mucosal tissues are heavily innervated, and many immune cells have receptors for neurotransmitters and are activated by nervous signals. Conversely, brain neurons can retain the memory of local mucosal reactions and recall them.[6] This suggests that the mucosal route of vaccination might create unique, long-lasting immune imprints via neural circuits, which we may become able to recall for improved protection. Thus, mucosal vaccination strategies must consider the neuronal control and memory of local mucosal immunity, for improving vaccine efficiency and ensuring their safety. Depending on the target mucosal tract and efficacy vs. safety considerations, different administration systems can be used, such as instillation (nasal mucosa), spray (pharyngeal) or aerosol/nebulization (lower respiratory tract) (Figure 1C).

In the case of TB, the only currently available vaccine is the attenuated *Mycobacterium bovis* strain BCG, administered intradermally. BCG is only partially protective, mainly in children, thus over 85% of TB cases occur in

adults.[7] Despite significant efforts, recent trials with newly developed vaccines did not show better protection than BCG. It is likely that the success of BCG as vaccine is partly due to its resemblance with *Mtb* in terms of interaction with the human host. For instance, aerosolized BCG can induce tissue-resident memory T (TRM) cells in the lung, while subcutaneous or intradermal routes generally do not. This distinction is mechanistically critical: pulmonary $CD4^+$ and $CD8^+$ *Mtb* antigen-specific TRM cells demonstrated multifunctional IFN-γ, TNF-α, and IL-2 expression, accompanied by heightened PD-1 expression,[8,9] accelerating local Th1 responses, and enabling cognate immunological synapses with infected macrophages, required for sterilizing bactericidal activity, while constraining Th1-driven immunopathology.[10] As a result, aerosolized vaccine delivery could accelerate protective immunity and potentially improve vaccination outcomes. Hence, protection appears to critically depend on the site of vaccination via routes that engage the respiratory mucosa to rapidly mobilize local immunity. On this line, the aerosol delivery of BCG in human adult subjects has been experimented with limited adverse events,[11] and two aerosol and one intranasal vaccine with non-replicating adenoviruses are currently in clinical trial.

However, several challenges remain. First, protection in adults remains suboptimal despite BCG vaccination in infancy, suggesting that mucosal boosters or heterologous prime-boost strategies may be necessary to overcome immune tolerance in chronic infection. Second, correlates of protection remain poorly defined, complicating vaccine development and clinical trial design. Third, latent TB infection in high-density populations and emerging drug resistance present epidemiological barriers that vaccination alone cannot address. These challenges underscore why mucosal immunization strategies must be integrated with improved diagnostics, shorter drug regimens, and global health infrastructures.

PRACTICAL NEEDS: VACCINE PLATFORMS AND CORRELATES OF INFECTION/PROTECTION

For mucosal immunization strategies, different platforms are being used/developed. These can differ depending on the target mucosal tract, and different adjuvants or muco-adhesive molecules can be used. Adjuvants are required for subunit vaccines, whereas particulate vaccines (e.g., whole inactivated or attenuated bacteria, adenoviral vectors; Figure 1D) reproducing the size and shape of the original pathogen usually induce an immune reaction without needing further "help". Thus, vaccine formulations must be designed on the chosen platform, the targeted mucosal area, the route of administration and the type of immunity that the vaccine should achieve. In this regard, a still unsolved issue is the identification of reliable and easily measurable correlates of infection/protection that would allow for a rapid and robust evaluation of the immunological status of the subjects, and the level and duration of vaccine-induced protection. This is particularly important for mucosal vaccines, in which the accepted biomarkers of immunization (the blood levels of specific antibodies) do not correlate with protection at the local level. A century ago, Charles Mantoux developed a simple test, the tuberculin skin test, to detect latent TB infection. Yet, we have not figured out any other suitable correlate of infection/protection. The current IGRA test is a modern version of the tuberculin skin test, with the disadvantage of being invasive (blood). Since for many mucosal tracts sampling is difficult and substantially invasive (e.g., biopsies), new correlates and surrogate markers are needed, non-invasive (e.g., nasal swabs, including specific IgA and TRM cells, saliva, urine, faeces, gut microbiota), easy to measure (rapid simple testing), robust and realistic, which need to be accepted by regulatory authorities in front of a comprehensive and well-substantiated dataset. A strong engagement is encouraged in research on these new dimensions of real and surrogate biomarkers.

CONCLUSIONS

Mucosal vaccines for preventing bacterial infections are technically possible and will have the advantage of enhancing protective immunity specifically at the pathogens' port of entry, thereby ideally affording sterilizing immunity.[12] Therapeutic vaccines/immunotherapy approaches are conceptually more difficult, in particular for chronic infections such as TB, as these should disrupt a well-established mutual interaction between the host and the pathogen. The recent advances in technologies have already enabled the rapid development of new vaccines and will soon form the basis for the immune profiling of human subjects before and after vaccination. In any case, the contribution of scientific knowledge, experience and conceptualization will be fundamental in exploiting technological tools in the most efficient and successful fashion: knowledge together with technology can make the difference. The challenges posed by diseases such as TB are huge and will require reliable and accessible diagnostic/prognostic markers, preventive strategies for blocking infection, treatments that prevent the evolution of latent TB to active infection and others that can treat active TB. The surge of antibiotic-resistant bacteria is dramatically limiting the efficacy of current drugs and makes even more urgent the need for preventive and therapeutic alternatives.

While great advances in fundamental knowledge are being made, and innovative technologies and platforms are developed, we still need a globally coordinated effort for the effective translation of knowledge into new technologically advanced and easily applicable preventive and therapeutic tools.[13] Mucosal immunization strategies are one of the most interesting strategies because of their ability to target the most relevant ports of entry of infectious microorganisms and for the ease of administration, which would facilitate acceptance and increase compliance and overall coverage. New approaches are also needed for evaluating the efficacy of new vaccines, since most animal models are insufficient and hardly predictive. The adoption of controlled human infection models (CHIMs), already possible in some countries, should be expanded and exploited for their highly reliable predictive power. While prone to substantial ethical concerns and requiring strict control of conditions, CHIMs are greatly helping in the design and testing of vaccines against many infections. A CHIM is being established for TB as well, using aerosol BCG as surrogate tuberculosis infection.[11]

Eventually, we vouch for a globalized effort toward freeing humankind from infectious diseases by implementing worldwide vaccination (implying coordinated actions in research, development, manufacturing and distribution across countries and without any kind of borders), privileging tools such as mucosal vaccines (e.g., oral or inhalation) that provide needle-free, physician-independent, easy and fast administration to a large number of subjects. Vaccine equity ("everybody must have access to vaccines") and one-health ("human health depends on environmental health") are the global goals that we should aim at. If we will implement an integrated global effort, we should be able to tackle the most difficult specific goal, i.e., eliminating tuberculosis, a disease that is almost an integral part of humankind.

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AUTHOR CONTRIBUTIONS

Diana Boraschi, Lazaro Moreira Marques-Neto and Ling Chen collectively wrote the manuscript, all other authors contributed to writing and critically revising it. All authors approved the final version.

DECLARATION OF INTERESTS

Diana Boraschi is co-editor in chief of The Innovation Life and was blinded from reviewing or making final decisions on the manuscript. Other authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

Not applicable.

DATA AND CODE AVAILABILITY

Not applicable.