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IMMUNOPROPHYLAXIS – NEW GENERATION VACCINES AGAINST THE NEW HUMAN CORONAVIRUS

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Throughout history, humanity faced many challenges, including deadly epidemics/pandemics caused by microbial/viral pathogens. These host-pathogen interactions contributed to human immune system development and evolution. In addition to pathogens, the autochthonous microbiota brings tremendous benefits to the host, with alterations in its composition being associated with susceptibility to microbial/viral infections, metabolic disturbances and even cancer. In addition to naturally acquired immunity, humans have used many protection means against pathogens of human or zoonotic origin: isolation or quarantine, natural products, drugs, and empirical immunization. The scientific basis of vaccination was stated at the end of the 19th century, the first vaccines being obtained by attenuating the pathogens' virulence. Such conventional vaccines were used in the past century against epidemics/pandemics. With the evolution of molecular biology technologies, conventional vaccines are now progressively replaced by new generation vaccines. While the search for vaccines against some pathogens continues (*e.g.*, HIV, HCV, Dengue, and MDR bacteria), 2020 was marked by the development and implementation of messenger-RNA vaccines against the new severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2), which demonstrated an unprecedented efficacy of 95% in clinical trials. Here, we highlight the importance of immunoprophylaxis against some pathogens that create major health problems at individual/population level.

Key words: Immunoprophylaxis, Conventional vaccines, New generation vaccines, SARS-CoV-2, mRNA vaccines.

INTRODUCTION

Throughout history, human kind faced many challenges, including aggressions by microbial and viral pathogens. These invaders contributed to human evolution and especially to its immune system, which is the result of human-pathogens interaction. In addition to pathogens, our normal microbiota (composed of bacteria, archaea, fungi, protozoa, viruses and bacteriophages) contributes to immune system development, mainly in the intestine, stimulating local defense mechanisms, acting as an anti-infectious barrier[1]. This intestinal microbiota actively modulates the immune system to maintain a mutually beneficial relation, the mechanisms involved in homeostasis maintenance being not yet completely understood[2]. The beneficial roles manifest when the microbiota is in an interspecific balanced status or *eubiosis*; the alternative condition is *disbiosis*, favoring the manifestation of the pathogenic potential of some normal microbiota members called opportunistic pathogens (microbial or viral), leading to local or systemic infections and inflammation, metabolic disturbances and even cancer[3]. The main cause of disbiosis is the drastic impact of the administration of broad spectrum antibiotics, this condition also favoring the amplification of antibioresistance and even opportunistic microbial or viral infections. The complex causal interactions between the microbiota and the host represent a topic of interest and active investigations[4,5,6]. Even if the role of the microbiota in acute viral infection is still poorly studied, there are already data about enteroviruses

which have functional and genetic relationships with the host and all intestinal microbiome components; there are proven bidirectional or even interkingdom connections, which is a new paradigm in intestinal immunity to viral infection. Along the intestinal tract, viruses face different conditions of pH, digestive enzymes, microorganisms, before the penetration of the mucus layer and adherence at specific cell receptors – the initial step of viral infection[7]. Last year some evidence emerged about the link between the microbiome and COVID-19 severity[8].

PATHOGENICITY AND VIRULENCE

Generally, the capacity of a pathogen to localize, colonize a host, trigger an infectious process and the consequent disease depends on the pathogen's capacity to overcome or neutralize physiological and immunological defense mechanisms. For an airborne pathogen, the survival in a host organism depends on its ability to overcome some anti-infectious physical traps, chemical and antimicrobial substances, locally active at mucosa surfaces, such as lysozyme, lactoferrin, antimicrobial peptides or defensins, secretory IgA, traps – such as the mucocilliary clearance mechanism[9]. That means that ciliary epithelial cells have a continuous vibrating activity and flow of the mucous secretion loaded with foreign particles from the inspired air, including microbial/viral ones[10]. This clearance process of pathogens from the airways is a major innate/physiological defense mechanism, affected by pollution (including smoking) and viral infections; there are known genetic defects in cilia structure or motility (the so called ciliopathies) which are frequently associated with airway infections, proving the important physiological role of this mechanism[9]. During the long host-pathogen coevolution process, all kinds of pathogens developed means to adhere (adhesins) and fix at host cellular substrates, avoiding expulsion, having also the capacity to colonize and resist host defense mechanisms. All these properties of pathogens are assured by a set of virulence factors[10].

Why do we have such specific receptors for different pathogens? In fact, these receptors have a normal, physiological role in our body (to link other cells, hormones, cytokines), but the pathogens were pushed to modify their genes and produce different virulence factors which attack host tissues or neutralize immune defense cells or molecules[11]. So, via this capacity to adhere and fix to host cell receptors, pathogens avoid the elimination; or maintenance of a pathogen in a host means colonization and an ongoing infection process, but also its persistence in nature[12, 10]. The antigenic variation of adhesins is another virulence factor and one of the main difficulties in producing efficient vaccines against some pathogens which have the capacity to reorganize specific genetic sequences for the synthesis of successive variants of surface components (bacterial adhesins or viral spikes) in order to deceive the host immune effectors produced against previous antigenic variants of the same pathogen. It is a means for the pathogen's survival, at least in a small number, but still able to multiply and spread to other susceptible hosts. For instance, SARS-CoV-2 is able to adhere to ACE-2 receptors as the entry receptor and to transmembrane protease serine 2 (TMRSS2), essential for S protein priming[13]. It is a similar situation at population level, when a novel or unknown pathogen enters an immunological naïve population lacking herd immunity, leading to an epidemic or pandemic infection with high morbidity and mortality rates. On the other hand, when the pathogen dissemination comprises a large part of the population, the risk for its genetic modification is increased, such is the case of hCoVs (human coronaviruses), all of them having a zoonotic origin (alpha and beta coronaviruses being parasites of bats and rodents); generally, the shift and leap from animals to the human host is due to genetic changes (by recombination or mutations) and leads to greater infectious capacity and increased virulence, even with lethal potential when they cross the species barrier and infect humans, all populations being immunologically naïve to this new pathogen)[14,15].

A SHORT HISTORY OF VACCINATION

EMPIRICAL PERIOD

The oldest „vaccination„ was a Chinese practice, derived from empirical observation, called "*variolation*"; the vaccine was produced from small-pox crusts taken from less severe cases of variola, crushed and inhaled, the procedure being efficient and proven at the next variola epidemic, but risky. This practice was accepted by the population and spread toward the Middle East and thereafter Europe and America (at the beginning of the 18th century), being used until an

English farmer had the idea to use for the variolation of his children instead of variola crusts, other crusts coming from vaccinia – a cow specific disease, with a similar manifestation as variola, but much less dangerous. It is the English physician E. Jenner's merit (hearing about the farmer's practice) to have demonstrated the protective role of this vaccine and to have published his results (1798), being recognized as the "father of Immunology"[16]. Today, the protective role of this vaccine and anti-cowpox antibodies to recognize and protect even against variola is called cross-immunity or heterologous immunity[17,18] and is also observed for the BCG vaccine[18].

SCIENTIFIC PERIOD

The inventor of vaccines, who built the scientific foundation of vaccination was L. Pasteur who experimentally established the principle of attenuation of pathogens' virulence and pathogenicity (in the late 19th century) by cultivation in suboptimal conditions[19,20]. After these attenuated vaccines, other types were also produced, using killed/inactivated pathogens and toxoids[16]. Today, all these vaccines are considered conventional vaccines and they were valuable tools to obtain immunoprophylaxis of some widely spread severe illnesses, reducing the morbidity and mortality indexes during the last century; but they also have adverse effects, some of them being reactogenic, others being able to induce hypersensitivity reactions or, in the case of attenuated vaccines, there was the danger of inducing the illness, instead of protection, if attenuation was not well done[21].

During the last decades researchers have been preoccupied with imagining new types of vaccines, well known at molecular level, able to induce a specific response without adverse reactions.

But, nowadays, some voices ask for conventional vaccines against SARS-CoV-2; but this is not always very simple; for instance, BCG was obtained after 13 years of successive passages of the pathogen. Although today there are efficient methods of attenuation, inactivation, immunogenic epitope selection, for SARS-CoV-2 one difficulty was linked to the requirement for virus cultivation in biosafety level 4 conditions.

In 2021, this above-mentioned BCG vaccine turns a century old, being used for the prevention of mycobacterial diseases, TB and also leprosy[16]; however, TB eradication is not yet achieved. The immune response to this vaccine shows some degree of cross-reactivity against other respiratory pathogens. This vaccine induces a certain level of protection, proved by clinical studies, to some unrelated diseases (autoimmune and inflammatory, some types of cancer) and pathogens. Even if this vaccine does not have direct antiviral activity, it induces in the host immune system a stimulatory/immunomodulatory effect, so that it alleviates the symptoms of many viral infections. The specialists expect (and we will see the results of upcoming meta-studies) that the frequency and severity of many microbial diseases, including COVID-19 (***Co**rona-**vi**rus **D**isease-20**19***), will be lower in countries with mandatory BCG vaccination programs[22].

The BCG vaccine has also the property of exerting an immune-modulatory effect and activating the ability of the immune system of an immunodeficient host to react to further exposure to other pathogens. This property, called trained immunity, is a relatively new concept about the innate immune memory, which determines an enhanced antimicrobial and improved non-specific response to subsequent infections; this phenomenon is explained by epigenetic reprogramming and sustained changes in gene expression and cell physiology[18].

The conventional or reference vaccines were great immunoprophylactic instruments over the last century saving millions of people worldwide by preventing severe infections (whooping cough, diphtheria, cholera, tuberculosis, variola, influenza etc.). But, until now, only one infectious disease was eradicated, due to a large scale vaccination campaign, the biggest one until now, organized in the past by W.H.O. It should be noted that the pathogen itself – the small pox virus - and its properties contributed to the success of this campaign: great genetic and antigenic stability, its specificity for the human host (so, a reservoir of infection in the animal world does not exist) and its great pathogenicity – which means that carrier hosts do not exist); moreover, the small pox virus is not highly transmissible[23,24].

For other viruses such as the influenza virus, which is very unstable due to its peculiar genetic features, namely its segmented genome, their frequent pseudorecombination (genetic and antigenic shift) of the eight segments (when two types of viruses – human and animal or two strains, during the replication and self-assembling of the new virions) and point mutations (genetic drift)

leading to a great genetic variability and consecutive antigenic variation[25]. It is proved that the anti-influenza immunity is a long-term one but, due to genetic instability, each year the new types of viruses are differently "dressed up" and are not recognized by the memory lymphocytes and circulating antibodies, previously produced against former strains[26]. In the absence of a universal flu vaccine, such genetic variations impose annual vaccination against influenza in vulnerable groups[27].

At the opposite pole, there are viruses such as the human immunodeficiency virus type 1 (HIV-1) and the hepatitis C virus (HCV), for which there are still no vaccines despite intensive research since their isolation in 1983 [28,29] and 1989, respectively[30]. HIV-1 is a retrovirus unique in its ability to integrate its genetic material into the genome of the host cells, mainly CD4+ T-cells, further behaving as a human gene, persisting in people living with HIV (PLWH) despite viral suppressive antiretroviral therapy (ART)[29]. HIV-1 is also unique in its genetic instability caused by multiple errors produced during the process of reverse transcription. Multiple vaccine trials were performed, with some increasing the risk of HIV acquisition (the STEP Merck HIV vaccine trial[31] by inducing T cells highly permissive to HIV in response to the adenoviral vector AD5[32], some showing partial efficacy (RV144 HIV clinical trial in Thailand[33]), and the most recent one in South-Africa being stopped for futility (https://www.sciencemag.org/news/2020/02/another-hiv-vaccine-strategy-fails-large-scale-study)[34].
While for HCV there is natural protection to disease, with a large fraction of individuals resolving infection without any intervention, such natural protection against HIV does not exist. Considering the idea that vaccines typically resume what natural infection can do in terms of immunological memory, it is still a matter of debate whether or not an HIV vaccine remains a realistic objective. At the opposite end, there are the SARS-CoV-2 vaccines, which demonstrated an unprecedented efficacy in clinical trials, as described below.

NEW GENERATION VACCINES

The evolution of molecular biology techniques offered the possibility to generate new types of vaccines targeting various old or emergent pathogens. Some of them produced by recombinant DNA technology, already used for mass immunization (anti-HBV, anti-HPV and others) or by other molecular technologies: anti-idiotype or subunitary vaccines – peptidic, ribosomal, anti-adhesins, and others were in the research phase, such as DNA and mRNA vaccines. The newest type, mRNA vaccines, was conceived and developed in the last decade for personalized cancer therapy (immunotherapies based on T lymphocytes and dendritic cells)[35] and infectious diseases[36]. These vaccines were first produced and used for large scale anti-SARS-CoV-2 immuno-prophylaxis upon a demonstrated success in Phase III clinical trials[37,38]. The vaccine consists of in vitro synthesized mRNA molecules – IVT (*in vitro transcribed* messenger RNA-based vaccines), included in nanoliposomes – artificial membranes, whose fusion with the muscle cells membrane is stimulated by a vaccine excipient – polyethilene-glycol/PEG. The message written in mRNA is translated in the cells of the immunized organism to immunogenic peptide molecules that correspond to sequences in the viral spikes. These peptides, once arrived in the regional lymph nodes are recognized by B lymphocyte receptors which, activated by this recognition step synthesize specific antibodies, able to recognize and bind viral spikes, neutralizing their infectious capacity[39]. The anti-spike antibodies induced by SARS-CoV-2 mRNA vaccines appear to last in organisms at least 3 months, as investigated to date[38]. Since the anti-spike antibodies persist at high titers at least 8 months in the plasma of convalescent people[40,41], together with cellular immunity[42], there is hope that the humoral immunity induced by SARS-CoV-2 mRNA vaccines will last as well[38,43]. Of note, antibody responses induced by mRNA vaccination are higher in people with pre-existent naturally acquired SARS-CoV-2 immunity[44]. Finally, considering the natural appearance of multiple SARS-CoV-2 variants[45] and the likelihood that SARS-CoV-2 will become endemic in the human population[46], seasonal vaccination may be required. In this context, it is important to stress that alternative vaccines, such as adenovirus-based vaccines, have received interim approval for large scale use; also, multiple other vaccines are still in clinical trials and may be available soon. These vaccines require adequate surveillance in multiple groups including people with autoimmune conditions[47,48] and caution in data interpretation. The final results of vaccination will be protection from disease and/or infection.

ABOUT THE NECESSITY OF ANTI-SARS-CoV-2 VACCINES

Nowadays there are a lot of factors which favor the community spread of pathogens: demographic expansion, long distance travel, migration, contact with wild animals (used as food, pets) and the infectious agents they carry favor the emergence of new microbial or viral pathogens of zoonotic origin[15], some of them having the potential to adapt to human host. For instance, CoVs generally determine zoonotic infections in birds and mammals (bats, rodents, other wild animals) but, in recent decades, have proved their capacity to also infect humans[49,50].

Specialists have observed that every few years a highly virulent strain appears. Until now, in the CoV family there have been identified seven hCoVs[51,52]; four of them are known to cause common cold in immunocompetent individuals, but the other three provoke serious illness: SARS-CoV (2002), Middle East respiratory syndrome (MERS - 2012) and the last one, which succeeded in leaping from animals to humans, the novel hCoV. This new emergent coronavirus has proved to be the cause of viral respiratory infection and pneumonia, with a large spectrum of symptoms, ranging from flu-like symptoms to acute respiratory distress syndrome and a cytokine storm[53]. Thereafter, the virus, identified by sequencing and officially called SARS-CoV-2, the etiological agent of the COVID-19 [52,54].

The new type of coronavirus, normally not very virulent, has high transmissibility, being very contagious and the cause of this pandemic which we have been facing for more than a year. There are some available antiviral drugs, but not very efficient; in old patients, with comorbidities – cardiovascular diseases, obesity, diabetes, ATS, autoimmune diseases – all these diseases are accompanied by inflammation and an increased level of pro-inflammatory cytokines (TNFα, IL-1β, IL-6), as well as circulating acute phase proteins, pro-coagulant factors, other cytokines, chemokines[55]. The inflammatory state is further exacerbated by the SARS-CoV-2 infection[56].

There were also observed severe forms apparently with no comorbidity, but the susceptibility to infection is also influenced by the host genetic profile and environmental factors – diet (macro- and micronutrients) and associated microbiota (with a huge impact on the host immune system), lifestyle (active or sedentary, stress), environmental pollution which is immunotoxic; there are also micropollutants (food additives, antibiotics etc.) which determine a certain level of secondary immunodeficiency, which can be masked, but revealed in confrontation with opportunistic pathogens. Severe forms of infection are also correlated with age and lymphocytopenia (especially T cells)[57] need to be hospitalized in intensive care units which are still overwhelmed.

Being a new pathogen and the population being immunologically naïve, the single active way to stop the large dissemination of this pathogen is via an efficient vaccine (accompanied by the well-known isolation and personal plus community hygiene methods). Whether such vaccines will stop the COVID19 pandemic remains to be demonstrated. Notably, the international research community invest tremendous efforts toward the identification of correlates of vaccine-mediated immune protection. Other aspects important to study will be the natural protection provided by pre-existent immunity in response to other CoV infections[58] and vaccines, such as BCG[59].

CONCLUSIONS

Nowadays there are a lot of factors which favor the community spread of pathogens, microbial or viral, of human or zoonotic origin. Despite the progress in biomedical sciences, hygiene methods, antibiotherapy and immunoprophylaxis, modern society with its new life style habits including the western diet, crowded cities and metropolitan areas, mass migration, intercontinental air transportation, as well as new chronic and immunosuppressive pathologies favor interindividual dissemination of pathogens.

In this context, it is impossible to conceive a modern society without well-organized public health services, including pathogen monitoring, vaccine-based immunoprophylaxis, specific drugs and immunotherapy/ monoclonal antibodies. These prophylactic and therapeutic agents are obtained via modern technologies, which can be quickly adapted to emergency situations. This SARS-CoV-2 pandemic revealed the unpreparedness of our societies in facing such threats. There are lessons to learn about how to improve public health through pathogen surveillance, biomedical research, and mass sanitary education for preventing infectious diseases as well as chronic comorbidities. Also, this pandemic points to the huge importance that all countries have their own

local capacity to produce medical protection materials, drugs and vaccines.

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