# Chapter 14
# Vaccine Development for Human Leishmaniasis

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**Abstract** The development of vaccines for human leishmaniasis is one of the most important approaches for effectively controlling and/or eradicating the several forms of the disease. Based on the knowledge obtained from the practice of leishmanization and its protective immune response, several strategies have been used to develop vaccines against *Leishmania* species, such as the use of whole killed and attenuated parasites, recombinant proteins, and DNA vaccines. An ideal vaccine should be safe, effective, and immunogenic. Although several candidates have achieved safety and some level of effectiveness, the current challenge in the development of prophylactic vaccines is to achieve long-lasting immune protection by generating a robust and irreversible Th1 adaptive immune response in the host, with rapid recruitment of memory and effectors T cells at key acute points of infection. However, despite all efforts over the years, due to the antigenic diversity of the parasite and the complexity of the host's immune response, human vaccine trials have been disappointing in mediating long-term immunity against sandfly-delivered infection. Therefore, more investments in this field should be carried out to translate preclinical findings from mice to humans through effective vaccine development strategies.

**Keywords** Human leishmaniasis · *Leishmania* · Vaccine development · Long-lasting immunity · Correlates of protection

## 14.1 History of Human Leishmaniasis Vaccines

Leishmaniasis has afflicted mankind from ancient to modern times. Even though *Leishmania* were only described as a new genus at the beginning of the C20th [1, 2], their presence has been reported in Egyptian mummies dated as early as

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2050–1650 BCE [3, 4]. In fact, ancient societies had already observed a key fact from cutaneous leishmaniasis (CL) that healed individuals achieved lifelong protection from new infections [5]. Especially in endemic areas of Asia and Africa, this knowledge would be later applied as the rationale for the first attempt of active immunization against *Leishmania* parasites [6].

This practice, known as leishmanization, was based on inoculating exudates from active lesions into a hidden part of the body of healthy individuals, which would produce a single self-healing lesion and consequently induce a protective response against future infections [6]. This type of immunization was used in several countries for decades, especially in hyperendemic areas [7–9]. Large-scale vaccination trials were conducted in conflict areas during the 1970s and 1980s, including one in which almost two million soldiers and refugees in Iran were immunized with live virulent *L. major* harvested from culture media [10]. Although leishmanization is considered to this day the most effective control measure against CL, concerns regarding vaccine safety, the lack of standardization, and numerous adverse effects caused it to be discontinued in most countries that still adopted this method [11]. Taking into account these limitations, attention has shifted into new approaches aimed at developing a safe and effective *Leishmania* vaccine for humans (Table 14.1). This includes a refinement of the leishmanization method, which will be discussed later in this chapter.

First-generation vaccines against leishmaniasis focused on whole killed parasites. This method is very attractive, since they are quite simple to produce at low cost, which is a prerequisite for wide distribution in developing countries [29]. The first trials of a vaccine against leishmaniasis using dead parasites took place in Brazil in the 1940s, using a polyvalent vaccine of 18 strains of *Leishmania*, and these trials had conflicting results [29–31]. These studies were resumed in the 1970s by other Brazilian research groups, through the evaluation of a pentavalent preparation without adjuvant known as Leishvacin® [12–15]. Other efforts were made worldwide, associating different *Leishmania* preparations with adjuvants such as BCG and aluminum hydroxide [16–20]. However, despite showing promising

**Table 14.1** Vaccines against leishmaniasis evaluated in human trials

| Vaccine | Classification | Candidate | Adjuvant | Phase reached | Reference |
|---|---|---|---|---|---|
| Leishvacin | First generation | Pool of five *Leishmania* isolates | None | III | [12–15] |
| Autoclaved *Leishmania* | First generation | Killed *Leishmania* spp. | BCG | III | [16–20] |
| Leish-F1 | Second generation | TSA, LmSTI1, and LeIF | MPL-SE | I | [21, 22] |
| Leish-F2 | Second generation | TSA, LmSTI1 and LeIF | MPL-SE | II | [23, 24] |
| Leish-F3 | Second generation | NH36 and SMT | MPL-SE and GLA-SE | I | [25, 26] |
| ChAd63-KH | Third generation | KMP-11 and HASPB | None | II | [27, 28] |

results regarding their safety and immunogenicity, overall, these vaccines failed to provide satisfactory levels of protection [32].

Second-generation vaccines then began to exploit purified or recombinant proteins as vaccine antigens. Associated with different adjuvants responsible for optimizing their immunogenicity [33, 34], vaccines using this method have advantages such as purity and ease of large-scale production [35]. Some second-generation vaccines against leishmaniasis that have reached clinical trials include LEISH-F1, LEISH-F2, and LEISH-F3 [36]. LEISH-F1, one of the first second-generation vaccines tested in humans, is made up of the fusion of the TSA, LmSTI1, and LeIF proteins (Table 14.1), associated with the adjuvant MPL-SE. Several phase I trials have demonstrated the vaccine's immunogenicity and safety, in addition to its therapeutic efficacy in patients with cutaneous and mucocutaneous leishmaniasis [21, 22]. Based on the positive results of phase I, the same group reformulated the vaccine, now called LEISH-F2. This time, the aim was to achieve a protein more like its wild-type version, by excluding the histidine tail present in its recombinant predecessor. After having its safety and immunogenicity evaluated in phase I, the vaccine entered phase II to have its therapeutic effects evaluated on CL patients [23, 24]. LEISH-F3 is composed of NH36 and SMT proteins (Table 14.1) fused in tandem, formulated with the adjuvant GLA-SE. Phase I trials have demonstrated its safety and immunogenicity in a healthy population in the United States and Bangladesh [25, 26].

In order to optimize the specificity of protein-based vaccines, third-generation vaccines began to explore the potential of coding DNA in their composition [37]. The advantages of this type of approach include ease of production and administration, stability, and immunogenic potential [38, 39]. While many *Leishmania* genes have been evaluated for their vaccine efficacy, only one candidate has reached the clinical trial stage [36]. This vaccine uses the ChAd63 adenovirus as a vector for expression of the KH gene, constituted by the KMP-11 and HASPB antigens of *L. donovani* (Table 14.1). The results of phase I trials demonstrated the safety and immunogenicity of the vaccine, which is currently being evaluated for its therapeutic effect in patients with post-kala-azar dermal leishmaniasis (PKDL). Preliminary phase II results reported that the vaccine induced a potent cellular immune response and was responsible for the emergence of mild adverse effects [27, 28]. Despite promising results, the level of protection obtained by DNA vaccines is still limited, so more studies should be carried out to increase their effectiveness.

## 14.2 Strategies to Vaccine Design: Where Are Good Candidates to Be Found and How Do We Explore Their Potential?

Since the vaccine development field started to focus on immunogenic fractions instead of whole parasites, screening methods to search for these candidates became crucial. Therefore, genome sequencing of *Leishmania* spp. was a key step to understanding the molecular biology of these organisms [40–43]. Although different *Leishmania* species exhibit variable numbers of chromosomes and some

species-specific genes, their genomes display a high degree of genetic conservation [44, 45]. This aspect becomes especially attractive when we consider the design of a pan-*Leishmania* vaccine.

Among other approaches to discover novel vaccine candidates, bioinformatics has been widely explored for its potential to process large amounts of data that are deposited on different databases. This interdisciplinary field combines computational techniques with biological data, supporting a large area of studies [46]. Regarding vaccine design, several tools and algorithms can be applied to predict a number of important antigen features, such as transmembrane domains, subcellular localization, secondary and tertiary structures, HLA recognition, and B- and T-cell epitopes [47–52]. Such characteristics not only help to understand the function of these molecules but also contribute to the search for dominant and therefore increasingly promising epitopes, which should be recognized by the human immune system and hopefully can stimulate a protective response. Furthermore, given the processing and analytical capabilities inherent to bioinformatics, this approach substantially reduces the time required for the simultaneous screening of thousands of targets [53]. On the other hand, a major limitation to this method is that the output data quality is highly affected by the accuracy of the annotations and predictions made upon them [54, 55].

A different approach to antigen discovery is based on bacteriophage libraries. In 1985, it was demonstrated that an exogenous gene could be fused to the gene from a capsid protein of the phage M13, resulting in the expression of a hybrid protein on the viral surface [56]. This technique, known as phage display, made it possible to create phage libraries composed of billions of phages capable of expressing different exogenous peptide sequences on their surface. These sequences can then be selected through their affinity for different types of ligands, such as enzymes, antibodies, and cell surface receptors [57]. An important aspect of this technology is the link between genotype and phenotype, since it is possible to find the selected peptide sequence through the nucleotide sequence fused to the viral genome [58].

Libraries constructed by random peptide sequences are the most common type of library used in phage display selection, often helping to identify epitopes [59], many of which have been evaluated as candidates for a *Leishmania* vaccine in experimental models [60–65] (Table 14.2). The application of this method in vaccinology explores both the role of the bacteriophage as an immunogenic carrier of antigens as well as the identification of mimotopes. These are peptides that, despite having a different sequence from that of the native epitope, are able to interact with the paratope in an analogous way, often mimicking conformational epitopes [66, 67]. Besides the ease of large-scale production, relatively low cost, and safety, one of the main advantages of phage display is the possibility of selecting mimotopes, since it is estimated that approximately 90% of B-cell epitopes are discontinuous in nature [35, 68]. Furthermore, the use of phages as antigen carriers is capable of inducing both the cellular and humoral arms of immunity, which is fundamental in orchestrating an effective response against intra- and extracellular pathogens [59, 69].

Although having good candidates is important while developing a promising vaccine, it is only the first step in a very long process. A fundamental aspect for a

**Table 14.2** Main phage display vaccine candidates against leishmaniasis

| Vaccine presentation | Adjuvant | Protection | Experimental model | Main findings | Reference |
|---|---|---|---|---|---|
| Mimotopes anchored to M13 bacteriophage coat proteins | Saponin | *L. infantum* *L. amazonensis* | BALB/c mice | 1. Specific Th1 immune response<br>2. Significant reduction of parasite burden in all organs evaluated | [60, 61] |
| Synthetic soluble peptides | Aluminum hydroxide | *L. infantum* | BALB/c mice | 1. Significant protection (up to 98%) induction of mixed Th1/Th2 response | [62] |
| Synthetic soluble peptides | None | *L. major* | BALB/c mice | 1. Up to 81.94% protection rate with peptide P2 | [63] |
| Mimotopes anchored to M13 bacteriophage coat proteins | None | *L. infantum* | BALB/c mice | 1. Specific Th1 immune response<br>2. Reduction of parasite burden (up to 65%) in all organs evaluated | [64] |
| Mimotopes anchored to M13 bacteriophage coat proteins | None | *L. amazonensis* | BALB/c mice | 1. Induction of specific Th1 immune response<br>2. Significant reduction of parasite burden in all organs evaluated | [65] |

successful vaccine is, in fact, how these candidates are explored. Despite peptide-based vaccines offer advantages like safety and ease for production, it is well-known that synthetic single peptides are poor immunogens and require some tweaks to be able to elicit a potent and hopefully long-lasting immune response [70]. Among commonly used approaches to overcome this issue and, in the right context, drive a protective immune response is the use of adjuvants, adenovirus vector, or chimeras [71].

Chimera vaccines are composed of multiple epitopes which can be repeated in tandem to enhance the immune response [72]. Several studies have demonstrated the potential of these vaccines against leishmaniasis in a murine model (Table 14.3), including polyproteins composed by conjugated antigens such as KSAC [82] and Q protein [73], T-cell epitopes for a specific protein [72, 74–81], and MHC I- and MHC II-specific epitopes from different proteins [83, 84]. Regardless of the specific target, multicomponent vaccines are especially interesting in the case of complex organisms such as *Leishmania* spp. that present an extensive antigen repertoire [76].

**Table 14.3** Main chimera vaccine candidates against leishmaniasis

| Candidate name | Composition | Adjuvant | Protection | Experimental model | Main findings | Reference |
|---|---|---|---|---|---|---|
| Q Protein | Lip2a, Lip2b, P0, and H2A proteins | None | *L. infantum* | Dogs | Clinical protection observed in anatomo-pathological and phenotypic levels. Early and intense long-lasting specific IgG2 antibody response | [73] |
| RCP (recombinant chimeric protein) | T-cell epitopes from LiHyp1, LiHyp6, LiHyV, and HRF proteins | Saponin | *L. infantum* | BALB/c mice | Significant reduction of parasite burden in all evaluated organs. Th1 cell-mediated immune response. Induction of predominantly IgG2a antibody isotype | [74] |
| F1F3 Chimera | T-cell epitopes from NH36 protein | Saponin | *L. amazonensis* | BALB/c mice | 84% reduction of skin lesions size and 99.8% reduction of parasite load. Th1 cell-mediated immune response. Induction of higher titers of IgA, IgG, and IgG2a antibodies | [75] |
| Recombinant Chimeric Protein | T-cell epitopes from LiHyS, SGT, and PHB proteins | Saponin | *L. infantum* | BALB/c mice | Significant reduction of parasite burden in all evaluated organs. Th1 cell-mediated immune response. Induction of predominantly IgG2a antibody isotype | [76] |
| ChimeraT | T-cell epitopes from PHB, EIF5a, LiHyp1, and LiHyp2 proteins | Saponin | *L. infantum* | BALB/c mice | Significantly reduction of parasite load in distinct organs. Th1 cell-mediated immune response. Induction of predominantly IgG2a antibody isotype | [77, 78] |
| LiChimera | T-cell epitopes from Cpn60, Gcvl-2, Eno, CyP2, CyP40, and HyP proteins | AddaVax | *L. infantum* | BALB/c mice | Reduction of parasite burden in short- and long-term vaccinated mice. Cellular responses dominated by central and effector multifunctional CD4+ and CD8+ T memory cells | [79] |
| Chimera A and Chimera B | Chimera A: T-cell epitopes from H2A, LiP2a, LiP0, LACK, and CPC proteins chimera B: T-cell epitopes from CPA, CPB, PSA-50S, and A2 proteins | Saponin alone or in association with MPL-A | *L. infantum* | BALB/c mice | Reduction of parasite burden in the spleen. Th1 cell-mediated immune response. Generation of central and effector memory T cells | [72, 80] |
| ChimT | T-cell epitopes from LiHyp1, LiHyV, LiHyC, and LiHyG proteins | Saponin or MPL-A | *L. infantum* | BALB/c mice | Reduction of parasite burden in all evaluated organs. Th1 cell-mediated immune response. Induction of predominantly IgG2a antibody isotype | [81] |

In addition to optimizing the chances of triggering an immunogenic response by recognizing at least one of its epitopes, vaccines composed of polyproteins demonstrate greater potential for mass application [85], especially when considering the genetic polymorphism of the mammalian immune system and the possible interactions of these antigens with different types of MHC [86]. Furthermore, the high level of conservation among the genomes of *Leishmania* spp. makes possible the development of a pan-effective vaccine against several species [87]. Despite these advantages, chimeric vaccines still need to be associated with adjuvants that are safe for use in humans and that together can stimulate the robust and long-lasting response associated with protection. This vaccine is yet to be developed.

Although whole parasite vaccines have the advantage of exhibiting the complete repertoire of antigens to the immune system [88], one of the biggest caveats about using attenuated organisms is the risk of reversion to virulence [89]. Particularly, older approaches such as maintaining the parasites in culture for long periods of time and exposure to chemical and physical attenuation did not ensure its safety. Random mutations and the return to a virulent state were often observed [90–92]. Fortunately, the use of attenuated strains gained a new momentum thanks to the progress made in genetic manipulation techniques. The discovery of the CRISPR-Cas9 system, for instance, proved to be of great importance for editing the genomes of several organisms, including different *Leishmania* species [93–95]. This system is based on two components: Cas9, an RNA-guided endonuclease, and a guide RNA sequence, which has the function of directing Cas9 to the complementary strand of the target DNA that will be cleaved [96]. Since genetic manipulation before CRISPR-Cas9 was largely based on homologous recombination with the use of antibiotics as selection markers [97–99], the development of this technology improved the ability to explore and edit the genome of a number of organisms. In addition to other possibilities, this method allows the precise deletion and insertion of genes in known locations, being able to introduce mutations, selection markers, and protein sequences of interest [94].

Several important genes for the survival of *Leishmania* spp. have been explored in vaccine development, such as those responsible for the expression of cysteine protease, biopterin transporter, p27, and centrin [100–109] (Table 14.4). Centrin is a constitutive protein of the eukaryotic cytoskeleton, responsible for the duplication and segregation of the centrosome. Deletion of the centrin encoding gene in *L. donovani* reduced the growth of the amastigote forms, although it did not interfere with the viability of the promastigotes [107, 110]. While multiplying inside macrophages, mutant amastigotes were unable to properly perform cell division, becoming multinucleated and entering a process of programmed cell death [107]. Immunization with this strain, called LdCEN$^{-/-}$, was able to provide protection against infection by *L. donovani* [108, 111, 112], *L. infantum* [113, 114], *L. mexicana* [115], and *L. braziliensis* [116] in mice, hamsters, and dogs. Immunity generated by vaccination was mediated by a CD4$^{+}$ and CD8$^{+}$ T-cell response, characterized by potent production of pro-inflammatory cytokines IL-12, IFN-ɣ, and IL-17 and reduction of IL-10 by macrophages [88, 116–118].

**Table 14.4** Main attenuated live parasites vaccine candidates against leishmaniasis

| Candidate name | Mutation target | Protection | Experimental model | Main findings | Reference |
|---|---|---|---|---|---|
| *L. donovani* BT1 null mutant | Biopterin | *L. donovani* | BALB/c mice | 1. Reduction of parasite burden (65%) when compared to wild-type infection<br>2. Induction of protective immunity | [100] |
| *L. mexicana* cysteine proteinase-deficient mutant | Cysteine proteinase | *L. mexicana* | Hamster | 1. Reduction of parasite burden<br>2. Reduction in the severity of lesions | [101] |
| *L. donovani* p27 gene knockout parasites (Ld27−/−) | p27 protein | *L. donovani*<br>*L. braziliensis*<br>*L. major* | BALB/c mice | 1. Significantly lower parasite burden in the liver and spleen<br>2. Induction of protective immunity<br>3. No parasite survival beyond 20 weeks after infection | [102] |
| *L. infantum* KHARON1 null mutant | KHARON1 protein | *L. infantum* | BALB/c mice | 1. Reduction of parasite burden<br>2. Unable to sustain infection in macrophages | [103] |
| *L. infantum* HSP70-II null mutant | Heat shock protein 70 | *L. major*,<br>*L. infantum*<br>*L. braziliensis* | BALB/c mice, C57BL/6 mice | 1. Reduction of parasite burden<br>2. Induction of long-term protection<br>3. Th1 cell-mediated immune response | [104–106] |
| *L. donovani* and *L. major* centrin deleted parasites (LdCEN−/−) | Centrin Protein | *L. donovani*<br>*L. infantum*<br>*L. mexicana*<br>*L. braziliensis* | BALB/c mice, hamster, and dog | 1. Reduction of parasite burden<br>2. Unable to sustain infection in macrophages<br>3. Protective immune response<br>4. Safe in immunocompromised mice | [107–118] |

Despite all benefits, the use of this strain as a human vaccine raises concerns regarding its potential for visceralization, which can be fatal. Furthermore, the method used to obtain the centrin gene knockout required the insertion of an antibiotic resistance marker gene, an inadmissible feature from a human vaccine candidate. In light of these limitations, an attenuated *L. major* centrin gene deletion mutant ($LmCen^{-/-}$) was generated using the CRISPR-Cas technique. This

technology eliminates the need for resistance markers, which facilitates the approval of this strain as a vaccine by regulatory agencies and makes its evaluation possible in human clinical trials. Another relevant aspect for the safety of this strain is that *L. major* is a dermotropic species and its infection, unlike *L. donovani*, remains in the skin and does not cause visceral disease. Evaluation of LmCen$^{-/-}$ in a murine model was able to prevent the appearance of lesions after challenge by *L. major*, in addition to having reduced the parasite load within internal organs and induced a protective immune response analogous to leishmanization. Moreover, inoculation of LmCen$^{-/-}$ was unable to generate pathology in susceptible and immunodeficient mice, proving the safety of this vaccine [109].

The pursuit for knowledge and the advancement of new technologies have facilitated the search for increasingly promising vaccine candidates against leishmaniasis. The support of bioinformatics and genetic manipulation techniques has allowed the design and evaluation of different types of vaccines, whether composed of parasite fractions or those that exploited genetically modified whole parasites. Even though many candidates have been evaluated in preclinical trials, few had a chance to reach human clinical trials. There is still no vaccine available against human leishmaniasis. However, scientific efforts made in recent decades have brought us closer to achieving a safe, immunogenic, and effective human vaccine.

## 14.3 Immunological Insights into Vaccine Development

The host's immunity during leishmaniasis is complex and varies according to parasite or host species, parasite load and sandfly, or needle challenge. In general, a protective immune response during *Leishmania* spp. infection involves the cross talk between the innate immune response, including neutrophils, monocytes/macrophages and dendritic cells (DCs), and subsequent activation of a Th1 adaptive immune response. Both CD4$^+$ Th1 and antigen-specific CD8$^+$ T-cell activation result in the production of IFN-γ and TNF-α cytokines that upregulate inducible oxide nitric synthase (iNOS) and reactive oxygen species (ROS) expression by macrophages, important molecules that have been associated with disease control and parasite clearance [119–121].

The resolution of a primary *Leishmania* spp. infection in humans who recover from the cutaneous manifestation, but maintain chronic infection in the skin, leads to long-lasting immunity mediated by CD4$^+$ T cells. Healed patients establish a strong Th1 memory response with low number of parasites due to immune regulation mediated by IL-10, known as concomitant immunity, which confers resistance to a secondary infection, the same protection observed in the practice of leishmanization [122, 123]. Thus, from the knowledge of concomitant immunity comes the idea of developing vaccine-mediated immunity against different forms of leishmaniasis using several approaches, such as the use of attenuated live parasites, whole killed parasites, parasite protein, recombinant vaccines, and DNA vaccines, among others. However, despite all efforts in this field, human vaccine trials have been

disappointing in mediating long-term immunity when compared to leishmanization.

Healed humans and mice from experimental models of CL showed that upon antigen presentation, different populations of memory and effector $CD4^+$ T cells are generated. Central memory T ($T_{CM}$) cells ($Ly6C^-CD62L^+CCR7^+Ki\text{-}67^+$) reside in lymph nodes and can survive for life, regardless of persistent antigen presentation. During concomitant immunity, they have the capacity to transition into effector T ($T_{EFF}$) cells after the period of antigen presentation and activation by DCs. Moreover, they are important for the production of IFN-γ and TNF-α [119, 124]. Effector memory T ($T_{EM}$) cells ($Ly6C^-CD62L^-CCR7^-Ki\text{-}67^+$) can also produce these Th1 cytokines and are longer lived than $T_{EFF}$ cells in the absence of antigen, but shorter lived than $T_{CM}$ cells. They can be found in secondary lymphoid organs, blood, or periphery [119, 125]. Tissue resident memory ($T_{RM}$) cells ($Ly6C^-CD62L^-CCR7^-Ki\text{-}67^+$) are a non-circulatory population of memory T cells found at the distal site to the primary infection that respond quickly upon restimulation, producing IFN-γ and recruiting $T_{EFF}$ cells [125, 126]. Along with $T_{EFF}$ cells, $T_{RM}$ cells are crucial in IFN-γ production at very acute time points of infection.

Regarding $T_{EFF}$ cells, studies in experimental mouse models have demonstrated that the constant presence of the parasite in chronic subclinical infection is the key factor in Th1 concomitant immunity. Therefore, Peters et al. have shown that persistent antigen presentation is crucial for the maintenance of circulating $T_{EFF}$ cells, short-lived $CD4^+$ T cells expressing $Ly6C^+CD44^+CD62L^-$ that are predominantly single producers of IFN-γ. These cells are rapidly recruited and responsible for IFN-γ production almost instantly after secondary challenge by sandfly bite, preventing the formation of a phagosomal pathogen niche and the development of the disease in mice [125, 127].

Several experimental vaccine formulations have been able to generate *Leishmania*-specific $T_{CM}$ and $T_{EM}$ cells and have successfully protected mice against needle challenge. However, although these memory T cells enhance Th1 response by cytokine production upon re-exposure to parasite antigen weeks to months after vaccination, the same vaccine formulations were ineffective in providing protection against sandfly bite-mediated challenge [127–131]. These observations highlight that the failure of *Leishmania* vaccines is not due to a lack of generating an appropriate Th1 memory response but due to a lack of generating $T_{EFF}$ and $T_{RM}$ cells, in addition to inflammatory conditions at the sandfly bite site that compromise the effector function of the memory response and should be considered when designing and testing vaccines. The human counterparts of $T_{EFF}$ cells in mice are not characterized yet, and understanding how to best induce generation of $T_{RM}$ and $T_{EFF}$ cells in humans during vaccination against *Leishmania* infection is one of the major challenges that remains undefined [132].

Immune protection against sandfly bite, rather than just the needle challenge, is the other big issue that needs to be overcome in successful vaccine design. Vector transmission of *Leishmania* by female sandfly bite delivers into the skin a low number of promastigote parasites and active molecules present in the saliva, inducing a robust local inflammatory response associated with the recruitment of neutrophils

and monocytes. This specific inflammatory response in vector transmission has an important impact in the context of vaccination. Studies have shown that neutrophil recruitment is an important factor in impairing IFN-γ production by $CD4^+$ T cells and vaccine efficacy, due to suppression of T-cell activation by macrophages and DCs that are engaged in both antigen presentation and efferocytosis (i.e., clearance of apoptotic cells) of infected neutrophils [119, 132–134]. In addition, another important aspect that must be taken into account is the shortage of antigen availability during vector transmission when compared to needle challenge in many experimental models. The low number of parasites delivered by sandfly bite can hamper the development of a protective immune response, including $T_{CM}$ activation in the draining lymph node. Despite the difficulty of maintaining sandfly colonies to reproduce the context of natural infection, efforts to replicate the low dose and inflammatory response conditions of vector transmission is an essential concern and should be used as the "gold standard" of preclinical research to interpret the effectiveness of protective immunity and vaccination [119, 123, 132].

## 14.4 Lessons from the COVID Era: What Have We Learned, and How Can We Translate It to *Leishmania* Vaccines?

Vaccine development is a lengthy process—a decade can easily pass by between the discovering phase and the start of clinical trials. In 2020, the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) pandemic shook the entire world, both for the speed with which it infected and killed millions of people and for the agility with which vaccines capable of containing the spread of the virus were developed. Coronaviruses are a group of large enveloped RNA viruses that usually cause mild disease in humans, the main reason why vaccination efforts were nonexistent up until recently [135]. This scenario dramatically shifted after the SARS-CoV and MERS-CoV (Middle Eastern respiratory syndrome coronavirus) outbreaks revealed a highly transmissible and pathogenic profile for these viruses [136].

Studies soon found a promising antigenic candidate for coronaviruses vaccines, a large surface protein responsible for receptor binding and cell invasion mechanisms known as "spike" protein [137]. Thankfully, due to the close relation between these pathogens, the discovery phase during vaccine design for SARS-CoV-2 could be significantly shortened and effective vaccines could be evaluated in clinical trials at an unprecedented speed. A pandemic like the one caused by SARS-CoV-2 justifies all the great scientific efforts and the number of financial investments made all over the world. It is also noteworthy that the success of different strategies explored during vaccine design brought attention not only to their advantages as a SARS-CoV-2 vaccine per se but more importantly to its capacity to be applied to vaccines against all types of etiologies, including leishmaniasis. Adenovirus (Ad) vector-based mRNA vaccines such as the ones developed by Johnson and Johnson and Oxford/AstraZeneca showed large potential as a platform for numerous infectious diseases. Aside from their high transduction efficiency and thermostability, Ad

vectors are especially attractive when we consider their ability to induce moderate levels of innate immunity, a key feature needed to activate adaptive immunity that is usually obtained only by the use of adjuvants [138]. This is one of many design approaches used in SARS-CoV-2 vaccines that we can draw experience from and that can be certainly translated to *Leishmania* vaccines.

A different kind of reflection provoked by the COVID-19 pandemic is what an effective vaccine looks like and what we should expect from it. Sterile immunity is often thought as the main goal for vaccination, despite being rather difficult to achieve. Admittedly, several vaccines including those against influenza, rotavirus, and the ones recently developed for SARS-CoV-2 fall under that category. However, the fact that these vaccines are unable to entirely block the infection does not mean they cannot prevent diseases or even reduce associated burden. We have witnessed first-hand COVID-19 vaccines significantly reducing hospitalization, morbidity, and mortality rates worldwide—while aided by important safety guidelines like social distancing and implementation of face mask obligation. Taking that into account, one can argue if we absolutely need to induce sterile immunity in a *Leishmania* vaccine, particularly since it is well-known that parasite persistence is required for long-life immunity. Furthermore, no vaccine alone can eradicate a complex multifactorial disease like leishmaniasis. Much like COVID-19, leishmaniasis control needs far more than an effective vaccine; it needs a One Health approach that encompasses vector control, reservoir vigilance, and environmental conservation programs.

## 14.5 Conclusions

The main concept of *Leishmania* long-lasting vaccination is to generate a robust and irreversible $CD4^+$ Th1 memory response and early IFN-γ-producing effector T-cell responsiveness at challenge site, which is crucial in preventing the establishment of a parasite niche, in addition to mediating parasite killing and infection control. Therefore, key points must be considered in vaccine evaluation: (a) cytokine production and cell differentiation by parasite-specific memory T cells ($T_{CM}$ and $T_{EM}$), (b) persistent antigen presentation to maintain circulating IFN-γ-producing $T_{EFF}$ cells required to mediate an optimal response, (c) induction of $T_{RM}$ populations at the inoculated inflamed skin, and (d) to replicate the low-dose/high-inflammation conditions of experimental sandfly challenge as the "gold standard" of preclinical research. In conclusion, understanding of aspects related to the protective immune response in leishmaniasis has made important advances over the years and is crucial for translating preclinical findings from mice to humans through effective vaccine development strategies. The current prophylactic vaccine approach against all forms of leishmaniasis aims to obtain immune protection through a rapid recruitment of IFN-γ-producing $T_{EFF}$ and $T_{RM}$ cells in key acute times of *Leishmania* infection. This outcome should be able to occur even after natural sandfly challenge, preventing the development of the disease.

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