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Narrative review

Non-specific effects of BCG in protozoal infections: tegumentary leishmaniasis and malaria

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ABSTRACT

Background: Leishmaniasis and malaria are major causes of illness in the poorest countries. In the absence of efficient strategies to prevent infections and to control the transmission of the parasites by insect vectors, treatment relies on drug therapy. Vaccine development continues on several fronts; however none of the candidates developed has so far been shown to provide long-lasting protection at a population level. Because the bacillus Calmette–Guérin (BCG) vaccine can induce heterologous protective effects, we hypothesize that BCG has beneficial effects in the control of tegumentary leishmaniasis (TL) and malaria.

Aims: In this review we describe evidence for the protective efficacy of BCG against tegumentary leishmaniasis and malaria in humans.

Sources: Relevant data from peer-reviewed scientific literature published on Pubmed up to January 2019 were examined.

Content: From experimental animal and various human studies with BCG and one recent randomized malaria trial there is evidence that BCG has beneficial effects in *Leishmania* spp. and *Plasmodium falciparum* infections. Although the precise mechanisms remain unknown, BCG can activate innate immune responses, and an increasing body of evidence demonstrates that the induction of trained innate immunity could explain its non-specific protective effects.

Implications: Despite many years of research to prevent and treat TL and malaria, these diseases remain a public health problem in tropical countries. Future studies are required to examine if BCG vaccination could be used as an effective treatment option. **J.C. dos Santos, Clin Microbiol Infect 2019;25:1479**

Tegumentary leishmaniasis and malaria

Tegumentary leishmaniasis (TL) and malaria are two of the world's most important vector-borne neglected diseases [1,2]. TL is caused by different species of intracellular protozoan parasites from the genus *Leishmania*, with an estimated 1 million new cases worldwide each year. The main species causing TL in the Old World are *L. major*, *L. tropica* and *L. aethiopica*, whereas in the New World it is most frequently caused by *L. mexicana, L. amazonensis*, *L. braziliensis*, *L. panamensis* and *L. guyanensis* [3]. Flagellated forms of *Leishmania* parasites, called promastigotes, are transmitted to humans via the bite of sandflies. Within the phagolysosome of macrophages, the promastigotes differentiate to become a round non-flagellated replicative form called amastigotes [4].

The spectrum of clinical manifestations range from single self-healing cutaneous lesions to chronic ulcers and mucosal involvement or chronic metastatic dissemination throughout the skin [5]. Localized cutaneous lesions (LCLs) are characterized by one or few ulcers in the skin with elevated borders, appearing mainly in exposed areas. Among severe cases, mucosal leishmaniasis (MCL) primarily affects the nasopharyngeal and oral mucosal epithelial barriers, often leading to ulceration and septum perforation. Disseminated leishmaniasis is characterized by more than ten papular and ulcerated lesions on at least two non-contiguous areas of the patient's body. Diffuse cutaneous leishmaniasis (DCL) is a rare but severe form of the disease characterized by non-ulcerative disseminated nodules that often affects the face, limbs and trunk of

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patients [6,7]. Leishmaniasis also comprises cases of visceral leishmaniasis, which can be mild, severe or fatal diseases [8]. The immunological spectrum observed in patients with TL range from individuals with a strong T-cell response, characterized by delayed type hypersensitivity (DHT) measured by the leishmanin skin test to individuals who lack a DHT response [9].

Malaria is a parasitic infectious disease caused by *Plasmodium* genus parasites, which affects more than 1 billion people and causes more than 1 million deaths annually. This disease is mainly associated with *P. falciparum*; however, humans can be infected by five different species: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* [10]. *Plasmodium* infection is initiated in humans by transmission of sporozoites from mosquitoes. These sporozoites establish asymptomatic infections in hepatocytes and multiply asexually to form merozoites, which are released into the blood stream. Red blood cells (RBCs) are infected by merozoites marking the start of the erythrocytic cycle. Once inside the RBCs, the parasites differentiate into trophozoites and then to multinucleated schizonts. The erythrocytic cycle ends when the RBCs are ruptured by the mature schizonts thereby releasing merozoites, which invade fresh RBCs. These repeated cycles from infected RBCs are crucial for the development of the clinical manifestations of the disease [11]. Malaria infection has a variable clinical phenotype, ranging from mild febrile illness to disease and death. In addition, asymptomatic cases of infection can also occur [12].

Protection against leishmaniasis and malaria is associated with early development of a strong, proinflammatory type 1 immune response mediated by Th1 lymphocytes. An effective Th1 response limits parasite growth. By contrast, high numbers of parasites are a consequence of low levels or absence of Th1 activation [13,14]. Infections with some species of *Leishmania* such as *L. amazonensis* result in an early development of a type 2 immune response that facilitates parasite growth and is consequently associated with disease susceptibility [15]. In addition, parasites such as *L. braziliensis* or *L. guyanensis* induce a strong Th1 response that is responsible for tissue destruction. The balance between proinflammatory and anti-inflammatory cytokines produced during the infections with both *Leishmania* sp. and *Plasmodium* sp. is an important determinant of disease outcome [13,16].

Measures currently available to reduce the burden of these two parasitic diseases are restricted to drug treatment programmes, and/or vector control. The emergence and spread of antimalarial and antileishmanial drug resistance represent an increasing public health threat. In addition to the oral drug treatment available for leishmaniasis, some patients require several drug injections that are toxic and painful [5,17]. Of note, there is not yet a safe, uniformly effective vaccine available to prevent or treat either disease [18].

Non-specific protective effects of vaccination

One of the great advantages of the discovery of vaccines is their pivotal role in prevention of infections, resulting in substantial reductions in morbidity and mortality [19]. Conventional vaccines target specific pathogens by enhancing antigen-specific adaptive immune responses. Proliferation and differentiation of lymphocytes induce a specific immunological memory, which is the basis of conventional vaccines [20,21]. This response confers acute and long-term protection against subsequent infection with the same pathogen through the activation of B and T lymphocytes.

On the other hand, epidemiological studies demonstrate that certain vaccinations induce non-specific effects through the modulation of immune response to infections that are not specifically targeted [11]. One of the best studied examples is bacillus Calmette–Guerin (BCG), which is a live attenuated vaccine known to induce non-specific effects in addition to its specific protective effect against infection caused by *Mycobacterium tuberculosis* [22,23]. Randomized trials have shown the beneficial effects of BCG for reducing mortality caused by sepsis, respiratory infections and fever in low-birthweight infants [24–26]. Furthermore, studies have described its role conferring protection against other types of pathogens or diseases [22]. Of note, potential therapeutic benefits for various oncological conditions have been reported. In mouse models, BCG immunization protected against malignant melanoma [27]. BCG is currently used as local immunotherapy in bladder cancer to reduce the risk of recurrence [28,29]. In mouse infection models, BCG reduced mortality by *C. albicans* [30]. Of particular interest for this review, vaccination with BCG reduced the incidence of visceral leishmaniasis caused by *L. infantum* in Brazilian children [31].

The non-specific effects mediated by BCG are attributable to the activation of the innate immune system. It is increasingly appreciated that innate immune cells can build a memory of previous encounters with pathogens and develop an increased immunological response upon reinfection with non-related pathogens, for which the term *trained immunity* has been adopted [22]. BCG is a striking example of a prototypical inducer of trained immunity. Mechanistically, trained immunity by BCG is defined by immunological, metabolic and epigenetic hallmarks [32,33]. These effects were recently demonstrated in a human trial where BCG reduced viraemia after vaccination with non-pathogenic, live attenuated yellow fever vaccine [34].

BCG-induced non-specific benefits during protozoal infections – focus on TL and malaria

Chronicity is a hallmark of parasitic infections, which is related to the capacity of protozoans to avoid or delay sterilizing immunity. Antigenic variation and immunosuppression are strategies developed by parasites to evade immunity during infections, aiming to prolong their survival in the mammalian host [35,36]. Protection against non-related pathogens by the induction of trained immunity is a potential additional strategy for treatment of TL and malaria. In situations where the immune system cannot eliminate chronic infection, the augmented immune response associated with trained immunity could provide additional treatment.

In support of this hypothesis, since the 1980s the group of Convit has treated Venezuelan TL patients with live BCG in combination with heat-killed promastigotes of *L. amazonensis*, using a protocol of three doses to stimulate immune response against the parasites. The researchers showed that BCG/promastigotes immunotherapy was as efficient as pentavalent antimonials (the conventional chemotherapy) to treat LCLs [37]. In another, high rates of cure were achieved in patients with LCLs and most of the patients with DCL presented improvement of the disease [38]. From 1990 to 1999 more than 5000 TL patients were treated using the same protocol. While studies confirmed the efficiency of BCG/*Leishmania* to cure LCLs with no relapses during the long-term follow-up of the patients, immunotherapy failures were observed in some patients presenting more severe diseases. Chemotherapy was needed for these patients, showing that in severe leishmaniasis a combination of immunotherapy and chemotherapy can be necessary to achieve cure [39]. BCG alone also leads to cure but in a lower percentage of patients with LCLs than BCG/*Leishmania* therapy, and with a longer mean time cure (around 43–51% of cure). What is most striking about these studies is that after cure no significant differences in the final levels of immune responses were detected among the groups of chemotherapy, BCG/*Leishmania* or BCG alone therapies. In all groups, similar numbers of T cells or *ex vivo* activation were detected as well as leishmanin skin test was similar in the patients and controls. BCG/*Leishmania* did not exacerbate the skin test reaction compared with BCG alone [38,40]. In mucosal leishmaniasis

(MCL) the combination of BCG/*Leishmania* with chemotherapy did not lead to increased Th1 immune response after cure when compared with treated LCL patients [41].

In accordance, beneficial non-specific effects of BCG can be detected in patients with DCL that do not present any *Leishmania*-specific immune response. Pereira et al. [42] reported a case of one Brazilian patient with DCL caused by *L. amazonensis*, who after several unsuccessful chemotherapy treatments and many relapses, started a immunotherapy with dead promastigotes of *L. amazonensis* and *L. braziliensis* (Leishvacin®) combined with live BCG. All lesions were healed with no relapses or new lesions for 9 months. This effect was associated with an increase in the numbers of proinflammatory monocytes and cytotoxic natural killer (NK) cells in the blood and NK cells at the lesion sites (Fig. 1). No specific T-cell immune response was detected *in vivo* (leishmanin cutaneous test) or *ex vivo* after culture of peripheral blood mononuclear cells (PBMCs) stimulated with *Leishmania* antigens. These data underscore the relevance of BCG to activate innate immune system for long-term responses, which can improve the life quality of DCL patients who are refractory to drug treatments and in the absence of specific T-cell immune responses.

Other case reports have shown the benefits of BCG in combination with heat-killed *L. amazonensis* to treat DCL patients in Argentina and Venezuela. In some cases of DCL, it was possible to break the tolerance of T-cell responses using immunotherapy with BCG/*Leishmania* [38,39]. Clinical improvement of patients with severe MCL or DCL was observed after the treatment of intradermal infections with a vaccine containing promastigotes of pasteurized *L. braziliensis* and viable BCG. Complete remission of lesions occurred, with no relapses or new lesions appearing for at least 10 months after the treatment [39]. Taken together, these data suggest that BCG can have a dual role in immunity against leishmaniasis: to generate an innate immunity memory and to contribute to specific immune memory development.

Several studies of animal models and human volunteers have tested candidate vaccines with a focus on BCG adjuvant properties to induce specific immune response and protection against *Leishmani* [43]. Since the 1990s, a candidate vaccine has been tested with BCG in Iran, using *L. major*/BCG in healthy volunteers. Injection with autoclaved *L. major* in combination with live BCG showed that the addition of BCG to the boosters (three injections) was needed to significantly increase the frequency and the magnitude of leishmanin skin tests [44]. In addition, a similar strategy of vaccination with *L. major*/BCG confirmed that low-dose BCG is not sufficient to induce specific cellular immune responses and protection against TL [45]. These studies reinforce the relevance of BCG as adjuvant to activate innate immunity and contribute to the development of specific immune responses to treat leishmaniasis caused by New World or Old world *Leishmania* infections.

There are limited data on BCG and the incidence of malaria from studies with humans. However, in endemic areas BCG vaccination is associated with reduced malaria-specific mortality [25]. To date, studies of the protective effect of BCG against malaria infections are largely restricted to rodent malaria models for which BCG administration reduces parasitaemia [46–48]. However, a recent randomized controlled phase 1 clinical trial has shed some light on the effects of BCG on human malaria. BCG vaccinated volunteers developed earlier clinical symptoms of malaria infection and reported a higher frequency of moderate or severe clinical symptoms than the non-vaccinated volunteers. Coinciding with the first appearance of blood stage parasites, researchers found an increase in the proportion of $CD56^{dim}$ NK cells expressing CD69 in half of the volunteers vaccinated with BCG. Moreover, the same subgroup of BCG vaccinated volunteers showed increased expression of HLA-DR and CD86 on $CD14^{+}$ $CD16^{-}$ monocytes. Of particular interest, BCG vaccinated volunteers presented an early increased activation of NK cells and monocytes that was inversely correlated with parasitaemia after malaria infection [49] (Fig. 1). This study has shown that BCG vaccination alters some of the clinical, immunological and parasitological outcomes of malaria. However, since the observation period was limited to 5 weeks, futures studies are required to determine the consequence of these effects in the development of immunopathology during a long-term infection.

In another study, it was shown that stimulation of human adherent PBMCs with *Plasmodium falciparum*-infected RBCs or the malaria crystal haemozoin induced increased production of proinflammatory cytokines after restimulation with TLR2 ligand. This hyper-responsiveness was further associated with epigenetic

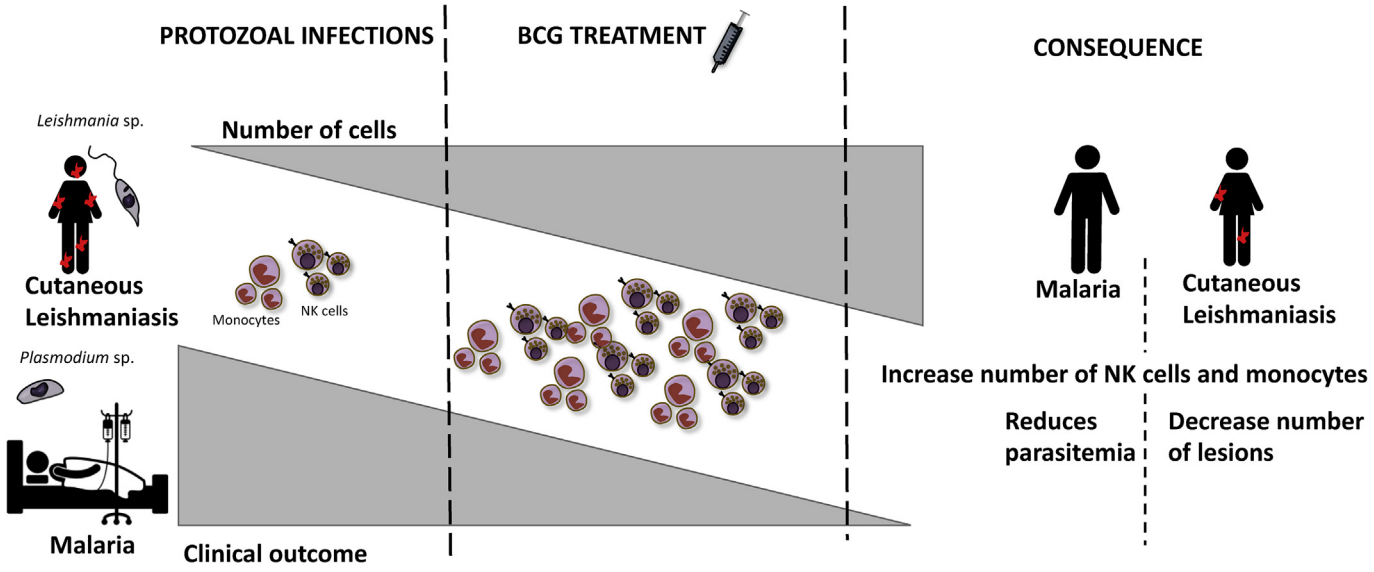

Fig. 1. Schematic overview of non-specific effects of bacillus Calmette–Guérin (BCG) in protozoan infections caused by *Leishmania* sp. and *Plasmodium* sp. Increased numbers of monocytes and natural killer (NK) cells observed in humans infected with *Leishmania* sp. or *Plasmodium* sp. after BCG treatment are associated with the reduction of parasitaemia in *Plasmodium* sp. infections and in the reduction of the number of lesions in tegumentary leishmaniasis.

Table 1
Selected studies on the effect of BCG on tegumentary leishmaniasis and malaria infection

Study	Study design	Result
Lima et al. [31]	Retrospective study of human VL performed in the state of Rio Grande do Norte, Brazil for the period 1990–2014	The decreased incidence of VL in children was associated with improved BCG childhood immunization
Convit et al. [37]	Randomized trial combining a vaccine consisting of live BCG and killed *Leishmania* promastigotes was compared with a regular antimonial chemotherapy in 94 LCL patients	The immunotherapy was as efficient as the regular antimonial chemotherapy. However, the side-effects were fewer in the immunotherapy group
Convit et al. [38]	The clinical efficacy of immunotherapy with a combination of killed *Leishmania* promastigotes and viable BCG was performed in a controlled clinical study of 217 TL patients	High therapeutic efficacy was observed using combined immunotherapy in patients with intermediate and DCL who were previously unresponsive to chemotherapy
Convit et al. [39]	Patients with ML (4) and with early DCL (3) were treated monthly with intradermal injections of a vaccine containing killed *Leishmania* promastigotes and viable BCG	Complete remission of lesions occurred after five to nine infections in patients with ML or seven to ten injections in patients with DCL. DCL patients developed a positive leishmanin skin reaction
Castes et al. [40]	The immune responses of LCL patients were evaluated before and after the immunotherapy treatment (39), chemotherapy (34) and in patients who had received BCG (14) alone	No differences were observed in the final levels of immune responses among the groups. In all groups, similar numbers of T cells or *ex vivo* activation was detected
Pereira et al. [42]	One patient with DCL from Amazonian Brazil was treated monthly with injections of a combination of two different species of killed *Leishmania* promastigotes associated with BCG	The immunotherapy treatment resulted in temporary healing of all lesions and an increased number of pro-inflammatory monocytes and NK cells were found infiltrating the lesions
Alimonhammadian et al. [44]	75 volunteers with no response to the leishmanin test were injected double-blind and randomly with either autoclaved *Leishmania major* parasites plus BCG or BCG alone	The addition of BCG to the boosters resulted in significantly increased the frequency and the magnitude of leishmanin skin tests. No differences in IFN-γ responses, a month and a year later were observed
Clark et al. [46]	Vaccinated or non-vaccinated BCG mice were infected with *Plasmodium berghei*, *P. yoelii* and *P. vinckei*	BCG conferred protection against *Plasmodium* spp. infections
Parra et al. [48]	Mice were vaccinated with BCG and challenged with *P. yoelii* parasites	BCG administration decreased the parasitaemia of *P. yoelii* in mice. In addition, an increased expression of antimicrobial peptides was observed
Walk et al. [49]	Randomized controlled phase 1 clinical trial in 20 healthy male and female volunteers vaccinated or not with BCG followed by a controlled human malaria infection	After malaria infection, BCG vaccinated volunteers present with earlier and more severe clinical adverse events. The parasitaemia in these BCG-vaccinated volunteers was inversely correlated with increased NK cell and monocyte activation

BCG, bacillus Calmette–Guérin; DCL, diffuse cutaneous leishmaniasis; IFN, interferon; LCL, localized cutaneous lesion; ML, mucosal leishmaniasis; NK, natural killer; VL, visceral leishmaniasis.

changes *in vitro* and in Kenyan children naturally infected with malaria. These findings highlight the capacity of malarial stimulation to induce trained innate immunity [50]. A selection of studies is summarized in Table 1.

Summary and future directions

Based on the evidence discussed in this brief review, administration of BCG seems to have non-specific beneficial effects for both leishmaniasis and malaria. A proof of concept was the improvement of the clinical outcome of patients with severe forms of TL after BCG administration and the low parasitaemia levels in BCG vaccinated volunteers challenged with *Plasmodium* sp. Moreover, previous studies have shown a potential role for BCG in the treatment of autoimmune diseases, which are diseases known to have a chronic inflammatory condition often seen in TL and malaria [51]. Although the cellular mechanisms of BCG-mediated protection against these parasites needs to be further unravelled, it is likely to be that trained immunity makes a considerable contribution to control of infection. A deeper understanding of the molecular mechanisms supporting the BCG-induced trained macrophage phenotype will provide new avenues for the development of effective treatment strategies for TL and malaria.

Preventative vaccines directed against neglected tropical diseases are needed as long-term solutions. The fact that both *Leishmania* sp. and *Plasmodium* sp. can activate the immune system supports the idea that a vaccine is possible for TL and malaria. So far, vaccine development for parasitic diseases has focused on generating long-term adaptive mediated immunity. Importantly, agents that activate innate immune memory might be candidates for long-term protection as well. Therefore, future studies should aim to explore the involvement of innate immune cells conferring protection against *Leishmania* sp. and *Plasmodium* sp. infections.

Since BCG vaccination is common practice in TL and malaria endemic countries as part of the WHO Expanded Programme on Immunization, potential efficacy against other pathogens underscores the need for investment in timely and correct BCG administration.

Transparency declaration

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