Vaccine 37 (2019) 3628–3637

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Vaccines and the regulatory arm of the immune system. An overview from the *Trypanosoma cruzi* infection model

Gabriel Cabrera *, Iván Marcipar

Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina
Facultad de Ciencias Médicas, Universidad Nacional del Litoral, Santa Fe, Argentina

ARTICLE INFO

Article history:
Received 3 August 2018
Received in revised form 13 April 2019
Accepted 3 May 2019
Available online 31 May 2019

Keywords:
Trypanosoma cruzi
Vaccine
Myeloid-derived suppressor cells
Foxp3+ regulatory T cells
Trans-sialidase

ABSTRACT

The knowledge that the immune system is composed of a regulatory/suppressor arm added a new point of view to better understand the nature of several pathologies including cancer, transplants, infections and autoimmune diseases. The striking discoveries concerning molecules and cells involved in this kind of regulation were followed by the elucidation of equally notable mechanisms used by several pathogens to manipulate the host immune system. Vaccines against pathogens are an invaluable tool developed to help the immune system cope with a potential infection or prevent disease pathology. Nowadays, there is accumulated evidence indicating that the powerful stimulation capacity of vaccines influences not only the effector arm of the immune system but also cells with regulatory/suppressor capacity, such as myeloid derived suppressor cells (MDSCs) and Foxp3+ regulatory T cells (Tregs).

Trypanosoma cruzi (*T. cruzi*) is a protozoan parasite with a complex life cycle that has evolved several strategies to influence the regulatory immune response. Although diverse vaccine formulations have been able to stimulate the effector response, achieving non-sterilizing protection against *T. cruzi*, the influence of the vaccine candidates on the regulatory machinery has scarcely been assessed. This fact may not only reveal important information concerning how vaccines may influence cells with regulatory/suppressor capacity but also open the possibility to analyze whether vaccines are able to disrupt the mechanisms used by some pathogens to manipulate the host regulatory circuits. The aim of this review is to summarize and discuss available data related to the role of cellular components, like MDSCs and Foxp3+ Tregs, during *T. cruzi* infection, and the potential utility of those populations as additional targets for the rational design of vaccines.

Contents

1. Introduction

Body health and immune homeostasis require the function of several tolerant mechanisms developed in order to avoid a reaction against self-components as well as non-self components that are not pathogenic, like the normal intestinal microbiota. Since

* Corresponding author at: Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.
E-mail address: galt132000@yahoo.com.ar (G. Cabrera).

https://doi.org/10.1016/j.vaccine.2019.05.015
0264-410X/

breakdown of tolerance may provoke a myriad of pathologies, active processes must be maintained to control the powerful mechanisms that are ready to react against danger signals provoked by internal or external agents. Notably, to persist in their hosts, several pathogens have evolved mechanisms that involve manipulation of some of the many regulatory mechanisms of the immune system. *Trypanosoma cruzi* (*T. cruzi*), the etiological agent of Chagas disease, is one of many of the pathogens endowed with the ability to take advantage of the regulatory pathways of its host. Several lines of evidence have shown that cellular immunosuppression plays a critical role during *T. cruzi* infection [1–4]. In this sense, cells like myeloid-derived suppressor cells (MDSCs) [5–7], Foxp3+ regulatory T cells (Tregs) [8–13], regulatory dendritic cells [14–16], γδ T cells [17], NK1.1 cells [17], and suppressive IL-10-producing neutrophils [18] have been mentioned among the cellular components with regulatory/suppressor capacity that may play a role during the infection process. In order to help the immune system in its fight against *T. cruzi*, numerous vaccine prototypes have been tested (live attenuated, inactivated, subunit and DNA vaccines). To date, achievements include an important stimulation of the effector immune response as well as an important level of protection, but without eliminating the parasite [19–22]. Interestingly, despite the well-known evidence of the ability of the parasite to alter regulatory circuits, few studies have evaluated the influence of vaccine candidates on the regulatory arm of the immune system.

The aim of this review is to discuss available information concerning the influence of vaccines on MDSCs and Tregs, with a special emphasis on the rational vaccine design against *T. cruzi*.

2. Myeloid-derived suppressor cells

The existence of non-T cells with suppressive capacity in the bone marrow was first described in the 1970s. At that time, it was also reported that foreign agents like Bacillus Calmette-Guerin (BCG) may influence this suppressive cell population [23,24]. However, this cell type only began to receive massive attention after its association with cancer pathology and the discovery of several molecular markers in mice and human [25].

Currently, MDSCs are described as a heterogenous population of myeloid origin, immature state and a remarkable ability to suppress T-cell responses [25]. Ample evidence has shown that MDSCs play an important role in regulating the immune system not only in the context of cancer pathology but also in viral, bacterial and parasitic infections, acute and chronic inflammation, traumatic stress, surgical sepsis and transplantation [25].

In mice, MDSCs are generally defined by the expression of both Gr-1 and CD11b markers. In addition, two subpopulations were described: G-MDSCs ($CD11b^{+}Ly6G^{+}Ly6C^{low}$, also called granulocytic MDSCs) and M-MDSCs ($CD11b^{+}Ly6G^{-}Ly6C^{high}$, also called monocytic MDSCs) [25]. In human peripheral blood mononuclear cells (PBMCs), the equivalent to G-MDSCs are defined as $CD11b^{+}CD14^{-}CD15^{+}$ or $CD11b^{+}CD14^{-}CD66b^{+}$ and M-MDSCs as $CD11b^{+}CD14^{+}HLA\text{-}DR^{-/low}CD15^{-}$ [26]. Several molecules and mechanisms have been associated with suppression by MDSCs, including arginase, nitric oxide (NO), reactive oxygen species (ROS), transforming growth factor-β (TGF-β), interleukin-10 (IL-10), carbon monoxide (CO), indoleamine 2,3-dioxygenase (IDO), heme oxygenase-1 (HO-1), prostaglandin E2 (PGE2) and depletion of cysteine [27].

Interestingly, it has been shown that MDSCs play a very important role during *T. cruzi* infection. In particular, it has been reported that the percentage of CD11b+ GR-1+ cells in total spleen cells may increase from nearly 2% to nearly 20% in acutely infected mice [5–7]. In addition, it has been shown that MDSCs also infiltrate the heart of *T. cruzi* infected mice [28]. The increase of these cells in the heart and spleen very likely contributes to immunosuppression of the response against *T. cruzi*, as has been widely reported in the literature [1–3,5].

Although it may be assumed that immunosuppression is detrimental to the host, in fact, it has also been shown that MDSCs are very relevant because their depletion results in nearly 100% of mortality of infected mice. Furthermore, after Tulahuén *T. cruzi* infection, C57BL/6 mice responded with lower increases of MDSCs than BALB/c mice and show higher mortality [6].

Thus, according to this information, several questions can be raised concerning the development of a vaccine against *T. cruzi*:

- Should MDSCs be considered in the rational vaccine design and assessment?
- Could a vaccine affect the MDSC response?
- Should a vaccine eliminate or decrease the MDSC response?

To date, the general influence of vaccines on MDSCs has been scarcely assessed, neither during the immunization process nor after immunization and subsequent challenge with the corresponding pathogen (Table 1).

Concerning only immunization, in a model of live attenuated vaccine it has been reported that intradermal BCG vaccination in mouse ears can recruit $CD11b^{+}Ly\text{-}6C^{int}Ly\text{-}6G$ myeloid cells (called imMDSC in the report) [29]. Those cells were able to block T-cell proliferation and dampened Ag-specific priming in the draining lymph node through NO production in vivo. The authors suggest that although imMDSC may play a beneficial role for the host, avoiding excessive tissue inflammation and controlling the BCG-induced T-cell response; in fact, the induction of those cells with suppressive capacity may also dampen the efficacy of the BCG vaccine. In this sense, it has been widely suggested that some pathogens are able to subvert the immune system by inducing regulatory/suppressor cells; then, it could be assumed that the use of that kind of microorganisms in live attenuated vaccines would induce a similar response. In this particular case, it would be interesting to analyze what response would be elicited after BCG immunization and Mycobacterium tuberculosis challenge, since to date, it has not been completely explained why BCG vaccine does not protect against the pulmonary forms of mycobacterium tuberculosis [29].

In another model of live attenuated vaccine, it has been reported that immunization of mice with live wild type *Salmonella* induced CD11b+ GR-1+ cells, whereas the use of a bivalent vaccine (comprising strains that lack or overproduce DNA adenine methylase) reduced the expansion of CD11b+ GR-1+ cells. Interestingly, the bivalent vaccine (instead of the wild-type that induced MDSCs) was able to confer protection against several serotypes of pathogenic *Salmonella*; a fact that also correlated with the presence of *Salmonella*-specific cross-reactive opsonizing antibodies and memory T cells [30].

In a model of nonhuman primate simian immunodeficiency virus (SIV) infection, it was shown that a protocol of immunization using a subunit vaccine based on peptides plus a boost with a live attenuated vaccine (modified vaccinia Ankara, MVA) expanded two populations of MDSC-resembling cells that were able to suppress a specific CD8+ T cellular response. The described MDSCs expressed the following markers: $Lin^{-}DR^{low}CD33^{+}CD11b^{+}$ (Lin^{-} MDSCs) and $CD14^{+}DR^{low}CD33^{+}CD11b^{+}$ (CD14+ MDSCs). Notably, a control group of mice, treated only with a combination of adjuvants (D-Type CpG oligodeoxinucleotides, PolyI:C, IL-15, α-GalCer, and B7-DC-Ig), did not develop the increase of MDSCs after treatment. In addition, after the SIV challenge, mice treated only with the adjuvant combination showed a lower increase of MDSCs than non-treated mice or mice receiving the peptide-prime/MVA boost vac-

Table 1
Studies of vaccines that have analyzed alterations in MDSC.

Pathogen model	Vaccine (type)	MDSCs after immunization	MDSCs after challenge	Protection	Ref.
BCG	BCG vaccine (live attenuated vaccine)	Increased	n.m.	n.m	Martino et al. [29]
Salmonella	Salmonella wt (live vaccine)	Increased	n.m	Not obtained	Heithoff et al. [30]
Salmonella	Salmonella bivalent vaccine* (live attenuated vaccine)	Decreased (compared to wt)	n.m	Obtained	Heithoff et al. [30]
Simian immunodeficiency virus (SIV)	Peptides/Modified vaccine Ankara (subunit vaccine + live attenuated vaccine)	Increased	Increased (similar to control non-vaccinated)	Not obtained	Sui et al. [31]
SIV	combination of adjuvants*1 (treatment)	Slightly increased	Decreased (compared to non-vaccinated)	Obtained	Sui et al. [31]
T. cruzi	TSf-ISPA (subunit vaccine)	Slightly increased	Decreased (compared to non-vaccinated)	Obtained	Prochetto et al. [7]

This table shows the influence of each type of vaccine on MDSC after immunization, after challenge with the corresponding pathogen and the correlate of protection obtained. n.m: not measured; *bivalent vaccine: comprise strains that lack or overproduce DNA adenine methylase; *1combination of adjuvants: D-Type CpG oligodeoxinucleotides, PolyI:C, IL-15, α-GalCer, and B7-DC-Ig.

cine, a fact that correlated with lower SIV loads and thus better protection [31]. Based on these results, the authors proposed that counteracting the vaccine-induced MDSCs could be important in designing an effective HIV vaccine [31].

According to these data, it seems that at least some live attenuated and subunit vaccines may increase MDSCs with suppressive capacity, which in turn may affect vaccine efficacy. We have recently reported that mice immunization with a subunit vaccine composed of a fraction of the *T. cruzi* trans-sialidase protein (TSf), adjuvanted with a cage-like liposomal adjuvant (ISPA) [32], influenced the effector arm of the immune system as well as cells with regulatory/suppressor phenotype [7]. In our study, immunization slightly increased spleen CD11b+ GR-1+ cells. In contrast, after challenge with *T. cruzi*, TSf-ISPA immunization partially prevented a high increase in the percentage and absolute number of CD11b+ GR-1+ cells in the vaccinated group as compared to non-vaccinated control infected mice [7]. Since immunized mice showed higher survival after the challenge, the results are in line with previous reports describing that a decrease of MDSCs correlates with a better protective capacity of a vaccine after challenge with the corresponding pathogen [31,33]. At the same time, the results suggest that a slight increase of MDSCs after vaccination does not invalidate the efficacy of a vaccine, which should be tested after the challenge.

Interestingly, it has been reported that a decrease in MDSCs during *T. cruzi* infection has been associated with higher mortality of mice. Since the TSf-ISPA vaccine decreased MDSCs but at the same time increased mouse survival, results strongly suggest that the vaccine candidate was able to beneficially modulate the host immune system, very likely in part as a consequence of the stimulation of the effector immune response. In this sense, we have shown that the TSf-ISPA formulation as well as other trans-sialidase (TS)-based vaccine formulations elicited several components that are compatible with a Th1 response, such as the generation of plasma antibodies with a ratio IgG2a to IgG1 that is greater than 1, an important delayed-type IV hypersensitivity response against the TS antigen, and increases of IFN-γ expression by spleen CD4+ and CD8+ cells [7,32,34,35].

On the other hand, we also found that only the subtype of G-MDSCs (CD11b+ Ly6G+ Ly6C^{low}) was decreased within the CD11b+ GR-1+ population after TSf-ISPA immunization and *T. cruzi* infection, suggesting that these cells may have a different role than that of M-MDSCs (CD11b+ Ly6G− Ly6C+). Interestingly, in the SIV model the treatment with the adjuvant combination resulted in a lower increase of the Lin− subtype of MDSCs than that of naïve infected mice. Since this alteration correlated with protection against SIV infection, the result also suggests that MDSC subtypes may play different roles, depending on the characteristics of each pathogen [31].

Much more research is required to better understand the scenarios where vaccines may significantly affect MDSCs, the mechanisms involved and the relevance of those cells in the protective capacity of vaccines. Nonetheless, in the particular case of *T. cruzi* infection, at least four possibilities may be proposed to explain the decrease in the MDSC response observed after TSf-ISPA immunization and *T. cruzi* infection (Fig. 1).

First, it is possible that TSf-ISPA immunization elicited a more effective immune response, decreasing parasitemia and then rendering the increase in MDSCs unnecessary. Second, it has been reported that the IL-17 cytokine is able to induce MDSCs in some settings [36]. Moreover, it has been proposed that a subpopulation of γδ T cells, producing high levels of IL-17, could be the candidate that modulates the numbers of MDSCs in the acute phase of *T. cruzi* infection [17]. Therefore, it is possible to speculate that if the TSf-ISPA formulation affected in some way the γδ T cell population and/or IL-17 levels, then the decrease of MDSCs could be correlated to the alterations in that cytokine; currently, we are performing experiments to assess the levels of IL-17 in our model [7]. Third, as will be discussed below, the induction of specific Foxp3+ Treg cells may replace in some way the immunosuppressor function of the MDSCs. Fourth, since it has been shown that certain proteins may induce MDSCs, such as the core protein of hepatitis C virus (HCV) [37] and the tat and gp120 proteins of human immunodeficiency virus (HIV) [38,39], it cannot be discarded that TS may play a similar role during *T. cruzi* infection. In support of this hypothesis, it is well-known that TS is a critical virulence factor that mediates several alterations of the immune response [40–42]. Thus, it is possible that TS also affects MDSCs by an unknown interaction; in this case, anti-TS antibodies would account for the lower increases of MDSCs observed in TSf-immunized mice.

The first alternative depicted in Fig. 1 could be used as an example of a vaccine that has the capacity to beneficially influence the regulatory arm of the immune system, without disrupting any mechanism of the pathogen and leading to a decrease in MDSCs and a better survival. On the other hand, the fourth alternative could be used as an example of a vaccine that may disrupt a mechanism evolved by a pathogen to manipulate the immune system, since has been widely shown that TS can favor *T. cruzi* survival in many ways [40–42]. Finally, it cannot be ruled out that both influences may take place at the same time, or combinations of the alternatives mentioned above. For instance, IFN-γ was found to be involved in *T. cruzi* induction of MDSCs in vivo [5]. However,

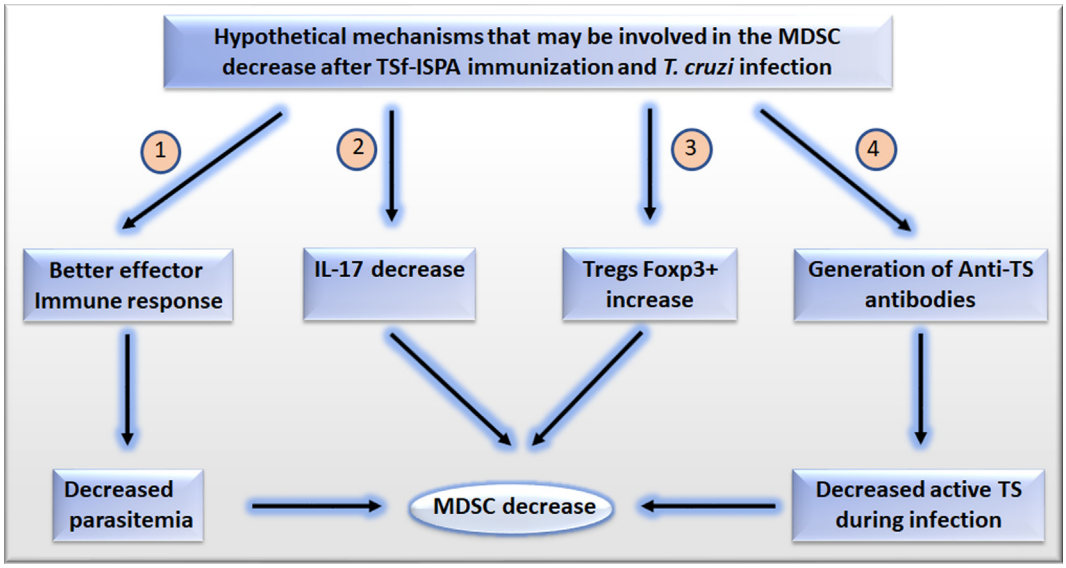

Fig. 1. Possible explanations for the decrease in MDSCs recorded after TSf-ISPA immunization and *T. cruzi* infection. (1) If TSf-ISPA immunization elicited a more effective immune response, a decreased parasitemia might induce a lower MDSC increase. (2) If the TSf-ISPA formulation affected in some way the γδ T cells and/or the IL-17 levels, then the decrease of MDSCs could be correlated to the alterations in that cytokine. (3) The induction of specific Foxp3+ Treg cells would replace in some way the immunosuppressor function of the MDSCs. (4) If TS affected MDSCs by an unknown interaction, anti-TS antibodies would account for the lower increases of MDSCs observed in TSf-immunized mice. Combinations of the four alternatives mentioned as well as the involvement of other immune circuits cannot be discarded.

we described that the TSf-ISPA vaccine candidate increased IFN-γ production at least in T cells without inducing MDSCs, suggesting that the vaccine influences mechanisms of MDSC induction [7]. Moreover, it has also been proposed that TS can manipulate the immune response by decreasing the production of IFN-γ and thus impairing the development of a Th1 response [42]. Interestingly, the TSf-ISPA vaccine also seemed to interfere with this pathogen manipulation by increasing IFN-γ and eliciting several components compatible with a Th1 response.

In summary, regarding MDSC role during *T. cruzi* infection, there are multiple mechanisms that remain to be elucidated, and experiments conducted to study this topic should be cautiously interpreted until additional mouse models and parasite strain combinations are assessed. However, results obtained up to date support that *T. cruzi* induces MDSCs with suppressor capacity during the acute phase of experimental infection. In this setting, we observed that our vaccine formulation, which prevents mouse death in acute infection, was able to significantly decrease the MDSC response after pathogen challenge. The fact that the blunted response of those cells correlated with better survival supports consideration of MDSCs as another potential vaccine target. Notably, it could be expected that the reciprocal influence of vaccines and MDSCs in different pathologies could only be assessed properly from a broad perspective that includes the characteristics of both the host and the pathogen, as well as the analysis of the effector immune response [7,33].

3. Foxp3+ regulatory T cells

The first evidence that a subtype of T cells may be important in regulating the immune response was also obtained during the 1970s [43]. However, interest in this kind of cells greatly increased only after the work of Sakaguchi et al., published in 1995, which identified a subpopulation of CD4+ T cells expressing the CD25+ marker with immunoregulatory capacity [44]. In 2001, a mutation in a gene called Foxp3 was found to account for a characteristic extensive multiorgan infiltration and elevation of numerous cytokines in the scurfy strain of mice. Then, a short time later, several groups showed that Foxp3 is the master regulatory gene for cell-lineage commitment of CD4+CD25+ regulatory T cells in the thymus and the periphery [45–47]. In mice, it was demonstrated that Foxp3 expression was necessary and sufficient to program regulatory T cells (Tregs) [48]. Studies in humans showed differences from those findings in mice; in particular, Foxp3 was shown to be inducible and expressed in activated T cells. Accordingly, the use of CD127 as an additional marker was proposed, and CD4+Foxp3+CD25^{high}CD127$^{low/-}$ is generally used to phenotypically differentiate human Treg cells [49]. According to the origin, Tregs are usually classified into three types: thymus-derived T regulatory cells (tTregs, previously called natural Tregs or nTregs); pTregs (peripheral Tregs), when Tregs are generated in the periphery from conventional T cells under appropriate conditions; and finally the name of iTreg (induced Tregs) has been proposed for those Tregs generated ex vivo [50].

The suppressor function of Tregs is exerted on a wide range of cells, including dendritic cells, natural killer cells, B cells and T cells [51]. Several molecules have been associated with the regulatory capacity of Tregs, including IL-10, TGF-β, Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4), granzymes, CD39, CD73 and IL-35 [51,52].

It is well-known that Tregs play a role in almost every biological context where the immune system is involved, including autoimmunity, cancer, acute and chronic infections, host-commensal interactions and inflammation at barrier sites, allergy, pregnancy, tissue repair, metabolic sterile inflammation, and allotransplantation [52]. Moreover, several reports have made clear that many pathogens are able to take advantage of Tregs in order to delay and/or impair the immune response, facilitating their chronic persistence. *Leishmania major*, *Plasmodium yoelii*, *Mycobacterium tuberculosis*, Hepatitis C virus, *Helycobacter pylori*, helminths, Japanese encephalitis virus, mouse mammary tumor virus and other pathogens have evolved complex strategies to manipulate the host immune system by inducing and expanding Tregs [53–61].

In the particular case of *T. cruzi* infection, several studies conducted in different mouse models have analyzed the role of Tregs during the acute phase of infection, highlighting differences in the influence of Tregs in clinical and immunological parameters of *T. cruzi*-infected mice (summarized in Table 2). Although the available data may seem contradictory, the comparison between

Table 2
Role of Tregs in different models of *T. cruzi* infection.

Mouse strain/*T. cruzi* strain/dose/route	Experimental Treatment	Conclusion about Treg influence on infection parameters	Conclusion about the influence of Tregs on survival	Highlighted Treg role	Ref.
A/J mice/Brazil strain/10,000 parasites/intraperitoneal (i.p.)	Anti-CD25 (PC61)	Tregs increased cardiac parasitemia	**Not measured**	**Tregs were able to favor the pathogen**	Bonney et al. [62]
C57BL/6 mice DEREG mice/Y strain (DTU II)/10,000 parasites/subcutaneous route (s.c)	Depletion with Diphtheria toxin or anti-CD25	Tregs decreased CD8+ response Tregs increased parasitemia	**Not measured**	**Tregs were able to favor the pathogen**	Ersching et al. [63]
C57BL6 mice/Tulahuén strain (DTU VI)/5000 parasites/i.p.	Adoptive transference of Tregs	Tregs increased parasitemia Tregs decreased CD8+ response	**Not measured**	**Tregs were able to limitedly favor the pathogen**	Araujo Forlán et al. [64]
BALB/c mice/Y Strain (DTU II)/1000 parasites/i.p.	Anti-CD25 (PC61) Anti-GITR	Tregs decreased miocarditis, Treg decreased parasitemia	**Tregs increased survival**	**Tregs were able to favor the host**	Mariano et al. [11]
C57BL/6 BALB/c mice/Y strain (DTU II)/50–2000 parasites/i.p.	No treatment	C57BL/6 mice have more Treg in heart and decreased parasitemia as compared to BALB/c mice	**Tregs increased survival**	**Tregs were able to favor the host**	Sanoja et al. [65]
BALB/c mice/H8 Yucatán strain/80,000 parasites/i.p.	Adoptive transference of rSSP4 specific Treg cells	Tregs increased parasitemia	**Tregs Increased survival**	**Tregs were able to favor the host**	Flores-Garcia et al. [66]
C57BL/6 mice/Tulahuen strain (DTU VI)/1000 parasites/s.c	IL-2+ dexamethasone	No difference in parasitemia	**Tregs increased survival**	**Tregs were able to favor the host**	González et al. [10]
C57BL6 mice/brasil strain/1000 parasites/i.p.	anti-CD25 (PC61 and 7D4)	**No influence**	**No influence**	**No influence**	Kotner and Tarleton [8]
C57BL/6, BALB/c and C3H/HeJ mice/Y strain (DTU II)/50 parasites/(i.p)	Anti-CD25 (PC61)	**No influence**	**No influence**	**No influence**	Sales et al. [9]
C57BL6 mice/Tulahuén strain (DTU VI)/100–1000 parasites/i.p.	anti-CD25 (PC61 and 7D4)	Tregs slight decreased the CD8+ response. Tregs did not influence parasitemia	**No influence**	**Tregs were able to limitedly favor the pathogen**	Kotner and Tarleton [8]
C57BL/6 BALB/c and C3H/HeJ mice/Colombian T. strain (DTU I)/50 parasites/i.p	Anti-CD25 (PC61)	Tregs increased parasitemia No difference in CD8 response	**No influence**	**Tregs were able to limitedly favor the pathogen**	Sales et al. [9]
Outbred Swiss mice/Colombian strain (DTU I)/1000 parasites/i.p.	Non-depleting Anti-CD25 (7D4)	Tregs increased parasitemia Tregs decreased CD8 response	**No influence**	**Tregs were able to favor the pathogen**	Nihei et al. [67]

This table shows the influence of Tregs on each model of study, concerning immunological parameters, survival and general role in the acute phase of infection.

the used models and the obtained results reveals some similarities underlying the divergent results. In particular, during host-parasite interaction Tregs never play a detrimental role in host survival, even if, most of the times, their influence favors an increase in parasitemia and restrains the CD8 response. This interpretation may be achieved if the experimental models are divided into three groups: (a) the models in which survival was not measured (light gray in Table 2); (b) the models in which survival was measured and was found to be changed (white in Table 2); (c) the models in which survival was measured and was found unchanged (dark gray in Table 2). Each group is fully described below.

Group (a): At least three mouse models may be included in this group. They were focused mainly on the influence of Tregs over parasitemia and/or the CD8 response. For instance, Bonney et al. 2015 reported that depletion of Tregs could lead to decreased cardiac parasitosis and inflammation in *T. cruzi*–infected mice, suggesting that Tregs may play a role in restraining the inflammatory and parasite-specific immune responses generated by *T. cruzi* infection [62]. In addition, a role of Tregs in the acute phase of infection might be inferred by a study conducted by Ersching et al., in 2016. In this case, the authors described that depletion of Foxp3+ cells improved the priming of specific CD8+ T cells and decreased parasitemia upon *T. cruzi* infection. Moreover, the authors reported that *T. cruzi*-exposed bone marrow derived dendritic cells (BMDCs) loaded with the specific SIINFEKL-OVA peptide were able to induce CD4+ Foxp3+ cells that dampen the OTI CD8+ response, suggesting that *T. cruzi* may take advantage of Tregs to impair an efficient CD8 priming by DCs [63]. Lastly, a recent study has described that a reduced frequency of Tregs in relation to effector cells is required to allow the generation of a CD8+ response against *T. cruzi* [64]. The fact that adoptive transference of Tregs can dampen the immune response suggests that an increase of those cells may favor the persistence of the parasite. Taken together, all mouse models in which survival was not measured mainly highlight that Tregs are able to play a role in restraining the immune response and/or increasing parasitemia during the acute phase of infection.

Group (b): At least four mouse models may be included in this group, in which survival measurement is reported. For instance, Mariano et al. 2008 described that Tregs may play a role in increasing mouse survival, since anti-CD25 or anti-GITR treatment may increase mortality after a challenge with *T. cruzi*. The same report demonstrated that treatment with anti-GITR augmented myocarditis, with increased migration of CD4+, CD8+, and CCR5+ leukocytes, TNF-α production, and tissue parasitism [11]. In the same line, a recent report showed that treatment with dexamethasone and IL-2 was able to increase Tregs and mouse survival during *T. cruzi* infection [10]. In addition, in a work that compared the immune response in BALB/c and C57BL/6 mice infected with the Y strain of *T. cruzi*, the authors described that C57BL/6 mice had increased numbers of Tregs in the heart, decreased cardiac parasitemia and increased survival after challenge [65]. Lastly a report showed that adoptive transfer of CD4+CD25+FOXP3+ T cells from rSSP4- (a recombinant *Trypanosoma cruzi* amastigote derived protein) immunized mice to syngeneic infected mice resulted in increased parasitemia but also increased survival [66]. Taken together, all these mouse models suggest that when survival is measured and found to be changed, Tregs always play a role in increasing the resistance to infection, regardless of the alterations in the CD8 response and parasitemia.

Group (c): At least five mouse models can be included in this group in which survival was measured but was found unchanged.

For instance, it has been reported that depletion of Tregs using an anti-CD25 monoclonal antibody before and during acute infection with a nonlethal strain (Brazil strain) neither improved nor worsened mouse survival, and only a slight increase of the CD8 response could be detected when the anti-CD25 antibody was administered prior to challenge with a lethal strain (Tulahuén strain) [8]. Similar results were obtained by another group that also injected a depleting anti-CD25 antibody before infection of mice with *T. cruzi*. When mice were infected with the Y strain, no differences in parasitemia or mortality rates were detected as compared to control non-depleted and infected mice [9]. In addition, the anti-CD25 antibody treatment decreased parasitemia without affecting mortality when the Colombian strain of *T. cruzi* was used [9]. Lastly, a study conducted using a non-depleting anti-CD25 antibody reported that administration of the monoclonal antibody 10 days before infection did not significantly affect survival but stimulated some components of the effector immune response and reduced parasitemia [67]. Results with this group of mouse models suggest that when survival is unchanged, Tregs may play role in favoring the parasite, although this influence could also be limited in some scenarios.

Thus, according to this classification, the results are not necessarily contradictory, since the first group of models highlights the functional capacity of Tregs, showing that those cells are able to dampen the immune response and increase parasitemia. In contrast, the results obtained from the second and third groups suggest that Tregs do not play a role in decreasing survival, which is favorable for the host if that parameter is considered the most important. In other words, the results may reflect the biological importance of Tregs, which would play a role (when necessary) in allowing host survival, even if they have to restrain the immune response with a resulting increase of parasitemia. It can be concluded that although the role of Tregs during acute infection seems contradictory according to the particular mouse and parasite strain combination employed in the studies, important similarities may be found when the reports are analyzed focusing on the assessment of survival. Notably, this role of Tregs seems to be a constant trend, considering that the experiments differ in mouse strains (BALB/c, C57BL/6, C3H/HeJ, A/J mice, outbred Swiss), DTUs of *T. cruzi* strains (Tulahuén, Y strain, Colombian, Brazil, Yucatán, RA), parasite doses (50, 100, 1000, 2000, 10,000, 80,000), experimental approaches used to assess the role of Tregs (anti-CD25 depletion, adoptive transference, IL-2+ dexamethasone treatment, depletion of Tregs with diphtheria toxin), and inoculation routes (intraperitoneal and subcutaneous).

Concerning the chronic phase of infection, accumulated evidence suggests that Tregs play a beneficial role. For instance, it has been described that individuals with the indeterminate (IND) clinical form of the disease have a higher frequency of $CD4^+$ $CD25^{high}$ Tregs than patients with the cardiac (CARD) clinical form [13,68]. According to the authors, an expansion of Tregs could be beneficial, controlling the balance between regulatory and effector T cells, which could be important in the progression and development of the disease [13,68]. Tregs from IND patients showed suppressive activity related to the increased production of IL-17, IL-10 and granzyme B [12]. In the same line, a study conducted by another group also found that patients with the IND clinical form of the disease had a higher percentage of CD4+CD25+ cells after culture with *T. cruzi* antigens; this finding reinforces the hypothesis that Treg lymphocytes may be able to control deleterious cytotoxic activity in individuals with asymptomatic chronic Chagas disease [69]. Another study suggested that high levels of macrophage-like cells ($CD14^+CD16^+$) and NK cells, together with high frequency of regulatory lymphocytes $CD4^+CD25^{HIGH}$ cells, may favor the establishment/maintenance of the lifelong indeterminate clinical form of the disease [70]. Moreover, in a mouse model, G-CSF administration increased CD3+Foxp3+ Tregs in the heart of chronically infected animals, a fact that correlated with decreased parasitism, inflammation and fibrosis [71]. Nonetheless, although most of the literature suggests that Tregs may play a beneficial role during the chronic phase of the disease, there are some contradictory data. In this sense, a recent report has described that cells cultured from CARD patients had higher frequencies of $CD4^+CD25^+Foxp3^+$ than IND patients (upon stimulation with EPI antigen) [72].

Thus, according to the information available, several questions arise concerning the potential influence of a vaccine on Tregs during *T. cruzi* infection.

- Could and should a vaccine induce an increase or decrease of Tregs during the acute phase of infection?
- Could a vaccine influence a hypothetical interaction between Tregs and MDSCs?
- Should a vaccine influence Tregs in order to favor a better outcome during the chronic stage of infection?

Accumulated evidence indicates that many kinds of vaccines (live attenuated, inactivated, subunit and DNA vaccines) can induce Treg increases with potential capacity to decrease the efficacy of vaccination, as previously reviewed [73,74]. Moreover, according to this evidence, there is an active search of adjuvants that target Tregs in order to avoid an increase of those cells after vaccination [75,76].

Interestingly, despite the active research on vaccines against *T. cruzi* as well as on the role of Tregs in the pathology associated with infection, the influence of vaccine candidates on Tregs in the particular case of *T. cruzi* infection has scarcely been assessed. We have recently reported that a subunit vaccine (TSf-ISPA) did not affect CD4+Foxp3+ Tregs after immunization [7]. On the other hand, after a challenge, immunized and infected mice showed an increased absolute number of spleen Tregs at day 21 of infection, which could be analyzed from different points of view [7]. Since Tregs are expected to reduce the immune response against a pathogen, as has been described for *T. cruzi* [8,9,63,67] and also in other models [77–82], it may be hypothesized that the Tregs increase may be detrimental to the host in the acute phase of infection. However, an equivalent number of reports described that those cells may play a beneficial role [10,11,62,65,66]. Moreover, the fact that the induction of Tregs by a vaccine may result in a better control of a parasite has also been reported in a mouse model of infection [83]. In addition, since the TSf-ISPA vaccine decreased the highly immunosuppressive MDSCs, and the depletion of those cells has been associated with increased mortality, it is possible that Tregs replaced at some extent the suppressive capacity of MDSCs, and even in a more specific manner. This observation also allows us to speculate that the huge increase of MDSCs during the acute phase of *T. cruzi* infection generates a systemic immunosuppression that renders assessment of the role of Tregs more difficult. This scenario may account, in part, for the divergence observed among the studies that report that Tregs plays a role during acute *T. cruzi* infection from those models that describe that those cells do not influence significatively the outcome of the of infection [8,9]; in addition, the different mouse models that have been used may contribute to the divergent results.

Similarly to MDSCs, several mechanisms may be proposed to account for Treg increases after TSf-ISPA immunization and *T. cruzi* infection (Fig. 2). First, a better immune response elicited by the vaccine may be paralleled by a higher increase of Tregs, which could help to balance the response. Second, the fact that MDSCs decreased at the same time as Treg numbers increased suggests the existence of a potential interaction between both populations of regulatory/suppressor cells, as suggested in tumor models [84–86]. Interestingly, the M-MDSC subtype, which was almost unaffected by the TSf-ISPA formulation, has been shown to expand

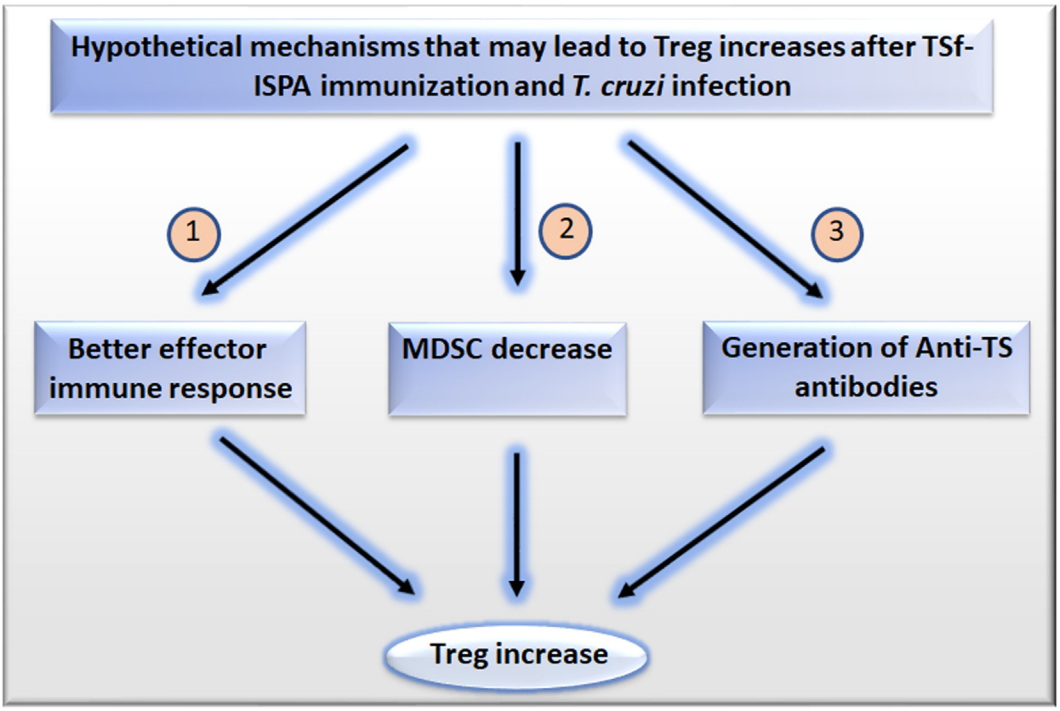

Fig. 2. Possible explanations for the increase of Tregs recorded after TSf-ISPA immunization and *T. cruzi* infection. (1) A better immune response elicited by the vaccine may be paralleled by a higher increase of Tregs that should help to balance the response. (2) A hypothetical interaction between Tregs and total MDSCs (or one MDSC subtype) may account for the Treg increase. (3) The generation of anti-TSf antibodies may result in thymus Treg protection after TSf-ISPA immunization and *T. cruzi* infection, allowing an increase of regulatory cells.

or recruit the Treg population [87,88], which raises the possibility that these cells play a role in the Treg increases observed in the spleen of immunized and infected mice [7]. Third, it has been reported that TS contributes to thymus atrophy [41] and that *T. cruzi* infection is able to cause a marked loss of thymus Tregs [89]. Thus, it may be speculated that the generation of anti-TSf antibodies may result in thymus Treg protection after TSf-ISPA immunization and *T. cruzi* infection. The finding that the use of TS-neutralizing antibodies apparently rescued normal thymocyte proliferation indexes, avoiding thymus alterations [90], supports this hypothesis.

Considering that thymus-derived Tregs are present in a basal state and are normally induced in several scenarios to balance the effector immune response, it seems noteworthy that a pathogen like *T. cruzi* does not induce clear increases of this population during the generation of the immunosuppression that is observed during the acute phase of infection. In this sense, it could be speculated that the parasite benefits much more from MDSCs, which increase from nearly 2% to nearly 20% in the spleen, than from Treg immunosuppression. Thus, it is possible that the balance between MDSCs and Tregs may represent a target for a vaccine designed to modulate the immune response or to interfere with the manipulating mechanism of *T. cruzi* or other pathogens. In any case, much more research is required to answer the questions concerning the influence of vaccines on Tregs during *T. cruzi* infection. In addition, it could be useful to determine whether vaccines that increase Tregs during the acute phase may be beneficial to avoid disease development during the chronic stage.

Nowadays, ample evidence indicates that some classical and widely used vaccines fail to prevent pathogen infection but protect against disease pathology [29,91]. The analysis of the influence of vaccines on the regulatory arm of the immune system may shed light about how this protection is achieved. Moreover, since alterations of cells with regulatory/suppressor capacity have been recorded after immunization with live attenuated vaccine [29,30], inactivated vaccines [83], subunits vaccines [77] and DNA vaccines [92], it cannot be discarded that many vaccine candidates against *T. cruzi* also influence the regulatory arm of the immune system after immunization and likely after parasite challenge. On the other hand, information from human beings concerning the role of cells with regulatory/suppressor capacity during the acute phase of *T. cruzi* infection is very scarce, since ethical guidelines and medical protocols indicate that any detected case should be treated with the available drugs (benznidazole and nifurtimox) in order to take advantage of the high probability of cure of the treatment during acute infection. Accordingly, even when there are similar populations of Treg Foxp3+ cells and MDSCs in both human and mice [48,49], the precise role of those populations during human *T. cruzi* infection cannot be predicted according to the information obtained from conventional animal models. Currently, humanized mice represent a valuable tool that may permit preclinical analysis of human immune responses [93,94]. Such kind of approach could be very valuable before clinical testing of a vaccine candidate that meets the criteria required for clinical testing, as previously described for Chagas disease [20].

4. Concluding remarks

Vaccination is a very powerful strategy developed with the aim of helping the immune system to protect against a potential, or even established, pathological condition. Although most vaccine studies address the effect of immunization on the effector arm of the immune system, ample evidence supports that the regulatory/suppressor counterpart may also be affected during immunization with many types of vaccines. *T. cruzi* infection results in a complex pathology that includes the generation of a strongly immunosuppressed state during the acute phase, representing a valuable

model to assess how a vaccine could influence regulatory components of the immune system or whether a vaccine could interfere with the mechanisms evolved by some pathogens to take advantage of the host regulatory response. Several populations could be assessed to this end, including MDSCs, Tregs, regulatory dendritic cells, γδ T cells, NK.1.1 and suppressive neutrophils IL-10+. The TSf-ISPA vaccine candidate has been shown to be able to influence simultaneously at least MDSCs and Tregs after the pathogen challenge, a result that correlated with increased mice survival. To our knowledge, that report was the first to address simultaneously two important regulatory/suppressor populations after immunization and the corresponding pathogen challenge. Much more research is needed to determine the importance of the regulatory response to the application of different vaccines and to understand the underlying mechanisms. Despite these limitations, the present results show the potential contribution of studies to determine if the regulatory mechanisms triggered by a given vaccine might influence its efficacy. This research would contribute new criteria to be applied in the rational design of vaccines.

Declaration of Competing Interest

None of the authors have a conflict of interest in relation to the content of the present work.

Acknowledgments

Gabriel Cabrera and Iván Marcipar are research career members of CONICET. The work of the authors related to this review was supported by ANPCyT (Argentine National Agency for the Promotion of Science and Technology) (PICT2014-620 and PICT 2015-2544) and SECTeI (Secretary of State for Science, Technology and Innovation of the province of Santa Fe) SECTeI 2016: 2010-099-16.

References

[1] Abrahamsohn IA, Coffman RL. Cytokine and nitric oxide regulation of the immunosuppression in *Trypanosoma cruzi* infection. J Immunol 1995;155:3955–63.
[2] Flávia Nardy A, Freire-de-Lima CG, Morrot A. Immune evasion strategies of *Trypanosoma cruzi*. J Immunol Res 2015;2015. https://doi.org/10.1155/2015/178947.
[3] Tarleton RL. *Trypanosoma cruzi*-induced suppression of IL-2 production. II. Evidence for a role for suppressor cells. J Immunol 1988;140:2769–73.
[4] DosReis GA, Fonseca MEF, Lopes MF. Programmed T-cell death in experimental chagas disease. Parasitol Today 1995;11:391–4. https://doi.org/10.1016/0169-4758(95)80011-5.
[5] Goñi O, Alcaide P, Fresno M. Immunosuppression during acute *Trypanosoma cruzi* infection: involvement of Ly6G (Gr1(+))CD11b(+)immature myeloid suppressor cells. Int Immunol 2002;14:1125–34.
[6] Arocena AR, Onofrio LI, Pellegrini AV, Carrera Silva AE, Paroli A, Cano RC, et al. Myeloid-derived suppressor cells are key players in the resolution of inflammation during a model of acute infection. Eur J Immunol 2014;44:184–94. https://doi.org/10.1002/eji.201343606.
[7] Prochetto E, Roldán C, Bontempi IA, Bertona D, Peverengo L, Vicco MH, et al. Trans-sialidase-based vaccine candidate protects against *Trypanosoma cruzi* infection, not only inducing an effector immune response but also affecting cells with regulatory/suppressor phenotype. Oncotarget 2017. https://doi.org/10.18632/oncotarget.18217.
[8] Kotner J, Tarleton R. Endogenous CD4(+) CD25(+) regulatory T cells have a limited role in the control of *Trypanosoma cruzi* infection in mice. Infect Immun 2007;75:861–9. https://doi.org/10.1128/IAI.01500-06.
[9] Sales PA, Golgher D, Oliveira RV, Vieira V, Arantes RME, Lannes-Vieira J, et al. The regulatory CD4+CD25+ T cells have a limited role on pathogenesis of infection with *Trypanosoma cruzi*. Microbes Infect 2008;10:680–8. https://doi.org/10.1016/j.micinf.2008.03.008.
[10] González FB, Villar SR, Fernández Bussy R, Martin GH, Pérol L, Manarin R, et al. Immunoendocrine dysbalance during uncontrolled *T. cruzi* infection is associated with the acquisition of a Th-1-like phenotype by Foxp3(+) T cells. Brain Behav Immun 2015;45:219–32. https://doi.org/10.1016/j.bbi.2014.11.016.
[11] Mariano FS, Gutierrez FRS, Pavanelli WR, Milanezi CM, Cavassani KA, Moreira AP, et al. The involvement of CD4+CD25+ T cells in the acute phase of *Trypanosoma cruzi* infection. Microbes Infect 2008;10:825–33. https://doi.org/10.1016/j.micinf.2008.04.009.
[12] de Araújo FF, Corrêa-Oliveira R, Rocha MOC, Chaves AT, Fiuza JA, Fares RCG, et al. Foxp3+CD25(high) CD4+ regulatory T cells from indeterminate patients with Chagas disease can suppress the effector cells and cytokines and reveal altered correlations with disease severity. Immunobiology 2012;217:768–77. https://doi.org/10.1016/j.imbio.2012.04.008.
[13] de Araújo FF, Vitelli-Avelar DM, Teixeira-Carvalho A, Antas PR, Assis Silva Gomes J, Sathler-Avelar R, et al. Regulatory T cells phenotype in different clinical forms of Chagas' disease. PLoS Negl Trop Dis 2011;5:e992. https://doi.org/10.1371/journal.pntd.0000992.
[14] Van Overtvelt L, Vanderheyde N, Verhasselt V, Ismaili J, De Vos L, Goldman M, et al. *Trypanosoma cruzi* infects human dendritic cells and prevents their maturation: inhibition of cytokines, HLA-DR, and costimulatory molecules. Infect Immun 1999;67:4033–40.
[15] Poncini CV, Soto CDA, Batalla E, Solana ME, González Cappa SM. *Trypanosoma cruzi* induces regulatory dendritic cells in vitro. Infect Immun 2008;76:2633–41. https://doi.org/10.1128/IAI.01298-07.
[16] Poncini CV, Ilarregui JM, Batalla EI, Engels S, Cerliani JP, Cucher MA, et al. *Trypanosoma cruzi* infection imparts a regulatory program in dendritic cells and T Cells via galectin-1-dependent mechanisms. J Immunol 2015;195:3311–24. https://doi.org/10.4049/jimmunol.1403019.
[17] Cardillo F, de Pinho RT, Antas PRZ, Mengel J. Immunity and immune modulation in *Trypanosoma cruzi* infection. Pathog Dis 2015;73. https://doi.org/10.1093/femspd/ftv082.
[18] Tosello Boari J, Amezcua Vesely MC, Bermejo DA, Ramello MC, Montes CL, Cejas H, et al. IL-17RA signaling reduces inflammation and mortality during *Trypanosoma cruzi* infection by recruiting suppressive IL-10-producing neutrophils. PLoS Pathog 2012;8:e1002658. https://doi.org/10.1371/journal.ppat.1002658.
[19] Rodríguez-Morales O, Monteón-Padilla V, Carrillo-Sánchez SC, Rios-Castro M, Martínez-Cruz M, Carabarin-Lima A, et al. Experimental vaccines against chagas disease: a journey through history. J Immunol Res 2015;2015:489758. https://doi.org/10.1155/2015/489758.
[20] Dumonteil E, Bottazzi ME, Zhan B, Heffernan MJ, Jones K, Valenzuela JG, et al. Accelerating the development of a therapeutic vaccine for human Chagas disease: rationale and prospects. Exp Rev Vacc 2012;11:1043–55. https://doi.org/10.1586/erv.12.85.
[21] Arce-Fonseca M, Rios-Castro M, Carrillo-Sánchez Sdel C, Martínez-Cruz M, Rodríguez-Morales O. Prophylactic and therapeutic DNA vaccines against Chagas disease. Parasit Vectors 2015;8:121. https://doi.org/10.1186/s13071-015-0738-0.
[22] Cazorla SI, Frank FM, Malchiodi EL. Vaccination approaches against *Trypanosoma cruzi* infection. Exp Rev Vacc 2009;8:921–35. https://doi.org/10.1586/erv.09.45.
[23] Duwe AK, Singhal SK. The immunoregulatory role of bone marrow. I. Suppression of the induction of antibody responses to T-dependent and T-independent antigens by cells in the bone marrow. Cell Immunol 1979;43:362–71.
[24] Bennett JA, Rao VS, Mitchell MS. Systemic bacillus Calmette-Guérin (BCG) activates natural suppressor cells. Proc Natl Acad Sci USA 1978;75:5142–4.
[25] Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–74. https://doi.org/10.1038/nri2506.
[26] Bronte V, Brandau S, Chen S-H, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun 2016;7:12150. https://doi.org/10.1038/ncomms12150.
[27] Zhao Y, Wu T, Shao S, Shi B, Zhao Y. Phenotype, development, and biological function of myeloid-derived suppressor cells. Oncoimmunology 2015;5. https://doi.org/10.1080/2162402X.2015.1004983.
[28] Cuervo H, Guerrero NA, Carbajosa S, Beschin A, De Baetselier P, Gironès N, et al. Myeloid-derived suppressor cells infiltrate the heart in acute *Trypanosoma cruzi* infection. J Immunol 2011;187:2656–65. https://doi.org/10.4049/jimmunol.1002928.
[29] Martino A, Badell E, Abadie V, Balloy V, Chignard M, Mistou M-Y, et al. Mycobacterium bovis bacillus Calmette-Guérin vaccination mobilizes innate myeloid-derived suppressor cells restraining in vivo T cell priming via IL-1R-dependent nitric oxide production. J Immunol 2010;184:2038–47. https://doi.org/10.4049/jimmunol.0903348.
[30] Heithoff DM, Enioutina EY, Daynes RA, Sinsheimer RL, Low DA, Mahan MJ. Salmonella DNA adenine methylase mutants confer cross-protective immunity. Infect Immun 2001;69:6725–30. https://doi.org/10.1128/IAI.69.11.6725-6730.2001.
[31] Sui Y, Hogg A, Wang Y, Frey B, Yu H, Xia Z, et al. Vaccine-induced myeloid cell population dampens protective immunity to SIV. J Clin Invest 2014;124:2538–49. https://doi.org/10.1172/JCI73518.
[32] Bertona D, Pujato N, Bontempi I, Gonzalez V, Cabrera G, Gugliotta L, et al. Development and assessment of a new cage-like particle adjuvant. J Pharm Pharmacol 2017. https://doi.org/10.1111/jphp.12768.
[33] Heithoff DM, Enioutina EY, Bareyan D, Daynes RA, Mahan MJ. Conditions that diminish myeloid-derived suppressor cell activities stimulate cross-protective immunity. Infect Immun 2008;76:5191–9. https://doi.org/10.1128/IAI.00759-08.

[34] Bontempi IA, Vicco MH, Cabrera G, Villar SR, González FB, Roggero EA, et al. Efficacy of a trans-sialidase-ISCOMATRIX subunit vaccine candidate to protect against experimental Chagas disease. Vaccine 2015;33:1274–83. https://doi.org/10.1016/j.vaccine.2015.01.044.

[35] Bontempi I, Fleitas P, Poato A, Vicco M, Rodeles L, Prochetto E, et al. Trans-sialidase overcomes many antigens to be used as a vaccine candidate against *Trypanosoma cruzi*. Immunotherapy 2017;9:555–65. https://doi.org/10.2217/imt-2017-0009.

[36] Kong X, Sun R, Chen Y, Wei H, Tian Z. γδT cells drive myeloid-derived suppressor cell-mediated CD8+ T cell exhaustion in hepatitis B virus-induced immunotolerance. J Immunol 2014;193:1645–53. https://doi.org/10.4049/jimmunol.1303432.

[37] Tacke RS, Lee H-C, Goh C, Courtney J, Polyak SJ, Rosen HR, et al. Myeloid suppressor cells induced by hepatitis C virus suppress T-cell responses through the production of reactive oxygen species. Hepatology 2012;55:343–53. https://doi.org/10.1002/hep.24700.

[38] Qin A, Cai W, Pan T, Wu K, Yang Q, Wang N, et al. Expansion of monocytic myeloid-derived suppressor cells dampens T cell function in HIV-1-seropositive individuals. J Virol 2013;87:1477–90. https://doi.org/10.1128/JVI.01759-12.

[39] Garg A, Spector SA. HIV type 1 gp120–induced expansion of myeloid derived suppressor cells is dependent on interleukin 6 and suppresses immunity. J Infect Dis 2014;209:441–51. https://doi.org/10.1093/infdis/jit469.

[40] Freire-de-Lima L, Fonseca LM, Oeltmann T, Mendonça-Previato L, Previato JO. The trans-sialidase, the major *Trypanosoma cruzi* virulence factor: three decades of studies. Glycobiology 2015;25:1142–9. https://doi.org/10.1093/glycob/cwv057.

[41] Nardy AFFR, Freire-de-Lima CG, Pérez AR, Morrot A. Role of *Trypanosoma cruzi* trans-sialidase on the escape from host immune surveillance. Front Microbiol 2016;7:348. https://doi.org/10.3389/fmicb.2016.00348.

[42] Ruiz Díaz P, Mucci J, Meira MA, Bogliotti Y, Musikant D, Leguizamón MS, et al. *Trypanosoma cruzi* trans-sialidase prevents elicitation of Th1 cell response via interleukin 10 and downregulates Th1 effector cells. Infect Immun 2015;83:2099–108. https://doi.org/10.1128/IAI.00031-15.

[43] Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 1970;18:723–37.

[44] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–64.

[45] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299:1057–61. https://doi.org/10.1126/science.1079490.

[46] Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003;4:330–6. https://doi.org/10.1038/ni904.

[47] Khattri R, Cox T, Yasayko S-A, Ramsdell F. An essential role for Scurfin in CD4 +CD25+ T regulatory cells. Nat Immunol 2003;4:337–42. https://doi.org/10.1038/ni909.

[48] Ziegler SF. FOXP3: of mice and men. Annu Rev Immunol 2006;24:209–26. https://doi.org/10.1146/annurev.immunol.24.021605.090547.

[49] Rodríguez-Perea AL, Arcia ED, Rueda CM, Velilla PA. Phenotypical characterization of regulatory T cells in humans and rodents. Clin Exp Immunol 2016;185:281–91. https://doi.org/10.1111/cei.12804.

[50] Shevach EM, Thornton AM. tTregs, pTregs, and iTregs: similarities and differences. Immunol Rev 2014;259:88–102. https://doi.org/10.1111/imr.12160.

[51] Benoist C, Mathis D. Treg cells, life history, and diversity. Cold Spring Harb Perspect Biol 2012;4:a007021. https://doi.org/10.1101/cshperspect.a007021.

[52] Plitas G, Rudensky AY. Regulatory T cells: differentiation and function. Cancer Immunol Res 2016;4:721–5. https://doi.org/10.1158/2326-6066.CIR-16-0193.

[53] Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 2002;420:502–7. https://doi.org/10.1038/nature01152.

[54] Hisaeda H, Tetsutani K, Imai T, Moriya C, Tu L, Hamano S, et al. Malaria parasites require TLR9 signaling for immune evasion by activating regulatory T cells. J Immunol 2008;180:2496–503.

[55] Trinath J, Maddur MS, Kaveri SV, Balaji KN, Bayry J. Mycobacterium tuberculosis promotes regulatory T-cell expansion via induction of programmed death-1 ligand 1 (PD-L1, CD274) on dendritic cells. J Infect Dis 2012;205:694–6. https://doi.org/10.1093/infdis/jir820.

[56] Hall CHT, Kassel R, Tacke RS, Hahn YS. HCV+ hepatocytes induce human regulatory CD4+ T cells through the production of TGF-beta. PLoS ONE 2010;5:e12154. https://doi.org/10.1371/journal.pone.0012154.

[57] Beswick EJ, Pinchuk IV, Earley RB, Schmitt DA, Reyes VE. Role of gastric epithelial cell-derived transforming growth factor beta in reduced CD4+ T cell proliferation and development of regulatory T cells during Helicobacter pylori infection. Infect Immun 2011;79:2737–45. https://doi.org/10.1128/IAI.01146-10.

[58] Grainger JR, Smith KA, Hewitson JP, McSorley HJ, Harcus Y, Filbey KJ, et al. Helminth secretions induce de novo T cell Foxp3 expression and regulatory function through the TGF-β pathway. J Exp Med 2010;207:2331–41. https://doi.org/10.1084/jem.20101074.

[59] Gupta N, Hegde P, Lecerf M, Nain M, Kaur M, Kalia M, et al. Japanese encephalitis virus expands regulatory T cells by increasing the expression of PD-L1 on dendritic cells. Eur J Immunol 2014;44:1363–74. https://doi.org/10.1002/eji.201343701.

[60] Boer MC, Joosten SA, Ottenhoff THM. Regulatory T-cells at the interface between human host and pathogens in infectious diseases and vaccination. Front Immunol 2015;6. https://doi.org/10.3389/fimmu.2015.00217.

[61] Cabrera G, Burzyn D, Mundiñano J, Courreges MC, Camicia G, Lorenzo D, et al. Early increases in superantigen-specific Foxp3+ regulatory T cells during mouse mammary tumor virus infection. J Virol 2008;82:7422–31. https://doi.org/10.1128/JVI.00102-08.

[62] Bonney KM, Taylor JM, Thorp EB, Epting CL, Engman DM. Depletion of regulatory T cells decreases cardiac parasitosis and inflammation in experimental Chagas disease. Parasitol Res 2015;114:1167–78. https://doi.org/10.1007/s00436-014-4300-3.

[63] Ersching J, Basso AS, Kalich VLG, Bortoluci KR, Rodrigues MM. A human trypanosome suppresses CD8+ T cell priming by dendritic cells through the induction of immune regulatory CD4+ Foxp3+ T cells. PLoS Pathog 2016;12:e1005698. https://doi.org/10.1371/journal.ppat.1005698.

[64] Araujo Furlan CL, Tosello Boari J, Rodriguez C, Canale FP, Fiocca Vernengo F, Boccardo S, et al. Limited Foxp3+ regulatory T cells response during acute *Trypanosoma cruzi* infection is required to allow the emergence of robust parasite-specific CD8+ T cell immunity. Front Immunol 2018;9:2555. https://doi.org/10.3389/fimmu.2018.02555.

[65] Sanoja C, Carbajosa S, Fresno M, Gironès N. Analysis of the dynamics of infiltrating CD4(+) T cell subsets in the heart during experimental *Trypanosoma cruzi* infection. PLoS ONE 2013;8:e65820. https://doi.org/10.1371/journal.pone.0065820.

[66] Flores-García Y, Rosales-Encina JL, Rosales-García VH, Satoskar AR, Talamás-Rohana P. CD4+CD25+FOXP3+ Treg cells induced by rSSP4 derived from *T. cruzi* amastigotes increase parasitemia in an experimental chagas disease model. Biomed Res Int 2013;2013. https://doi.org/10.1155/2013/632436.

[67] Nihei J, Cardillo F, dos Santos WLC, Pontes-de-Carvalho L, Mengel J. Administration of a nondepleting anti-CD25 monoclonal antibody reduces disease severity in mice infected with *Trypanosoma cruzi*. Eur J Microbiol Immunol (Bp) 2014;4:128–37. https://doi.org/10.1556/EuJMI.4.2014.2.6.

[68] de Araújo FF, da Silveira ABM, Correa-Oliveira R, Chaves AT, Adad SJ, Fiuza JA, et al. Characterization of the presence of Foxp3+ T cells from patients with different clinical forms of Chagas' disease. Hum Pathol 2011;42:299–301. https://doi.org/10.1016/j.humpath.2010.10.002.

[69] Braz SCde M, Melo AS de, Cavalcanti M de GA de M, Martins SM, Oliveira W de, Silva ED de, et al. Increase in the expression of CD4 + CD25+ lymphocytic T Cells in the indeterminate clinical form of human chagas disease after stimulation with recombinant antigens of *Trypanosoma cruzi*. J Clin Immunol 2014;34:991–8. https://doi.org/10.1007/s10875-014-0092-6.

[70] Vitelli-Avelar DM, Sathler-Avelar R, Massara RL, Borges JD, Lage PS, Lana M, et al. Are increased frequency of macrophage-like and natural killer (NK) cells, together with high levels of NKT and CD4+CD25high T cells balancing activated CD8+ T cells, the key to control Chagas' disease morbidity? Clin Exp Immunol 2006;145:81–92. https://doi.org/10.1111/j.1365-2249.2006.03123.x.

[71] Vasconcelos JF, Souza BSF, Lins TFS, Garcia LMS, Kaneto CM, Sampaio GP, et al. Administration of granulocyte colony-stimulating factor induces immunomodulation, recruitment of T regulatory cells, reduction of myocarditis and decrease of parasite load in a mouse model of chronic Chagas disease cardiomyopathy. FASEB J 2013;27:4691–702. https://doi.org/10.1096/fj.13-229351.

[72] Almeida MS, Lorena VMB, Medeiros C de A, Junior WO, Cavalcanti M da GAM, Martins SM, et al. Alternative Th17 and CD4+CD25+FoxP3+ cell frequencies increase and correlate with worse cardiac function in Chagas cardiomyopathy. Scandinavian J Immunol n.d.;87:e12650. https://doi.org/10.1111/sji.12650.

[73] Ndure J, Flanagan KL. Targeting regulatory T cells to improve vaccine immunogenicity in early life. Front Microbiol 2014;5. https://doi.org/10.3389/fmicb.2014.00477.

[74] Berod L, Puttur F, Huehn J, Sparwasser T. Tregs in infection and vaccinology: heroes or traitors? Microb Biotechnol 2012;5:260–9. https://doi.org/10.1111/j.1751-7915.2011.00299.x.

[75] Batista-Duharte A, Téllez-Martínez D, Fuentes DLP, Carlos IZ. Molecular adjuvants that modulate regulatory T cell function in vaccination: a critical appraisal. Pharmacol Res 2018;129:237–50. https://doi.org/10.1016/j.phrs.2017.11.026.

[76] Bayry J. Regulatory T cells as adjuvant target for enhancing the viral disease vaccine efficacy. Virusdisease 2014;25:18–25. https://doi.org/10.1007/s13337-013-0187-3.

[77] Lin P-H, Wong W-I, Wang Y-L, Hsieh M-P, Lu C-W, Liang C-Y, et al. Vaccine-induced antigen-specific regulatory T cells attenuate the antiviral immunity against acute influenza virus infection. Mucosal Immunol 2018. https://doi.org/10.1038/s41385-018-0004-9.

[78] Tang C-L, Yang J, Cheng L-Y, Cheng L-F, Liu Z-M. Anti-CD25 monoclonal antibody enhances the protective efficacy of *Schistosoma japonicum* GST vaccine via inhibition of CD4+CD25+Foxp3+ regulatory T cells. Parasitol Res 2017;116:2727–32. https://doi.org/10.1007/s00436-017-5581-0.

[79] Wen Z, Wang X, Dong K, Zhang H, Bu Z, Ye L, et al. Blockage of regulatory T cells augments induction of protective immune responses by influenza virus-like particles in aged mice. Microb Infect 2017;19:626–34. https://doi.org/10.1016/j.micinf.2017.08.013.

[80] Fedatto PF, Sérgio CA, Paula MOe, Gembre AF, Franco LH, Wowk PF, et al. Protection conferred by heterologous vaccination against tuberculosis is

dependent on the ratio of CD4(+)/CD4(+) Foxp3(+) cells. Immunology 2012;137:239–48. https://doi.org/10.1111/imm.12006.
[81] Brezar V, Ruffin N, Richert L, Surenaud M, Lacabaratz C, Palucka K, et al. Decreased HIV-specific T-regulatory responses are associated with effective DC-vaccine induced immunity. PLoS Pathog 2015;11:e1004752. https://doi.org/10.1371/journal.ppat.1004752.
[82] Knuschke T, Rotan O, Bayer W, Sokolova V, Hansen W, Sparwasser T, et al. Combination of nanoparticle-based therapeutic vaccination and transient ablation of regulatory T cells enhances anti-viral immunity during chronic retroviral infection. Retrovirology 2016;13:24. https://doi.org/10.1186/s12977-016-0258-9.
[83] Pereira Silva Bezerra I, Amaral Abib M, Rossi-Bergmann B. Intranasal but not subcutaneous vaccination with LaAg allows rapid expansion of protective immunity against cutaneous leishmaniasis. Vaccine 2018;36:2480–6. https://doi.org/10.1016/j.vaccine.2018.03.020.
[84] Schlecker E, Stojanovic A, Eisen C, Quack C, Falk CS, Umansky V, et al. Tumor-infiltrating monocytic myeloid-derived suppressor cells mediate CCR5-dependent recruitment of regulatory T cells favoring tumor growth. J Immunol 2012;189:5602–11. https://doi.org/10.4049/jimmunol.1201018.
[85] Wang J, Yang L, Yu L, Wang Y-Y, Chen R, Qian J, et al. Surgery-induced monocytic myeloid-derived suppressor cells expand regulatory T cells in lung cancer. Oncotarget 2017;8:17050–8. https://doi.org/10.18632/oncotarget.14991.
[86] Huang B, Pan P-Y, Li Q, Sato AI, Levy DE, Bromberg J, et al. Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res 2006;66:1123–31. https://doi.org/10.1158/0008-5472.CAN-05-1299.
[87] Ren JP, Zhao J, Dai J, Griffin JWD, Wang L, Wu XY, et al. Hepatitis C virus-induced myeloid-derived suppressor cells regulate T-cell differentiation and function via the signal transducer and activator of transcription 3 pathway. Immunology 2016;148:377–86. https://doi.org/10.1111/imm.12616.
[88] Luan Y, Mosheir E, Menon MC, Wilson D, Woytovich C, Ochando J, et al. Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion. Am J Transp 2013;13:3123–31. https://doi.org/10.1111/ajt.12461.
[89] González FB, Calmon-Hamaty F, Nô Seara Cordeiro S, Fernández Bussy R, Spinelli SV, D'Attilio L, et al. *Trypanosoma cruzi* experimental infection impacts on the thymic regulatory T cell compartment. PLoS Negl Trop Dis 2016;10:e0004285. https://doi.org/10.1371/journal.pntd.0004285.
[90] Mucci J, Hidalgo A, Mocetti E, Argibay PF, Leguizamon MS, Campetella O. Thymocyte depletion in *Trypanosoma cruzi* infection is mediated by trans-sialidase-induced apoptosis on nurse cells complex. Proc Natl Acad Sci USA 2002;99:3896–901. https://doi.org/10.1073/pnas.052496399.
[91] Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. Proc Natl Acad Sci USA 2014;111:787–92. https://doi.org/10.1073/pnas.1314688110.
[92] Wang S-M, Tsai M-H, Lei H-Y, Wang J-R, Liu C-C. The regulatory T cells in anti-influenza antibody response post influenza vaccination. Hum Vaccin Immunother 2012;8:1243–9. https://doi.org/10.4161/hv.21117.
[93] Akkina R. Human immune responses and potential for vaccine assessment in humanized mice. Curr Opin Immunol 2013;25:403–9. https://doi.org/10.1016/j.coi.2013.03.009.
[94] Legrand N, Ploss A, Balling R, Becker PD, Borsotti C, Brezillon N, et al. Humanized mice for modeling human infectious disease: challenges, progress, and outlook. Cell Host Microbe 2009;6:5–9. https://doi.org/10.1016/j.chom.2009.06.006.