Mechanobiology of immune cells: Messengers, receivers and followers in leishmaniasis aiding synthetic devices

Shweta Khandibharad, Prajakta Nimsarkar, Shailza Singh [*]

National Centre for Cell Science, NCCS Complex, Ganeshkhind, SP Pune University Campus, Pune, 411007, India

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ABSTRACT

Cytokines are influential molecules which can direct cells behavior. In this review, cytokines are referred as messengers, immune cells which respond to cytokine stimulus are referred as receivers and the immune cells which gets modulated due to their plasticity induced by infectious pathogen leishmania, are referred as followers. The advantage of plasticity of cells is taken by the parasite to switch them from parasite eliminating form to parasite survival favoring form through a process called as reciprocity which is undergone by cytokines, wherein pro-inflammatory to anti-inflammatory switch occur rendering immune cell population to switch their phenotype. Detailed study of this switch can help in identification of important targets which can help in restoring the phenotype to parasite eliminating form and this can be done through synthetic circuit, finding its wider applicability in therapeutics.

1. Introduction

In order to maintain the cellular homeostasis, the shape, migration, and differentiation of cells are altered as a result of the mechanical and other biophysical features of their surroundings. Through the process of mechanotransduction, cells transform external biophysical inputs into internal biochemical signals that can then alter gene regulation, cytoskeletal integrity, and/or chromatin's epigenetic configuration (Zhang et al., 2020). Cells in turn affect the structure and dynamics of the microenvironment by secreting, digesting, and modifying the matrix components. This dynamic mechanobiological interaction plays a key role in the progression of disease and tissue homeostasis. In this review, we have discussed about how signal transduction emanate immune response to infection by Leishmania spp. Mechanobiologists have a more complete understanding of how a signal transduction works. Due to the frequent nonlinear connections between protein activity and cellular function as well as the crosstalk with the other signaling pathways, many concerns have proven difficult to resolve. In the same time frame, new methods for comprehending and manipulating the cell behavior have been developed in the fields of synthetic biology, which tries to build *de novo* signaling systems from biological components, and systems biology, which seeks to characterize cell behavior holistically. With the goal of creating novel synthetic signaling pathways from protein and nucleic acids, synthetic biology offers a potent toolkit for engineering cellular behavior to treat diseases or deepen our understanding of biological processes. High-throughput assays, computational biology, and statistics are all used in systems biology to examine the relationships between many inherent features of the biological complexity on a systemic level.

Through the years definition of cytokines have advanced, it states that cytokines are small secreted proteins released by the cells to have a specific effect on the interactions and communication among cells (Zhang and An, 2007), whereas some describe them as instructions conferring communicators among immune and non-immune cells (Arango Duque and Descoteaux, 2014). Another group has also defined them as tiny secreted proteins (40 kDa) that nearly every cell produces to regulate and influence immune responses (Kany et al., 2019). In a nutshell, we are defining them as influential messengers that functions as a telephone which carries details to receiver end on how to comport. A message can be double-edged which will evoke deterministic response from receiving end such as, pro-inflammatory cytokines can induce a cell to eliminate foreign agents like bacteria, parasites, helminths (Muñoz-Carrillo et al., 2018) although they can also help the cancer cells to grow and proliferate through induced cell proliferation (Kartikasari et al., 2021). Cytokine is double-edged which makes apprehension of their roles in different diseases a concern.

The parasite that causes leishmaniasis is intracellular and can be transferred to humans by the bite of a sand fly. Asia, Africa, the

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Americas, and the Mediterranean region are all inhabited to this species (Torres-Guerrero et al., 2017). Phagocytic cells such as macrophages, neutrophils, and dendritic cells phagocytose the promastigotes. Because of intricate defense mechanisms, promastigotes can survive in macrophages and undergo dimorphic change to amastigote form (Maspi et al., 2016). There are broadly two forms of responses post infection, T Helper Cell Type 1 (Th1) and T Helper Cell Type 2 (Th2) which helps in parasite killing and survival respectively (Costa-da-silva et al., 2022). The immune response will be discussed in the subsequent sections.

2. Messengers and their role in leishmaniasis

Cytokines can function in a variety of ways, including autocrine, paracrine, and endocrine mechanisms. Different cell types frequently release the same cytokine, or a single cytokine may act on multiple cell types (pleiotropy). Cytokines are redundant in their activity, which means that different cytokines can trigger comparable processes. They are frequently produced in a cascade, with one cytokine stimulating the production of additional cytokines by its target cells (Zhang and An, 2007). The appellation for cytokines is indiscriminate (Kany et al., 2019) based on the type of immune response succeeding infection
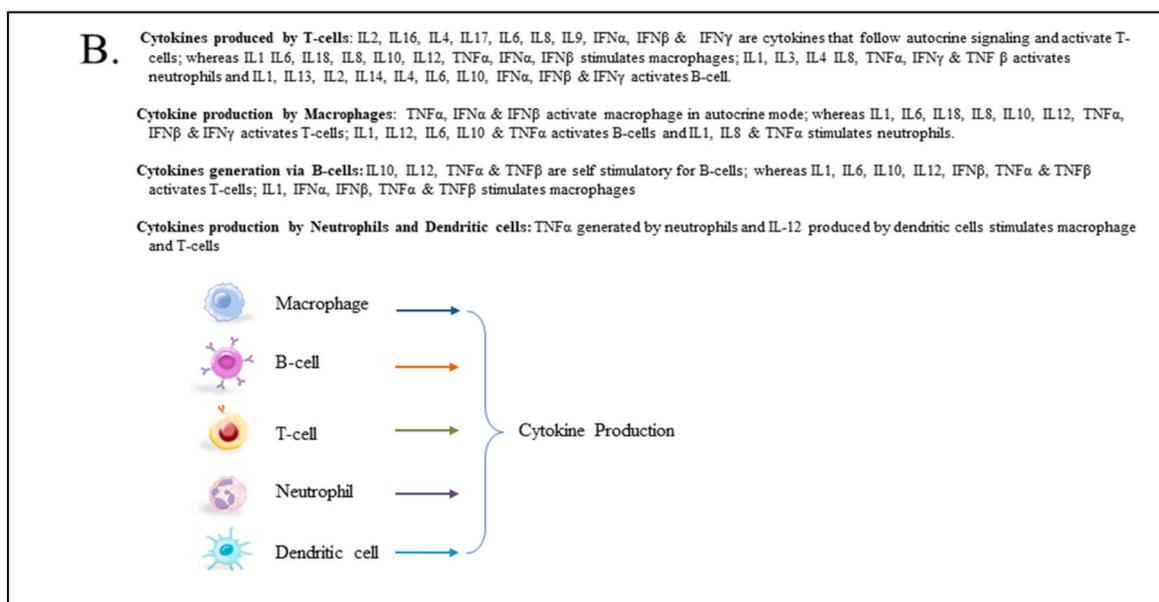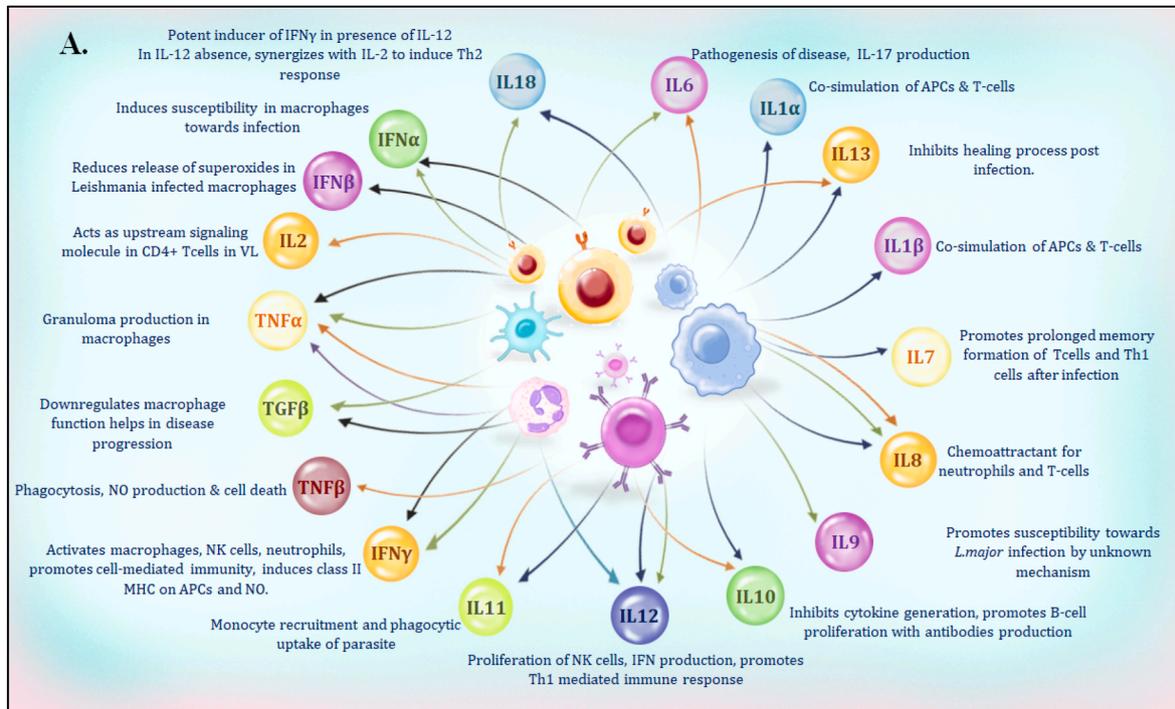

Fig. 1. Collaborative production of cytokine by immune cells during leishmaniasis: A: Cytokine and immune cells network. B. Figure legends of cytokine crosstalk among immune cells and their activity.

predominantly; cytokines are divided in two categories namely, pro-inflammatory cytokines and anti-inflammatory cytokines. As inflammation is useful for pathogen clearance and protection against infection, pro-inflammatory cytokines functions as regulators of host responses to infection, inflammation, but they can also exacerbate disease in pathological settings (Luo and Zheng, 2016). This group primarily consist Tumor necrosis factor alpha (TNF-α), Interleukin (IL-lα) and (IL-1β), Interleukin12 (IL-12), Interferon-gamma (IFN-γ), and Interleukin (IL-6). Anti-inflammatory cytokines, such as Interleukin10 (IL-10), Interleukin (IL-4), and Transforming growth factor beta (TGF-β), on the other hand, have been shown to reduce inflammation (Dayakar et al., 2019) (Gogos et al., 2000).

Every message is unique and the intention behind sending it is twice more special. Individual cytokine response contributes to a deterministic response of immune cells in leishmaniasis (Fig. 1) For instance, IL-6 is usually thought to be a pro-inflammatory cytokine although it is also reported to be important in the pathogenesis of *L. donovani* infected C57BL/6 mice. When compared to Wild Type (WT) mice, the rate of parasite elimination and control over infection was greater in IL-6−/− animals also the IL-6 −/− mice showed a higher Th1 response hence, it was inferred that IL-6 may have an immunosuppressive effect on the parasite-infected liver and could be a therapeutic target (Murray, 2008). In Cutaneous leishmaniasis (CL), IL-6 may impede suppression of parasite growth and survival in early stages of infection whereas it may also promote and enhance the disease severity (Soni et al., 2018). IL-6 can also act as gatekeeper cytokine for developmental regulation of CD25⁻ FoxP3⁻ IL-10⁺ CD4⁺ T regulatory (Treg) cells *in vivo* and is a crucial cytokine that regulates the therapeutic efficacy of adoptively transplanted Dendritic cells (DC) against *L. donovani* infection (Stäger et al., 2006). It can stimulate the production of IL-17 producing CD4⁺ T cells (Th17) in combination with TGF-β, which have been found to be pathogenic in a number of experimental models, while inhibiting Treg cells production (Kling et al., 2011). Significant effect of pentavalent antimony and amphotericin B was observed in WT C57BL/6 v/s IL-6 −/− C57BL/6 in parasite proliferation which was due to the inhibitory and deactivating effect of IL-6 on macrophages in a model of visceral leishmaniasis (VL), indicating that it could be a target for therapeutic intervention (Murray, 2008).

TNF-α participates in inflammation by induction of nitric oxide (NO). It functions by activating Th1 immune response through increasing macrophage activity, and is required for the generation and maintenance of granulomas (Bosch-Nicolau et al., 2019). Importance of TNF-α was also observed in *L.major* infected C57BL/6 TNF−/− mice as it lead to development of fatal form of VL despite highly active Th1 response (Fromm et al., 2015). In CL, TNF-α can directly activate macrophages derived from mouse and humans which can lead to suppression of disease or elimination of parasites (Tumang et al., 1994).

Acute and chronic inflammation is predominantly related with the IL-1 family of ligands and receptors. The cytokines IL-1α and IL-1β, which are encoded by two different genes, are referred to as IL-1. They possess potent pro-inflammatory properties and plays a key role in the host's response to external and endogenous deleterious stimuli (Gabay et al., 2010). Beyond inflammation, IL-1 is a downstream cytokine of the inflammasome, a cell injury sensor. It is vital for regulating inflammation and tissue damage (Kaneko et al., 2019). Role of IL-1 in leishmaniasis is debatable. In BALB/c mice, dose-dependent effects of IL-1 were found to impact the pathogenesis and made them susceptible to murine leishmaniasis (Voronov et al., 2010) whereas some members of the IL-1 family are anti-inflammatory or antagonistic to inflammatory receptors, suggesting therapeutic potential (Amorim et al., 2021).

IL-12 impacts the cell fate which shapes immunological responses after antigen presentation of developing naive T cells. Murine leishmaniasis is an infectious disease model in which IL-12 has been investigated the most (Gately and Mulqueen, 1996). The parasite invades macrophages silently, triggering a stress response, whereas DC become activated and produces IL-12, that prepare antigen-specific T cells (Scott

and Novais, 2016). IL-12 performs two important roles in leishmaniasis primarily it promotes development of Th1 response and it helps in prolonging IFN-γ synthesis (Mullen et al., 2001). It has also been reported to act as a major contributor for the maintenance of resistance to *L.major* infection. The decrease of Th1 cells is connected to the loss of a protective response in *L.major*-infected IL-12-deficient mice. IL-12 reduces Th2 cell growth mediated by IL-4 signaling determining Th1 cells enrichment (Park et al., 2002). Studies have also reported that in *in vivo* IL-12 neutralization at early and late stages of *L.donovani* infection may lead to substantial downregulation of IFN-γ, TNF-α and iNOS where, early IFN-γ production is dependent on IL-12 production (Engwerda et al., 1998) (Rostami and Khamesipour, 2021).

IFN-γ has long been known as a principal pro-inflammatory cytokine in the pathogenesis of inflammatory diseases that acts as a master regulator of immune responses (Zhang, 2007). Experimental L.*major* infections in C57BL/6 and BALB/c inbred mouse strains were linked to the production of IFN- γ by CD4⁺ T cells, whereas uncontrolled infections were linked to the absence of IFN-γ in the C57BL/6 and BALB/c mice, respectively (Kima and Soong, 2013). The finding that VL patients' peripheral blood mononuclear cells (PBMCs) do not respond to stimulation with leishmanial antigen is a crucial immunological aspect of the disease. Leishmania-driven IFN-γ showed inhibitory effects on parasite development in patients with active VL. This suggests that IFN-γ can be a possible additional medication in the treatment of VL (Kumar et al., 2014). IFN-γ induced activity are modulated by effector molecules produced by leishmania parasites such as L-like cysteine proteinases genes (lmcpb) modulates STAT-1α and AP-1 to block NO production mediated by IFN-γ (Kima and Soong, 2013) which represses parasite clearance machinery.

Pro-inflammatory cytokines as highlighted may help to eliminate infection. Although it may initiate life-threatening "cytokine storms" and may contribute to the fast systemic organ failure seen in some critically ill patients (Saha and Silvestre, 2021). The anti-inflammatory messengers can provide insufficient control over pro-inflammatory activity in immune-mediated diseases, overcompensate under pathologic conditions and suppress the immunological response, exposing the host at risks of infection (Opal and DePalo, 2000). In COVID-19 patients, when immunologic problems such as cytokine storm emerge, antiviral therapy alone is insufficient and must be coupled with anti-inflammatory therapy (Soy et al., 2020). It was observed that patients suffering from VL showed a cytokine storm characterized by an increase in levels of pro-inflammatory (IFN-γ, TNF-α, IL-2, IL-6, IL-12, IL-17, and macrophage migration inhibitory factor (MIF)), anti-inflammatory (IL-4, IL-5, and IL-13), and regulatory cytokines (IL-10) (Santos-Oliveira et al., 2011). Cytokine-based immunotherapy is a promising strategy for treating leishmaniasis, as cytokines restore host resistance and immune effector function, ultimately leading to the elimination of parasites (Saha and Silvestre, 2021).

The anti-inflammatory cytokine IL-10 is produced by activated immune cells (Ip et al., 2017). A prime regulatory cytokine for suppressing inflammatory pathways is IL-10. In addition, IL-10 has been found to promote in the spread of leishmania parasites. IL-10−/− mice on a BALB/c origin were able to suppress infection with *L.major*, while IL-10−/− mice on a C57BL/6 origin established sterile immunity to this parasite, unlike their wild-type littermates (Schwarz et al., 2013) that IL-10 has two functions in CL, first; it has been also reported to reduce immunological responses to leishmania parasites and enhance CL susceptibility. On the other side, it is important in speeding up the wound healing process. As a result, in the absence of IL-10 in CL, serious tissue damage may develop (Abdoli et al., 2017).

The hallmark cytokines of the type II inflammatory response are IL-4 and Interleukin13 (IL-13). They play an important role in the inflammatory response that is caused by an invading parasite or an allergen (Junttila, 2018). The quintessential Th2 cytokines IL-4 and IL-13 suppresses protective Th1 response and increases infection susceptibility of *L.major* in CL (Hurdayal and Brombacher, 2014). With infection of *L.*

donovani in, IL-4R −/−, IL-4 −/−, and IL-13 −/− mice on a BALB/c origin have shown that the Th2 cytokines IL-4 and IL-13 play important but not fully overlapping roles in suppressing primary infection in VL (McFarlane et al., 2019). IL-4 increases CL pathogenesis by boosting both Th2 and pathogenic CD8$^+$ T cell immunological responses through induction of GATA3 and IFN-γ inducing transcription factor (TF) Tbet which overall contributes to pathogenicity of leishmaniasis (Poudel et al., 2020). In vaccine development studies, due to higher levels of IL-4, leishmanial antigens (LAg) in conjunction with two routinely used human-compatible adjuvants, alum and saponin, failed, whereas simultaneous elevation of IL-4 and IL-10 aggravated the disease, in mice injected with saponin + LAg immunised (Bhowmick et al., 2014).

TGF-β is mostly known for its immunosuppressive properties. It was shown that *L. infantum* suppression was achieved by inhibiting the TGF-β pathway, which is a negative regulator of the innate immune response (Serafim et al., 2021). TGF-β is a potent regulatory cytokine that inhibits the expression of inducible NO synthase (iNOS) and IFN-γ, as well as the development of Th1 and Th2 cells (Gantt et al., 2003). In murine leishmaniasis, BALB/c mice treated with neutralizing anti-TGF1 antibody showed inhibition in formation of lesions within 5 weeks and was maintained thereafter (Barral-Netto et al., 1992). Therefore, in order to modulate anti-inflammatory messengers, therapeutic strategies are being devised which can lead to expression of pro-inflammatory cytokines without majority of them being suppressed.

3. Role of receivers in leishmaniasis

Innate immune cells secrete cytokines, which are important regulators of the immune response. These soluble regulatory signals that trigger and restrict inflammatory responses to infections derive from these intercellular messengers. In immunology, cytokine release pathways, molecules, and mechanisms are still a "black box" (Lacy and Stow, 2011). Macrophages and lymphocytes are the principal producers of cytokines (Arango Duque and Descoteaux, 2014). The cells which receive messengers (cytokines) and differentiate into different types and sub-types to respond towards infection are referred as receivers. These include macrophages, T cells, B cells, DCs and NK cells.

Macrophages are prime immune cells target of cytokine reception and act as receivers by interacting with the messengers from infected macrophage and initiating cytokine signaling that modulates cell pattern changes and mediates specified cytokines secretion. When macrophages phagocytose the parasite, its antigens are processed and placed on the plasmalemma's outer surface, where T helper cells can recognize them. Based on the functionality, macrophages are divided into two types namely, M1 classically activated that produces NO mediated by Th1 and NK cells and M2 alternatively activated that produces polyamines by upregulating arginase mediated by Th2 and Treg cells of which M1 helps in parasite clearance and M2 in parasite survival (Liu and Uzonna, 2012). M2 macrophage is further divided to four sub-types; M2a, M2b, M2c, and M2d based on signal stimulus and secreted factors. Based on messengers molecules that the macrophage receives; polarization occurs respectively such as, M2a is activated by macrophage colony-stimulating factor (M-CSF), IL-4 and IL-13 and mainly induces arginase and IL-10 expression, M2b is induced by IL-1βRa, LPS and TLR stimulators which induces IL-10, TNF-α and IL-6, M2c activation is a resultant of IL-10, TGF-β signaling which expresses high levels of arginase,IL-10 and TGF-β, induction of M2d occurs by IL-6 and it expresses elevated levels of IL-10 and show reduced expression of IL-12 (Tomiotto-Pellissier et al., 2018). T cells release cytokines that activate B cells, and activated B cells secrete antibodies specific to the antigens provided by the macrophage as a result of this identification. These antibodies bind to antigens on the parasite or cells infected by the parasites and macrophages phagocytose these antibody-bound complexes more readily (Arango Duque and Descoteaux, 2014). This elucidates the process highlighting the exchange of messengers between immune cells controlling their phenotype.

Dendritic cells (DCs), as a part of the innate immune system, use Pathogen Recognition Receptors (PRR) mainly Toll like Receptors (TLRs) to detect and respond to external pathogenic stimuli. They can detect opsonized leishmania parasite and then phagocytose them and in response to it secrete IL-12 (Donaghy et al., 2010). Innate protective cytokines such as type I and III interferons are secreted in lesser concentrations (Agrawal et al., 2017). DCs specializes in processing and presenting antigens to T cells, as they secrete IL-12 which can polarize naïve T cells to Th1 cell, thus Th1 cell can further induce infected macrophage to produce IFN-γ (Liu and Uzonna, 2012). Potent DC activation results in the release of cytokines that block Treg induction. In the presence of specific cytokines, DCs are more likely to expand and differentiate into Tregs. The cytokines IL-10 and TGF-β are particularly crucial in the induction of Tregs by DCs (Okeke and Uzonna, 2019).

T cell activation generates a powerful inflammatory response, which contributes in the development of cutaneous lesions. CD8$^+$ T cells have been found to protect against infection. Although on the other hand, they are also reported to exacerbate illness (Novais and Scott, 2015). Th1 and Th2, two counter-regulatory CD4$^+$ T cell populations control resistance and susceptibility to parasite through production of IL-12 and IL-4 (Alexander and Brombacher, 2012). Generation of Tregs by Regulatory Macrophages (Mregs) to has been shown to include the generation of reactive oxygen species (ROS), which is induced by IL-10 and TGF-β activity. Induction of Tregs by macrophages is hampered when the NAPDH-oxidase complex, which is involved in ROS generation, is disrupted. Mreg/Treg cross-talk is an important immunological tolerance mechanism that can be investigated for better understanding cell to cell crosstalk. γδT cells have both inflammatory and regulatory functions. Pro-inflammatory T cells are divided into two groups based on whether they produce IFN-γ or Interleukin-17 (IL-17) (Okeke and Uzonna, 2019).

B cells are the only cell type that can produce antibodies and are the only cell type whose selective depletion can ameliorate a wide spectrum of immune-mediated inflammatory diseases. B cell's ability to operate as cytokine-producing cells explains their properties of regulating monocyte activity and thus disease etiology (Fillatreau, 2018). In the priming of parasite-specific T lymphocytes, B cells and antibodies play a role. Dendritic cells could be stimulated to release high quantities of cytokines and increase their ability to prime naive CD4$^+$ T cells by coating them with the antibodies (Wanasen et al., 2008). It was reported that, stimulating endosomal TLRs, *L. donovani* amastigotes can activate B cells, resulting in the production of different cytokines such as IL-10 and IFN Type 1 cytokines (Silva-Barrios et al., 2016).

Although NK cells have seemed to be non-essential for the long-term control of CL and VL and can have immunosuppressive functions, they are a significant source of IFN-γ (Bogdan, 2012). IL-18 enhances NK cell activation in *L. infantum*-infected C57BL/6 mice. In the presence of leishmania infection, NK cells are part of the early inflammatory infiltrate at the infection site and co-localize with myeloid cells *in vivo*. NK cells are now also known for their potential to lyse or activate macrophages infected with Leishmania (Prajeeth et al., 2011).

Hence in response to various infection, immune cells functions as per the assigned roles in the immune system. Leishmaniasis enables modulation of host cells expression (Afrin et al., 2019) and tampering with the cell plasticity (Lecoeur et al., 2022), making it indispensable to study reciprocal expression of cytokines due to parasitic modulatory effects and thus observe how the cells behave (see Fig. 1).

4. Influence of cytokines reciprocity in leishmaniasis

The biological function of pro-inflammatory and anti-inflammatory cytokines is influenced by three major factors which includes the cytokine's local concentration, the stage of disease at which it is secreted, and its interaction with the other cytokines (Shachar and Karin, 2013). These may lead to changes in cytokine expression paradigm of the cell which can be referred as reciprocity. Reciprocity of cytokines here refers to anti-inflammatory cytokines produced in response to

pro-inflammatory cytokines in order to regulate disease which promotes the parasite survival. Reciprocity may be governed by different mechanisms such as epigenetic remodeling, transcription regulators such as activators and repressors, regulatory RNAs such as microRNAs and biochemical process of Arginine metabolism (Fig. 2) (see Fig. 5).

Leishmania spp cause a spectrum of illness ranging from asymptomatic mild response to progressive and ultimately fatal infection. *Leishmania major* infection in mice is similar to *L.donovani* infection in humans. In resolving or progressing infection in murine leishmaniasis, IFN-γ and IL-4 are produced reciprocally, supporting the theory that different T-Helper subsets modulate the spectrum of this infectious illness. Furthermore, passive injection of a neutralizing antiIL-4 mAb slowed the progression of leishmania infection in susceptible mice, implying that the lymphokine's actions encourage infection progression (Heinzel et al., 1989). BALB/c mice infected with *L. major* produced IL-4 mRNA, while infected C57BL/6 mice did not. IFN-γ mRNA was higher in the draining nodes and spleen of C57BL/6 mice than in BALB/c mice suggesting mRNA decay or modulation of transcription of cytokines to regulate disease progression (Maspi et al., 2016).

Two key cytokines that exceedingly affect the outcome of leishmaniasis are IL-12 and IL-10. The authors offered two models, one in a healthy state and the other in a diseased state, to demonstrate how IL-12 secretion is responsible for parasite elimination and how IL-10 can threaten parasite elimination and encourage parasite persistence. The fate of parasites can be determined by epigenetic changes in the host IL-12 and IL-10 promoters. It was discovered that IL-12 and IL-10 have a reciprocal interaction, which is largely regulated by the transcription factor NFAT5 from the Rel family of transcription factors which induces epigenetic remodeling at nucleosomes of respective gene promoters. It may be feasible to control the release of potent pro-inflammatory cytokines and thereby reduce parasite survival by targeting this transcription factor at the cellular level (Khandibharad and Singh, 2021). HDAC3 has a role in histone deacetylation of the Il12b promoter mediated by IL-10. Reports suggest that HDAC3 mediates the homeostatic impact of IL-10 in macrophages by deacetylating histones on the Il12b promoter (Kobayashi et al., 2012). Also, IL-10 targets an enhancer, nuclear factor interleukin 3-regulated (NFIL3) to 10 kb upstream of IL-12p40 promoter to suppress its expression (Ma et al., 2015). Hence, inhibitors of IL-12 transcription which are induced by IL-10 may play an essential role in mediating reciprocity.

Another immune axis which has gained attention in recent times is IL-17 and Interleukin-23 (IL-23) axis. Where, IL-6 and TGF-β can induce Th17 cell differentiation, IL-17 and IL-23 can promote inflammation. This is quite enticing as to how harmonically cytokines creates an environment for cell differentiation. For plasticity and stability of Th17 cells transcriptional players have an important role such as RORγt (Gaffen et al., 2014). Hence, IL-17/IL23 immune axis can be investigated to identify crosstalk points which may link and synchronize innate and adaptive immunity (Schön and Erpenbeck, 2018). This axis has been explored in inflammatory diseases such as Psoriasis, Ulcerative colitis Guillain Barré Syndrome (GBS) Spondyloarthritis (Schön and Erpenbeck, 2018) (Noviello et al., 2021) (Debnath et al., 2018) (Tsukazaki and Kaito, 2020). It would be interesting to investigate this axis in leishmaniasis as host cytokine machinery is put in a toss by the parasites.

In leishmaniasis, protein tyrosine phosphatase SHP-1 has been reported to control the CD40-induced p38MAPK activation threshold and
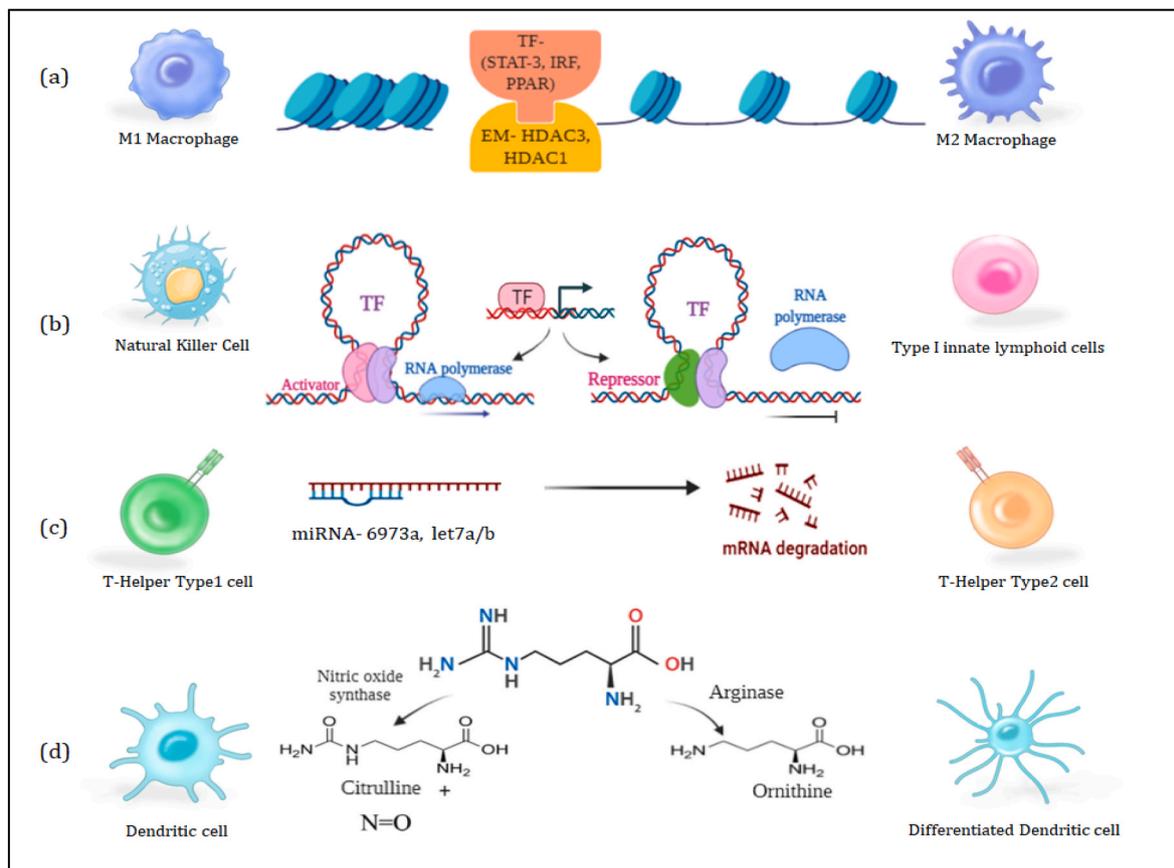

Fig. 2. Mentioned strategies in the above figure can be used by the immune cells to undergo reciprocity – (a) changes induced by Transcription factors and Epigenetic modifiers (EM) such as Histone modifying enzymes can govern silencing of important genes required for maintaining parasite eliminating phenotype, (b)Activators and repressors may bind to different genes and induce reciprocity, (c) non-coding RNAs can degrade mRNA derived from transcriptionally active genes, (d) Metabolic usage of nitrogen may change and can get directed toward different metabolic pathway in this case L-Arginine instead of undergoing citrulline pathway for NO production gets directed to ornithine cycle.

reciprocal ERK-1/2 activation, establishing itself as a crucial regulator of CD40 signaling reciprocity and mechanistically re-emphasizing its importance as a potential target against illnesses involving CD40. *Leishmania* uses these characteristics to develop the SHP-1–targeted immune evasion strategy, which ensures the parasite's survival in a mammalian host (Khan et al., 2014). SHP-1 may act as a point of IL-10 and IL-12 reciprocal intervention in leishmaniasis, and a peptide-based treatment strategy may specifically target this protein (Khandibharad and Singh, 2022).

IL-12, released by DCs, activates NK cells, which can inhibit parasite spread until a specific T-cell response is mounted. In fact, IFN-γ is critical for enhancing killing mechanisms in macrophages, which are leishmania's principal target cells. It's likely that a strong first-line cytokine response is enough to stop the parasites from spreading and growing, whereas in symptomatic people, this atypical response is overwhelmed by parasite infection or is weak due to genetic factors. Due to the suppression of macrophage activation and subsequent intracellular replication of the parasite, the Th2 response with IL-4 and IL-10 production results in vulnerability to infection and the development of severe illness (Saporito et al., 2013).

For killing leishmania parasites macrophage requires IL-12 because it promotes the development of CD4$^+$ T cells to Th1/Th2 and the production of IFN-γ. It also allows for upregulation of iNOS or NOS2 and NO synthesis, as well as parasite elimination. Other regulatory cytokines, such as IL-10 and TGF-β, are also produced in response to leishmania infection, interfering with the macrophage effector activities in favor of parasite survival and disease progression (Rostami and Khamesipour, 2021).

Depending on how macrophages are activated, they can either provide residence or kill leishmania, and L-Arginine's metabolic products play a role in both. The oxidation of L-arginine catalyzed by the iNOS2 produces NO, a key leishmanicidal ingredient. When L-arginine is hydrolyzed by arginase, however, polyamines are produced, which are critical nutrients for leishmania growth. Arginase activation and polyamine production have also been identified as an important contributors in the progression of leishmaniasis (Vendrame et al., 2007). IFN-γ, TNF-α, IL-6, IL-17, IL-12 are known inducers of NO whereas IL-4 and IL-10 are inducers of arginase (M Modolell et al., 1995).

In a nutshell, pro-inflammatory cytokines are secreted to eliminate parasite through NO production mediated by iNOS whereas, anti-inflammatory cytokines secretion promotes parasite survival where, arginine is harbored by arginase towards polyamine production which gets utilized by the parasite for growth and proliferation. The above mentioned reports suggest that both types of cytokines regulate each other's expression. Reciprocity of IFN-γ and IL-4 has already been reported and recently through computational systems level approach reciprocity between IL-10 and IL-12 has also been reported. It is apparent to speculate that reciprocity might also exist between the other members of pro-inflammatory and anti-inflammatory cytokines which should be explored by studying immune axis, genetic as well as epigenetic factors.

Translational repression not only allows cytokine production to be inhibited more rapidly but also protects mRNA (Anderson, 2008). On numerous levels, the unfolded protein response regulates cytokine production, from pattern recognition receptor stimulation to control of inflammatory signaling pathways and regulation of cytokine transcription factors (Smith, 2018). Hence, identification of key players governing the reciprocity needs to be identified; the type of control it provides for the cytokine switch to take place compiled with uniqueness of mode of action of each might help to provide newer insights about the encoded black box of cytokines. A realm of reciprocal regulation of cytokines can thus help in designing better therapeutics, vaccine and prevent cytokine storm.

5. Modulation of followers in parasite proliferation and disease progression

Immune cells utilize their receptors to read the environment and then change how they utilize the genes encoded in their DNA. Some gene groups are switched on, while others are turned off (Nicholson, 2016), resulting in differentiation of cell types to cope with the infection (Fang et al., 2018). Upon reciprocal regulation of cytokines, phenotypic changes might occur in the cells modulating their behavior and hence these cells are here referred as followers.

Within *in vitro* systems, monocytes/macrophages have been classified as M1, M2, and deactivated macrophages, with the latter two subtypes being associated with suppression of cell-mediated immunity, which confers susceptibility to intracellular infection (Bogdan, 2020). Macrophages develop a high expressing effector function against intracellular pathogens and flip to the M1 phenotype when stimulated with Th1-associated cytokines, particularly IFN-γ. The NF-κB-NLRP3 axis, which regulates the production of pro-inflammatory cytokines like IL-1β and IL-18, is regulated by a novel immune subversion mechanism based on histone H3 post-translational modifications in parasite which results in modulation of macrophage phenotypic plasticity, which is necessary for intracellular parasite survival (Lecoeur et al., 2020). In J774 macrophages and BALB/c mice, *L. donovani*-induced expression of histone lysine methyltransferases Ash1l, Smyd2, Ezh2 and histone lysine demethylases Kdm5b and Kdm6b directed reciprocal changes in histone lysine methylation/demethylation of M(LPS + IFN-γ)/M(IL-10) genes that further guided macrophage polarization towards disease resistance and susceptibility (Parmar et al., 2020). In the presence of Th2-associated cytokines, such as IL-4, IL-13, Interleukin 33 (IL-33), TGF-β, and IL-10, as well as microbial stimuli they polarize to M2 phase. Cardinal genes such as iNOS, arginase 1 (ARG1) govern the differentiation of M1 and M2 monocytes/macrophages (Mukhopadhyay et al., 2015). Although M2 macrophages can transition to the M1 phenotype, the reverse happens rarely; under certain circumstances such as inflammation reverse switch from M1 to M2 may happen (Italiani and Boraschi, 2014). Therefore, to resolve diseased state, M2 cells can be engineered to turn M1 and this population enrichment might contribute towards parasite killing.

Even though not all T cell subsets are terminally differentiated cells, the bulk of them are plastic (Geginat et al., 2014). MicroRNAs have been reported to control gene regulation which governs T cell proliferation and differentiation, leading to Th1/Th2 or Th17/Treg immune responses during human VL by controlling immunological signaling, cytokine synthesis, and immune cell migration (Pandey et al., 2016). Thus, based on infection pattern by leishmania parasite, cytokines secretion and gene regulators; T cells are differentiated and their plasticity is modulated which can favor parasite survival.

Disparities in the tissue cytokine milieu and exposure to certain pathogens, as well as the lineage origin of various tissue DCs, may cause functional differences among them (Liu et al., 2001). The ability of DCs to drive the formation of either a Th1 or a Th2 response, consequently determine the outcome of infections, and is reliant on its activation (Moll, 2003). DCs exerts significant plasticity which has been observed in research experiments (Huang et al., 2001). Their function is also influenced by pro-inflammatory and anti-inflammatory stimuli (Liu et al., 2001). In migratory DCs, classical Nuclear factor kappa B activated transcription (NF-κB) target genes including IL1b, IL1a, IL-6, IL-12b, and Tnfsf1a are inhibited, revealing context-specific NF-κB-regulated gene networks during DC maturation (Liu et al., 2021). Anti-inflammatory cytokines such IL-10, TGF-β, and other signals promote immature DCs to enhance Th2 differentiation or inhibit Th1 differentiation (Jonuleit et al., 2000) (King et al., 1998). The epigenetic and transcription factor landscapes of host cells are altered by leishmania infection, resulting in the production of a distinct anti-inflammatory host cell phenotype. After infection, DC maturation is caused by a complicated regulatory interaction between epigenetic and

transcriptional gene expression regulation (Lecoeur et al., 2022).

Reports suggest that NK cells may show plastic behavior. TGF-β promotes the transformation of NK cells into type 1 innate lymphoid cells (ILC1) intermediates (Gao et al., 2017). By cytotoxic death of infected cells and release of pro-inflammatory cytokines (e.g. IFN-γ, TNF-α), NK cells serve as the first line of defense against infections (Nylén and Gautam, 2010). Therefore, by understanding plasticity phenomena, we can assume that parasite may mediated cytokine modulation and impact the NK cells making them to differentiate and inhibit pro-inflammatory cytokine expression.

Functional plasticity of immune cells can be explored to reprogram them and enrich the desired population of M1, Th1, DCs, and NK cells so that the parasite is eliminated. Another concern might be the regulation of proliferation of these populations. To further advocate this, one way can be through synthetic biology and development of inducible synthetic constructs.

6. Synthetic biology as a therapeutic strategy for leishmaniasis

Synthetic biology is an emerging field with the two subfields. The idea is to create artificial life by using synthetic chemicals to recreate emergent phenomena from natural biology. The other aims are to assemble systems that function in an unnatural way using interchangeable elements from natural biology. Eric Kool and other presenters at the American Chemical Society's annual meeting in San Francisco in 2000 reintroduced the term 'synthetic biology.' The word was used to denote the creation of artificial organic compounds that work in living systems in this context. The word has also been used to refer to initiatives to 'redesign life' in a broader sense (Benner and Sismour, 2005). While there is no agreed-upon international definition, the key features of synthetic biology, according to the Secretariat of the Convention on Biological Diversity (2015), include "the *de novo* synthesis of genetic material and an engineering-based approach to develop components, organisms, and products" (Shapira et al., 2017). In the year 2000, the first genetic circuits were developed, including a genetic toggle switch and an oscillating network. Both circuits were built using simple mathematical models and consisted of well-known DNA regulatory components. In 2002, the poliovirus genome was successfully produced as the first synthetic genome. This study demonstrated that *de novo* chemical synthesis of genomes from existing genomic sequences is possible without the use of natural templates. These scientific advances mark a change in biology research from the study of life to the development of organisms with specific phenotypes (Wang and Zhang, 2019).

An engineered cell should have the property of localization to subcellular organelle; they must possess the abilities of cell growth and proliferation, an appropriate cell cycle and DNA replication. To achieve replication of synthetic circuit in engineered cells, single-protein DNA polymerase (DNAP) viruses are used and through random binomial partitioning and active molecular machinery, the replicated synthetic circuits get segregated in daughter cells (Olivi et al., 2021). According to research findings, homologous recombination would be most active during the S and G2 phases, both of which are quite transient in a normal cell cycle. Consequently, the capability to halt cells in S and G2 has the potential to improve the accuracy of mammalian genetic integration methods. Additionally, it has been demonstrated that cells that are briefly arrested in the G1 phase stably produce transgenes from episomes, suggesting that this approach to gene therapy may be an alternative to genome interruption (Wei and Smolke, 2015). A group reported that programmable drug-mediated regulation of cytokine expression from a synthetic ribozyme switch in the engineered T-cell proliferation regulatory system guided *in vivo* can regulate transcriptional machinery (Chen et al., 2010). Hence, the synthetic circuits can replicate and express through generations and retain its modularity and dynamicity. Another study discovered that synthetic circuit can be stable and resist external perturbations by maintaining robustness in two-cell (macrophage and fibroblast) system through computational

and experimental based observations where they monitored the enrichment of engineered macrophage population and stability through growth factors (Zhou et al., 2018), it may be possible that the Leishmania infected macrophage retain its dynamicity through cytokine signaling and inducer of the circuit.

7. Types of synthetic circuits and their applications in therapeutics

Immunotherapy for leishmaniasis is premised on the theory that a non-protective anti-leishmania immune response can be shifted to a protective phenotype. Immunomodulatory molecules could be used to achieve this (Ikeogu et al., 2020). Systems level understanding of host-parasite interactions may aid in interaction driven modulation of synthetic biology driven device construction (Mol et al., 2015). Considering murine experimental leishmaniasis model which suggests that Th1 cell mediated immune responses produced are mainly by APCs producing IL-12, IFN-γ by CD4$^+$ T cells, NO and ROS by macrophages, therefore a regulatory synthetic gene circuit can be designed to eliminate the intracellular parasites (Mol et al., 2014). Living designer systems with the potential to detect and correct human illnesses provide tremendous opportunities for twenty-first-century medicine in this setting (Sedlmayer et al., 2018). Based on the molecular design principles gene switches are customized to control distinctive properties of gene expression (Re, 2017).

Primarily, synthetic circuits can provide control at transcriptional, post transcriptional, translational and post translational level (Figs. 3–5). The most common type of synthetic circuit is one based on transcriptional control, which consists of an actuator component that allows positive or negative transcription regulation and a DNA-binding portion that recognizes a promoter DNA sequence.

Synthetic biologists have developed a wide range of genetic circuits, including toggle switches, oscillators, logic gates, dose-response linearizers, and multicellular systems, by creating unique transcriptional regulatory topologies (Cheng et al., 2017). To boost or reduce the flux, DNA-binding proteins can either recruit or block RNA Polymerase (Brophy and Voigt, 2014). Zinc finger domains particularly binds to DNA at specific target nucleotide sequence where promoter can be engineered to recruit basal transcription machinery and also it interacts with adjacent Transcription factors (Khalil et al., 2012). DNA binding domain of Transcription activator-like effectors (TALEs) is interchangeable. It generally functions in genome editing although when fused with KRAB or mSin3 interacting domain TALEs can inhibit mammalian transcription. TALEs can also promote transcription activation when fused with VP-64 (Moore et al., 2014). Hence, genomic sequences can be targeted directly to upregulate or downregulate expression.

A CRISPR-based transcriptional regulator is made up of two components mainly, a shortguide RNA (gRNA) with a 50-sequence specific for target DNA and a deactivated Cas9 (dCas9) protein that can be coupled to a transcription regulatory domain (Jusiak et al., 2016). For transcriptional activation and inhibition, two important strategies are available namely CRISPRa and CRISPRi (Santos-Moreno and Schaerli, 2020). CRISPRa has the potential to work on a wide variety of target genes (Fontana et al., 2020). Experiments in mammalian cells showed that fusing dCas9 to VP64 (four tandem repeats of the viral VP16 transcriptional activator) or the p65 component of NF-κB (Gilbert et al., 2013)(Perez-Pinera et al., 2013)(Maeder et al., 2013)(Mali et al., 2013) (Farzadfard et al., 2013). The fusion of the repressive Krüppel-associated box (KRAB) domain with dCas9 increases dCas9's repression abilities with the highest effect (up to 100-fold) shown when the gRNA was positioned 50–100 bp downstream of the transcription start point (TSS) (Margolin et al., 1994)(Gilbert et al., 2013)(Gilbert et al., 2014). Therefore, sgRNA can be synthesized in particular to upregulate or downregulate a gene although, the lack of inducible transcription mechanisms in leishmania parasite infection is a significant bottleneck
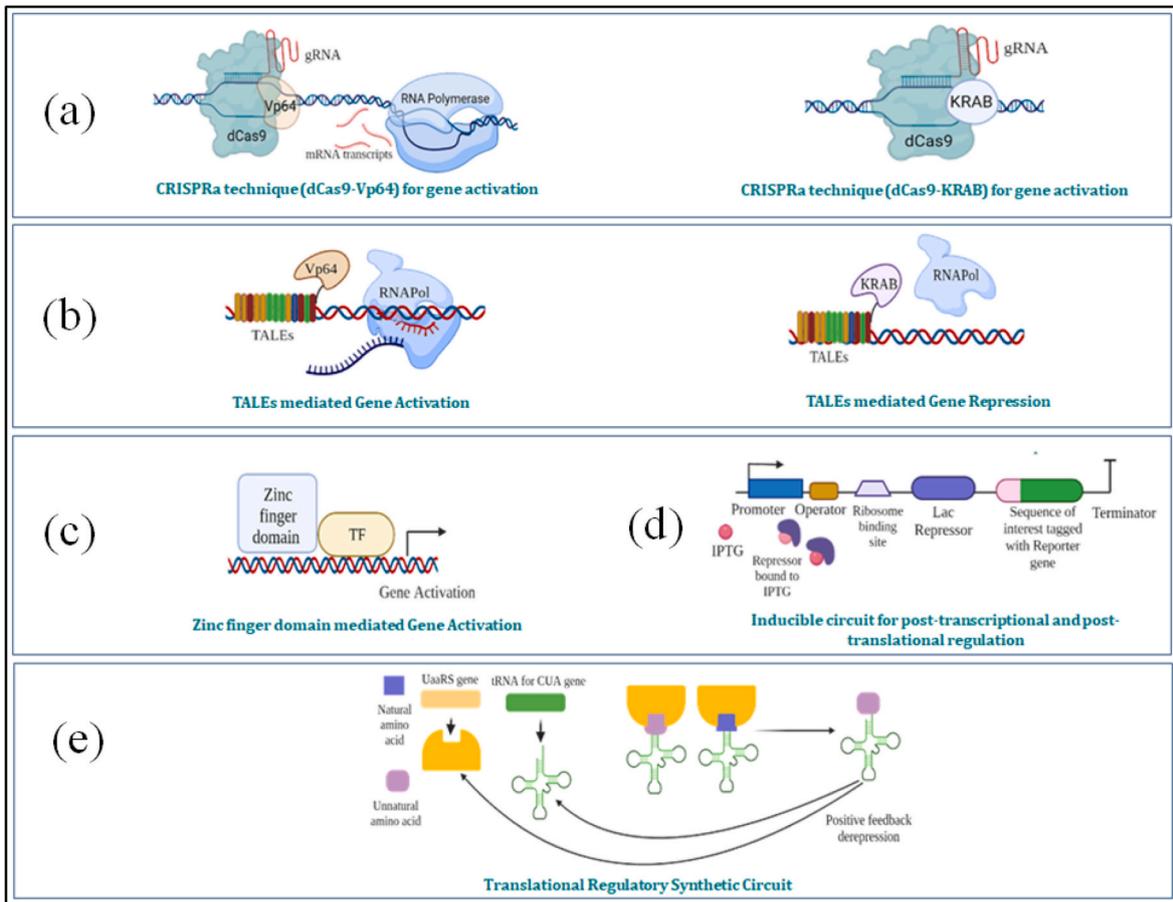
Fig. 3. Types of synthetic circuits used to exert control at different levels of expression: (a) CRISPRa and CRISPRi are used for transcriptional regulation, (b) and (c) Engineered TALEs and Zinc finger domain are used for transcriptional control, (d) Inducible Lac operon based toggle switch for post transcriptional and post translational control, (e) Unnatural amino acid based construct for translational control.
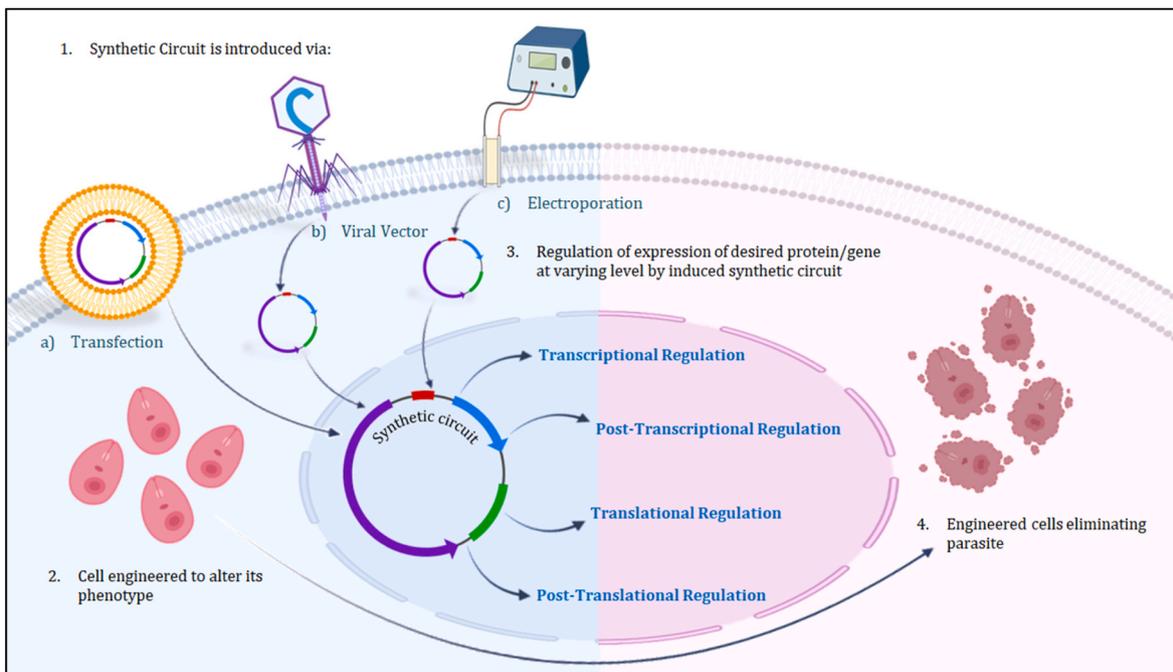

Fig. 4. Machinery of synthetic circuit introduced and expressed in target cells to modulate their phenotype to eliminate leishmania amastigotes.
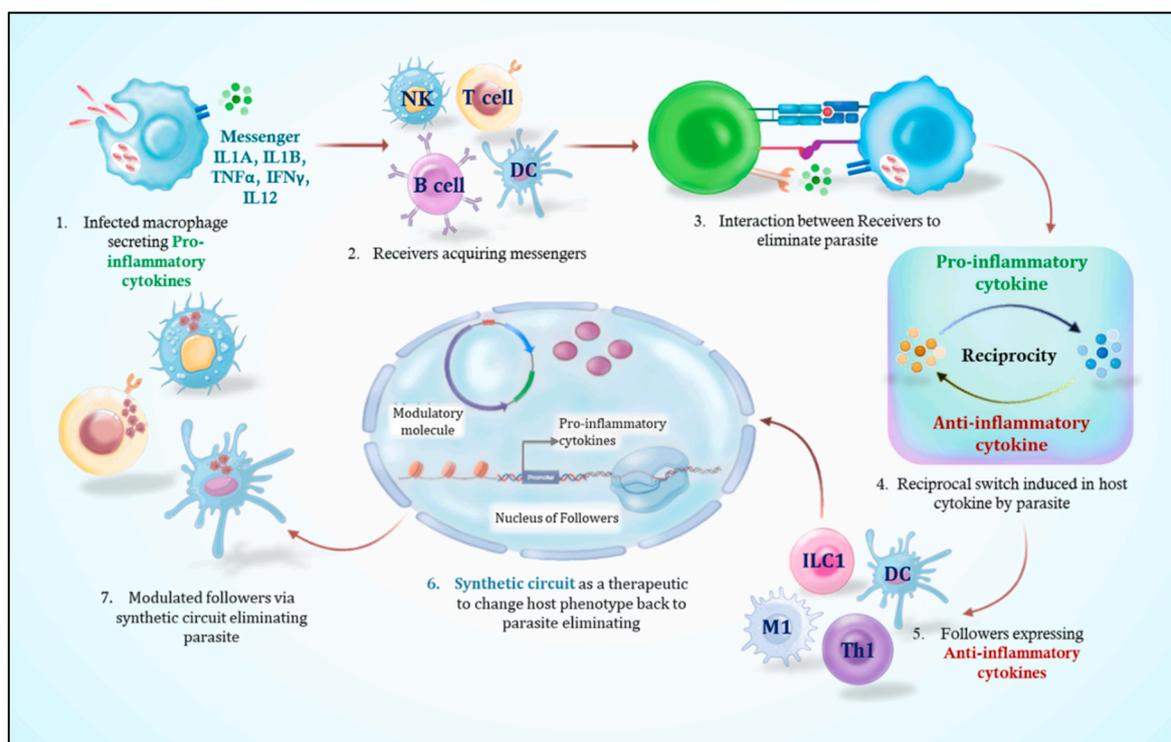
Fig. 5. Graphical abstract highlighting cytokine signaling, cell phenotype flexibility and impact of reciprocity on induction of phenotypic changes to further dictate cell fate with the disease progression followed by restoration of fine balance of cytokine through synthetic circuits which enables disease discouragement.

(Bryant et al., 2019).

Isaacs et al. published a post-transcriptional gene regulation system in 2004 that combined two non-coding mRNA sequences to modulate gene expression (Isaacs et al., 2004). Using a CIS element containing noncoding region and ribosome binding site (rbs) and TRANS element with a 4bp overlap with rbs and complementary to Cis element there was inhibition of translation observed showing post transcriptional control of any gene of interest (Ceroni et al., 2012). One classic example of post transcriptional regulatory synthetic circuit was microRNA (miRNA) regulatory construct used for leishmania infected cells. Many target genes, including NF-κB, IBK, IKK, and regulators in the NF-κB signaling pathway, are regulated by miRNAs, which operate as post transcriptional regulators of gene expression, generating positive or negative complex feedback loops (Barral et al., 1995)(Nimsarkar et al., 2020). The working mechanism of the designed circuits was based on the Lac operon system, in which Lac R stays bound to the operator region in the OFF state, inhibiting the expression of mir146a, while Lac R binds to the inducer in the presence of inducer (IPTG), switches the circuit in the ON state, expressing mir-146a and GFP as the reporter (Nimsarkar et al., 2020). Riboregulators such as riboswitches bind to regulatory motif of specific gene upstream on ribosomal binding site where it provides post translational regulation. In L.major, a novel theophylline binding motif was identified which was likely to control RNA Polymerase III subunit 1 indicative of riboswitch activity where this element can be used to further design novel therapeutic against leishmaniasis (Bejugam and Singh, 2018).

To provide translational control in synthetic circuits, unnatural amino acids has been used such as (Uaa) which has also shown to control target gene expression by getting incorporated at UAG stop sites in ribosomal synthesized proteins (Kato, 2019). This construct has a positive feedback derepression for tightness where it suppresses leakage of translation without the presence of Uaa when placed under the control of araBAD promoter and the araC regulator achieved 3×10^2-fold than their parent system (Kato, 2018). Also, to improve the binding of RNA Binding Proteins (RBPs) to mRNA and to achieve a stable RNA scaffold

aptamer can be used (Ono et al., 2020). Certain RBPs can repress their own translation by directing their mRNA to bind at their functional targets. Proteins such as L30, S15 and U1A possess this property. Assessing this natural role can be utilized in a synthetic feedback control strategy that might be quite fascinating (Stapleton et al., 2012). Not much has been reported about synthetic circuit being used to control infectivity or proliferation of leishmania parasite at translation level. Engineering of a repressilator model system can be done to express parasite specific inositol phosphorylceramide synthase (IPCS) in a genetic switch was deciphered (Mandlik et al., 2013). Hence, exploring translational synthetic circuit can provide a scope of better therapeutics in infectious disease such as leishmaniasis. All the mentioned strategies can be used to construct a translational regulatory circuit in order to uniquely target and control leishmaniasis.

For post translational regulatory circuit a generic framework for the fast and flexible coupling of genetic circuits using instinctual 'queueing' processes such competitive protein degradation where protease competitiveness was used to manipulate linker between protein and its degradation tag on the formulated basis that overloading a limited supply of intracellular proteases, protein degradation can elicit a precise response to stress (Prindle et al., 2014). Taking into consideration the evolvability of synthetic circuit, peptide based therapeutic approach was presented by (Soni and Singh, 2021), not to be visualized as Rube Goldberg machine and to treat *L.major* infection by modulating IL-6 production at cellular level. Biomolecules post-translational repertoire has been used to create logic devices by adapting Boolean logic operations making cells direct localization of activator molecule among subcellular spaces (Razavi et al., 2014). Rewiring of signaling pathway is also achieved by engineering chimeric proteins to target ligand receptor interaction. PKC is a protein kinase C (PKC) that is incorporated inside a negative autoregulatory synthetic circuit for immunological modulation via NF-κB activation, which could lead to phenotypic changes in leishmania infected macrophages (Mol et al., 2018).

Based upon the expression profile of infected cells one can design a synthetic circuit to have expressional control to govern the reciprocal

switch and have a variable target to get desired expression of parasite eliminating genes, thus, paving a way and a hope to design better therapeutics which can not only take care of the disease but also have time based operation.

8. Conclusion

Extensive execution of synthetic circuits has been presented in infectious disease such as leishmaniasis. Even though modern medicine has a long way to go we cannot neglect the fact that host-parasite interactions are complicated and fragile. To attune the balance and throw the ball in host's court, cytokines axis needs to be taken into consideration. Parasite is fabled for its notorious nature; it may alter the immune axis to favor its survival by manipulating hosts cells to go against their nature of eliminating them and rather use them for growth and proliferation. Identification of common crosstalk points between the two types of cytokines may help in restoring the balance at spatio temporal level through synthetic circuit by adopting above mentioned strategies. If correct message is delivered to the followers by receivers mediated by synthetic circuit, then parasite clearance may be possible. As synthetic circuits provide huge opportunity for molecular expression, with right efficiency and speed, it may enable insights into the resolution of disease. One of the benchmark bioengineered cells which changed the therapeutics world is Chimeric Antigen Receptor (CAR)-T cells therapy. In a similar fashion, these circuits may also revolutionize the comprehending link between molecular events and phenotypes in order to increase sensitivities and to study cellular events at different time scales.

CRediT authorship contribution statement

Shweta Khandibharad: Writing – original draft, Formal analysis, Investigation, Writing – review & editing, literature analysis, Conceptualization, figures, Contribution to writing and conceptual framing of the article: All the authors critically reviewed and approved the final manuscript. **Prajakta Nimsarkar:** Writing – original draft, Formal analysis, Investigation, Writing – review & editing, literature analysis, Conceptualization, figures, All the authors critically reviewed and approved the final manuscript. **Shailza Singh:** Writing – original draft, Formal analysis, Investigation, Writing – review & editing, literature analysis, Conceptualization, figures, Contribution to writing and conceptual framing of the article: All the authors critically reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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