Discussion

MAP Kinases play a pivotal role in the body's host defense by modulating cellular and humoral responses. As our previous studies reported, during *L. major* infection in macrophage p38MAP Kinase signalings hampered by inducing IL-10 through ERK-1/2 activation. Due to reciprocity in signaling, CD40-mediated immune response is altered, producing anti-inflammatory IL-10 but reducing pro-inflammatory IL-12 cytokine (34). As we have previously reported, MAPK10 a *Leishmania major* gene showed promise against experimental cutaneous leishmaniasis in BALB/c mice, a susceptible host (117). Here, we argue that *Leishmania major* MAPK10 offers cross-protection against heterologous species of *L. donovani* infection. This is quite interesting in terms of *Leishmania* infection because of a single gene capable of controlling the heterologous species of *Leishmania* parasite. This is a great advancement as MAPK10 is proven to be a potent antigenic vaccine candidate.

It has been observed that *Leishmania* infection is associated with resistance and susceptibility. BALB/c a susceptible model against *Leishmania* infection whereas C57BL/6 a resistant model in *Leishmania* infection. Resistance and susceptibility in mice are associated with variations in alternate forms of gene or allele. To date, many studies have been performed for the advancement of *Leishmania* vaccine but none of them met the true success. As it is reported, cloned leishmania antigen administration into BALB/c mice, a susceptible host, polarizes T_H2 into T_H1 inducing pro-inflammatory cytokine IL-12, IFN-γ, TNF-α by suppressing IL-4 and IL-10 pro-parasitic cytokine. There are 15 MAP Kinases reported in *Leishmania*. Out of these, we select MAPK10. Herein, we have selected MAPK10 as a vaccine candidate because of its homology with ERK-2 that favours parasite replication. MAPK10 is a cytosolic signaling intermediate protein that plays a crucial role in *Leishmania* infection. MAPK10 DNA along with protein vaccination shows significant protection in experimental BALB/c mice against *L.major* infection. Due to its protective efficacy against

cutaneous leishmaniasis, we tested in *L. donovani* infection. We found that *L. major* derived M10 reduced the parasite burden in visceral organ predominantly spleen and liver. Because *L. donovani* infection is responsible for splenomegaly and hepatomegaly, our *L.major* derived M10 DNA immunization in primed BALB/c mice leads to a reduction in parasite load in the visceral organs. As $CD4^+$T-cells contribute to check or arrest the growth of the parasite in *Leishmania* infection, we intended to study in an in vitro macrophage T-cell co-culture system.We found that $CD4^+$T-cells leads to decrease in amastigotes/cell count in an infected macrophage in M10 primed BALB/c *L. donovani* infected group. From the same co-culture supernatant, we also noticed elevated IL-12, IFN-γ and TNF-α, but suppressed IL-10 and IL-4 cytokine. As nitric oxide is known for leishmanicidal function, we assessed this by taking culture supernatant from an infected macrophage T-cell co-culture system. We observed that M10 immunized mice have a higher amount of nitric oxide than control or empty DNA. It indicates in vitro peritoneal macrophages also killing the *Leishmania* parasite in culture. We further tested by collecting blood, later on serum isolation from the same, before infection and post-infection study in M10 immunized group show heightened IgG2a; however, suppressed IgG1 and IgM response. These findings support that LmjMAPK10 DNA immunization induces a humoral response against *L. donovani* infection. We further confirmed by CSA stimulated the M10 group in splenocytes culture supernatant. We found M10 stimulated the group to have higher IL-12, IFN-γ but less IL-4 and IL-10 exactly inverse in control DNA group. These findings also give clue to M10 DNA immunization eliciting a cellular response. In terms of vaccination paradigm, T_H1-mediated immune response is superior to T_H2. Therefore, protective function by controlling the disease progression is secured by T_H1 cells. To achieve a good response against infectious disease, the immune response model systems are very important aspects. Because BALB/c is a susceptible host against *Leishmania* infection, we next confirmed in T-cell subsets, especially $CD4^+$T-cell by

checking all the T-cell phenotype. We found a higher double-positive population of T-bet and IFN-γ, i.e., T_H1 followed by the reduced population of GATA-3 and IL-4, i.e., T_H2. In addition, the T_H17 population, i.e., IL-17A and RORγT were elevated in the M10 group followed by suppressing the population of Treg by checking through Foxp3-IL10 or GITR-IL10 or GITR-Foxp3. These studies clearly indicate that phenotypic profiling expression reveals towards protective phenotype towards T_H1 based immunity. Next, we confirmed in an *in vitro* ex-vivo splenocytes by isolating RNA and quantitated at the gene level by specific primer through real-time PCR. In M10 immunized *L. donovani* infected group IL-12 and IFN-γ expression were higher although IL-10 and IL-4 were lower. In addition, T-BET a T_H1 type transcription factors were upregulated whereas the GATA-3 T_H2 type transcription factor was down-regulated at the gene level. Corroboratively, all these studies either at the gene level or protein level mostly oriented towards T_H1 type. Hence, we can conclude that MAPK10, a gene from *Leishmania major*, offers protection against *L. donovani* infection.

In another study, we want to address IL-7 has an anti-leishmanial function in BALB/c mice in association with *Leishmania major* MAPK10 DNA. As IL-7 is produced from non-hematopoietic stem cell precursor from thymic and bone marrow environment. IL-7 are not produced from T-cell, B-cell or NK cell rather than DC. As previously reported in literature IL-7 dose given to murine peritoneal macrophage following challenge with *L. major* reduces the amastigote count in vitro (118). In contrast, in vivo IL-7 treatment followed by *L. major* challenge to BALB/c mice, a susceptible host leads to the death of the animal (119). Based on these previous studies, we administered *L. major* MAPK10 antigen along with recombinant murine IL-7 in BALB/c mice followed by *L. donovani* infection. We found that M10+IL-7 immunized mice significantly reduced the parasite burden in vivo in spleen and liver respectively. Later on, the infected macrophage T-cell coculture system, $CD4^+$T-cell reduce the parasite burden in vitro. These findings suggest that M10+IL-7 regulates anti-leishmanial

function by reducing the parasite burden both in vitro and in vivo. Next, we confirmed that in an infected macrophage T-cell co-culture system by looking at pro and anti-inflammatory cytokine response. We found heightened IL-12, IFN-γ but reduced IL-10 and IL-4 production. However, M10+IL7 immunization also leads to enhance NO production. As we confirmed in in vitro infected macrophage T-cell co-culture system, then next we assessed in the serum sample. As it is well established that IgG2a induce T_H1 response so we check the M10+IL-7 immunized group, and we found that heightened immune response at the same time IgG1 and IgM levels were reduced. It suggests that M10+IL-7 modulate anti-leishmanial function by inducing a humoral response. We further check at the cellular level by stimulating CSA with the M10+IL-7 group then we found that higher IL-12, IFN-γ followed by down-regulation of IL-4 and IL-10 compared with the control DNA+IL-7 group. It suggests that M10 DNA+IL-7 priming regulates anti-leishmanial function by inducing a cellular and humoral response by inducing the T_H1 phenotype by down-regulating the T_H2 phenotype.

As it is reported that central memory T-cell generated during chronic infection with *L. major* the expression of IL-7R was enhanced. In addition, T_H1 based effector cell population was also increased by inducing T-bet and IFN-γ expression during *L. major* infection. Based on that study, we checked the T-cell phenotype in $CD4^+$T-cell subsets. We observed that T_H1 and T_H17 populations were enhanced in M10+IL-7 immunized group however, Treg and T_H2 cell populations were reduced. In addition, the effector memory & central memory T-cell population were enhanced. Corroboratively, all these studies we conclude that *Leishmania major* MAPK10+IL-7 regulates host-protective function by modulating T_H1 type immune response.

These results indicate that immunization with M10 DNA induces T_H1 response but down-regulates T_H2 response. M10 DNA priming, followed by boosting with M10 protein,

significantly reduced the severity of challenge *L. major* infection (117). The elicitation of the observed host-protective immune response against *L. donovani* infection in L. major-derived M10-primed BALB/c mice could be due to a strong homology in MAPK10 in these two parasite species. Although we did not use the prime-boost strategy, the M10 DNA-immunized BALB/c mice were significantly protected from *L. donovani* infection. We propose an independent study analyzing finer specificities of the responding T cells so that prime-boost strategy can be adopted for both *L. donovani* and *L. major* infections using the same antigen.