Discussion
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CD40 signaling proceeds by reciprocal regulation of p38MAPK and ERK1/2 as function of CD40 stimulation. Weak CD40 signal induce ERK1/2 dependent IL10 synthesis whereas strong signal induce p38MAPK dependent IL12 production. p38MAPK and ERK1/2 therefore have counter regulatory actions(34). Leishmania major skews CD40 signaling towards ERK1/2 inducing IL10 which inhibits activation of CD40 induced p38MAPK and expression of inducible nitric oxide synthase2 (iNOS-2) and IL12( 8 ).

In this report, we studied whether CD40 regulate various MAPKAPKs downstream of MAPKs results in counteractive effector functions .It was observed that anti CD40 stimulation of BALB/c mouse macrophages in three different doses resulted in MAPKAPKs activation. CD40 cross linking with αCD40, augments p38 phosphorylation in macrophages at higher dose and inhibits ERK1/2 activation. While at lower dose of αCD40 stimulation in macrophages ERK1/2 phosphorylation is higher and p38 phosphorylation is down regulated (34). The increase in MK2 phosphorylation was observed similar to its upstream p38 kinase at higher dose of αCD40. While RSK1/2 showed differential residual phosphorylation at different doses of αCD40.

We further studied effect of SB203580-p38MAPK inhibitor and PD098059 –ERK1/2 inhibitor , when treated with macrophages along with αCD40 stimulation , showed both p38 and ERK1/2 inhibition , inhibits MK2 phosphorylation but RSK1/2 phosphorylation is not affected.

Leishmania major protozoan parasite skews CD40 signaling in macrophage towards ERK1/2 dependent IL10 production and inhibits anti parasitic IL12 production (8). So we further checked the activation of MAPKAPKs in Leishmania infected macrophages and we found phosphorylation and activation of MK2 like as its upstream kinase p38 was reduced. While phosphorylation and activation of RSK1/2 unlike ERK1/2 were reduced in infection too.

Inhibition of MK2 phosphorylation using MK2 inhibitor showed decrease in phosphorylation of both p38 and ERK1/2 and also decrease in synthesis of IL12 and IL10. MK2 silencing using MK2siRNA gave similar results. The treatment of MK2 inhibitor to macrophages along with anti CD40, leads to decrease in parasite load in vitro. Thus inhibition of MK2 phosphorylation using MK2 inhibitor showed inhibition in phosphorylation of p38 and ERK1/2 . MK2 has direct role in IL10 synthesis as use of
MK2 inhibitor almost nullifies the phosphorylation of ERK1/2 in infected macrophages decreasing IL10 synthesis to great extent and thus doesn’t allow *Leishmania major* to survive in the macrophages. This was further studied by using MK2 inhibitor in BALB/c infected with *L. major* along with administration of αCD40. Disease progression was observed for 6 weeks. It was observed that there is significant decrease in footpad thickness and parasite load in mice treated with MK2 inhibitor and αCD40 together.

We further checked whether MK2 silencing using MK2shRNA would enhance CD40 induced anti-leishmanial effect in *L. major*-infected BALB/c. Administration of anti-CD40 together with MK2 shRNA resulted in reduced footpad thickness and parasite load in *L. major*-infected BALB/c mice, which indicates that MK2 silencing can skew CD40 signaling by reducing IL10 production and help in enhancing CD40-induced anti-leishmanial effects *in vivo*. Thus *Leishmania* targets MK2 in order to manipulate CD40 towards ERK1/2 dependent IL10 production.

Inhibition of RSK using SL0101 in macrophages decreases αCD40 dependent phosphorylation of RSK residues. Also decrease in the phosphorylation of p38 and synthesis of IL12 and TNFα while increase in phosphorylation of ERK1/2 and thus IL10 synthesis was observed.

When we further studied effect of SL0101 in macrophages infected with *L. major*, phosphorylation of p38 was decreased and ERK1/2 phosphorylation was more. This was further confirmed when inhibition of RSK using SL0101 favors *Leishmania major* survival in macrophages in vitro observed using Giemsa stain in parasite load assay.

When checked for parasite burden, RSK inhibitor and αCD40 treated macrophages showed almost similar number of parasites as the untreated macrophages. When treated invivo RSK inhibitor-SL0101 with αCD40 administration, in BALB/c infected with *L. major* and disease progression was observed for 6 weeks. It was observed that there is significant decrease in footpad thickness and but no significant decrease in parasite load in mice treated with RSK inhibitor-SL0101 and αCD40 together.

Thus we conclude that CD40 stimulation activates MAPKAPKs. Both p38 and ERK1/2 inhibition, inhibits MK2 phosphorylation but RSK1/2 phosphorylation is not affected. MK2 inhibition and MK2 silencing enhances CD40 induced anti leishmanial effect. While RSK inhibition has shown to have anti inflammatory role, but not anti leishmanial role. This study for the first time reveals the role of MAPKPKs in CD40-
regulated immune responses. The findings reveals a novel parasite-devised immune evasion strategy and an effective target to redirect CD40-regulated immune responses.