### 8. MHC-II signaling in APCs regulates T cell function.

#### 8.1 G protein inhibition in macrophages leads to inhibition of T cell function.

MHC-II is known to present antigen to T cells, which then either undergo proliferation or apoptosis or anergy. But little is known about the events happening in APCs influencing the T cell function. In order to investigate the events happening in macrophages during antigen presentation and their effects on T cell function, peritoneal macrophages were preincubated with Suramin sodium salt for two hours then washed and co cultured with column pass T cells and stimulated with SEB for seventy-two hours.

*Figure 8.1 PMΦ were preincubated with Suramin sodium salt (40µM, 80µM, 120µM) for 2hrs and cocultured with column pass T cells then these cells were stimulated with SEB for 72hrs. ELISA and proliferation was done from supernatant and cells respectively.*
In the case of TSS, T cells proliferate in large amounts and produce TNF-$\alpha$ that causes shock. IL-2 is the key factor which mediates the proliferation of these T cells and induces TNF-$\alpha$ as well. Therefore T cell proliferation and IL-2 production was analyzed. Dose dependent inhibition in IL-2 production and T cell proliferation was observed. Since macrophages were washed after pretreatment with Suramin sodium salt therefore the effect observed was due to modulation of macrophages, which indicates that macrophage modulation can modulate T cell function [Fig 8.1].

8.2 TNF-$\alpha$ converting enzyme blockage in macrophages diminishes the T cell function. As described in chapter one that G protein leads to the activation of TACE.

*Figure 8.2* PMΦ were preincubated with TAPI2 (20µM, 30µM, 40µM) for 2hrs and cocultured with column pass T cells then these cells were stimulated with SEB for 72hrs. ELISA and proliferation was done from supernatant and cells respectively.

To investigate the effect of this signaling pathway on T cell function, macrophages were pretreated with different doses of TAPI-2 for two hours and then cocultured with T cells
and stimulated with SEB for seventy-two hours. Thymidine was added at sixtieth hour of stimulation and at the same time IL-2 was assessed by ELISA from supernatant. Proliferation was done after seventy-two hours of T cells stimulation. Dose dependent inhibition of T cell proliferation and IL-2 production was observed suggesting that TACE plays a important role in the regulation of immune function [Fig 8.2].

8.3 EGFR inhibition in macrophages also affects T cell function. To investigate the effect of EGFR inhibition in macrophages on T cell, macrophages were pretreated with different doses of PD-153035 for two hours and then cocultured with T cells and stimulated with SEB for seventy-two hours.

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Figure 8.3 PMΦ were preincubated with PD-153035 (20nM, 30nM, 40nM) for 2hrs and cocultured with column pass T cells then these cells were stimulated with SEB for 72hrs. ELISA and proliferation was done from supernatant and cells respectively.

Thymidine was added at sixtieth hour of stimulation and at the same time ELISA assessed IL-2 from supernatant. Proliferation was done at seventy-two hours of stimulation. Concentration dependent partial inhibition was seen of both proliferation of T
cells and IL-2 production, suggesting that EGFR does play a role in the regulation of T cells. It is shown in chapter one that first peak leads to the proliferation of T cells by increasing CD25 expression, since EGFR is downstream of the first peak EGFR, therefore did not fully inhibited T cell function [Fig 8.3].

8.4 Effect of inhibition of p38MAPK in macrophages on production of cytokines from T cells. Peritoneal macrophages were preincubated with SB-203580 for 2hrs and then the cells were washed and co cultured with T cells and were stimulated with SEB. Supernatant was collected after 60hrs of stimulation and ELISA was done for IL-2.

![Figure 8.4 PMΦ were preincubated with SB-203580 (4µM, 6µM, 8µM) for 2hrs and cocultured with column pass T cells then these cells were stimulated with SEB for 72hrs. ELISA and proliferation was done from supernatant and cells respectively.](image)

Partial dose dependent inhibition of proliferation of T cells and IL-2 production was observed, suggesting that p38MAPK; a signaling molecule in macrophages can modulate the immune system by affecting the T cell function and hence will be a good
target for immunotherapy. Since p38MAPK is present in T cells also hence using the inhibitor of it *in vivo* will help in suppressing both APC as well as T cell function. Thus it can be a good target for therapy against autoimmune diseases [Fig 8.4].
8.5. Conclusion

Different inhibitors such as Suramin (G protein inhibitor), TAPI2 (TACE inhibitor), PD153035 (EGFR inhibitor), SB-203580 (p38MAPK inhibitor) and CAPE (NFκB inhibitor) in vitro were used to elucidate the effect these inhibitors on the course of T cell function. We found that only Suramin could completely modulate the activation of T cells other inhibitors also modulated the T cell activation but not fully. This could be because of the reasons that, cAMP elevating agents lead to the better presentation of antigen and better effector T cell function. Moreover cAMP also leads to the increased expression of CD80 and hence helps in increasing the costimulation, which is the necessary factor in determining the T cell effector function. Other molecules are downstream of G protein and have no connection with cAMP and hence do not influence CD80 expression. Costimulation is very important in T cell function and induction of TSS, inhibition of which will inhibit T cell induced TSS (Saha et al., 1996).