9. Regulation of TSS at APC level.

Mechanism of SEB induced TSS is regulated both at the level of APC and T cell.

9.1 G protein regulates the induction of TSS.

Figure 9.1 BALB/c mice were injected intraperitoneally with Suramin (700 µg/mouse) one hour before D-galactosamine injection. After one hour of D-galactosamine injection mice were challenged with lethal dose of SEB and mice were bled one hour after SEB challenge to assess serum level of TNF-α and same mice were observed for mortality.
It has been well documented that SEB induced TSS is caused by CD4⁺, but for the first time it is shown in this study that it can be regulated at APC level also. G proteins are known to mediate pathological symptoms in case of *Vibrio cholrae* caused food poisoning, which includes severe diarrhoea and vomiting basically water and salt loss from the body of the host (). SEB induced TSS also produces these symptoms; therefore it is possible that SEB induced effects are mediated by G proteins. To investigate this, BALB/c mice were injected with Suramin sodium salt one hour prior to D-Gal sensitization and then these mice were challenged with lethal dose of SEB and were monitored for mortality. These mice were bled after one hour to assess serum TNF-α level [Fig 9.1]. As demonstrated in chapter one, G proteins are involved in macrophage signaling, and we got complete protection of TSS along with inhibition of TNF-α in serum with the injection of G protein inhibitor in mice. This further explains that T cells are regulated by APC. Since TCR has not been shown to work through G proteins, therefore we can say that the protection we got was because of APC induced modulation of T cell function and not direct inhibition of T cell function by the G protein inhibitor.

**9.2 TACE regulates T cell effector function.** TACE stands for TNF-α converting enzyme, which cleaves membrane, bound pro-TNF-α to soluble TNF-α, which then produces its effects by binding to TNF-R. TACE is responsible for the induction of endotoxic shock (Solomon *et al.*, 1999). Its role in enterotoxin induced TSS is being worked out in this study for the first time. In order to determine its role in SEB induced TSS, BALB/c mice were injected with TAPI2 a specific inhibitor of TACE one hour prior to D-Gal injection then the mice were challenged with lethal dose of SEB. Mice were bled after one hour of SEB challenge to assess the serum TNF-α level. Then these mice were observed for mortality. Around 60% of mice were protected against TSS with TAPI2 suggesting that TACE plays an important role in the induction of TSS [Fig 3.2]. TACE was reported to be implicated in endotoxin induced shock and hydroxamate based inhibitor prevents endotoxin induced shock.
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Figure 9.2 BALB/c mice were injected intraperitoneally with TAPI2 (50 µg/mouse) one hour before D-galactosamine injection. After one hour of D-galactosamine injection mice were challenged with lethal dose of SEB and mice were bled one hour after SEB challenge to assess serum level of TNF-α and same mice were observed for mortality.

9.3 EGFR in APCs also modulates the course of TSS induction. EGFR a receptor tyrosine kinase that is responsible for neoplastic phenotype (Genther et al., 2005) in cancer it is for the first time shown in this study that it can also regulate TSS induction. To further prove this, mice were injected with PD-153035 one hour before D-Gal sensitization and challenged with lethal dose of SEB after one hour of D-Gal sensitization and observed for mortality.
Figure 9.3 BALB/c mice were injected intraperitoneally with PD-153035 (5 µg/mouse) one hour before D-galactosamine injection. After one hour of D-galactosamine injection mice were challenged with lethal dose of SEB and mice were bled one hour after SEB challenge to assess serum level of TNF-α and same mice were observed for mortality.

These mice were bled after one hour of SEB injection to assess TNF-α in serum. Mice showed some resistance against TSS but were not protected from TSS and subsequent partial decrease in serum TNF-α explains this effect. The results reveal that EGFR does play a role in the induction of TSS and hence in the modulation of T cell response [Fig 3.3].
9.4 p38MAPK regulates induction of TSS both at T cell and APC level. p38MAPK is a ubiquitous molecule present in both T cell and APC and has been implicated to protect organisms against many disease either by inducing iNOS2 or by inducing IL-12 a TH1 regulatory cytokine or by both (Awasthi et al., 2003, Mathur et al., 2004). But in TSS case TH1 is deleterious; therefore we need to inhibit TH1 in order to save an organism. Therefore mice were injected with SB-203580 one hour prior to D-Gal sensitization and were challenged with lethal dose of SEB.

![Graphical representation of TNF-α levels and survival rates](image)

**Figure 9.4** BALB/c mice were injected intraperitoneally with SB-203580 (50µg/mouse) one hour before D-galactosamine injection. After one hour of D-galactosamine injection mice were challenged with lethal dose of SEB and mice were bled one hour after SEB challenge to assess serum level of TNF-α and same mice were observed for mortality.
Mice were bled one hour after SEB injection and then these mice were observed for mortality. Mice survived longer in comparison to untreated mice. This suggests that p38MAPK does play a role in TSS induction and can be a good target for autoimmune disease [Fig 9.4].
9.5. Conclusion

Data in Chapter 1 suggests that MHC-II ligation on APCs results in activation of various molecules such as G proteins, TACE, EGFR, p38MAPK and NFκB. We used different inhibitors such as Suramin (G protein inhibitor), TAPI2 (TACE inhibitor), PD153035 (EGFR inhibitor) and SB-203580 (p38MAPK inhibitor) in vivo to elucidate the effect these inhibitors on the course of TSS induction. We found that only Suramin fully protected the mice against TSS other inhibitors gave resistance to the mice against TSS induction.

TACE is implicated in LPS induced shock and inhibition of TACE with hydroxamate based inhibitors leads to the protection against LPS induced shock. TACE specific inhibitor TAPI2 also protected mice against TSS. EGFR and p38MAPK are shown for very first time in this study to be involved in the course of TSS induction.