9. Summary

The adaptive immunity of mammals is highly evolved to discriminate self from non-self. Immune response is elicited against a myriad of invading pathogens. Further, it also keeps tolerance against body's self components. However, in some cases immunopathological condition may arise due to the unwanted immune response owing to organ transplantation, autoimmunity and allergy. In all these cases the activation of immune system leads to the destruction of body's own components. The current therapy to treat aberrant immune response is by immunosuppressive drugs. Unfortunately, these drugs need to be administered continuously to control the superfluous immune response. Further, immunosuppression due to treatment of drugs leads to susceptibility to opportunistic infections and induction of tumorigenesis. Hence, there is an urgent need to develop a new therapeutic regime that can efficiently tune and tame the unrestrained immune system without inflicting much harm to the host. Tregs are a subset of CD4 T cells that induces peripheral tolerance and suppress immune response. These cells can restrain a particulr type of immune response by suppressing antigen specific effector T cells. Further, they also possess the anti-inflammatory activity. Consequently, due to this unique property endowed with these cells, they are being explored to suppress the immune response and induce antigen specific tolerance. The limitation associated with Tregs therapy is the availability of their restricted number. Currently, many procedures are being exploited to generate optimum quantity of Tregs for therapeutic purpose. Unfortunately, till date Tregs generated *in vitro* are not quite stable and therefore fails to meet remedial prerequisite.

For above mentioned reasons, we utilized CaeA to check its potential to generate Tregs. We observed that CaeA can successfully expand the pool of Tregs in conjunction with TGF-β. Further, it was noticed that CaeA treatment reduces the disease symptoms associated with experimentally induced arthritis. The increase in the frequency of

Foxp3+ CD4 T cells in CaeA treated animals suggests the enhancement in Tregs population, which may play an important role in amelioration of arthritis. In addition, on treatment with CaeA we observed prolongation in the survival of skin allografts in mice and decline in the disease symptoms in the animals suffering from asthma. Furthermore, CaeA also suppressed the generation and activity of Th1 and Th17 cells. The role of Th1 and Th17 cells is considered to be quite imperative in predisposing towards arthritis and other autoimmune disorders and in rejection of organ transplants.

The mechanistic insight of CaeA action revealed that it suppresses the IFN-γ and IL-6 mediated STAT1 signaling. T-bet and Smad7 expression is under the control of STAT1 molecule, expression of these genes is repressed by CaeA. In addition, CaeA treatment enhances the TGF-β mediated Smad3 signaling. Our experiments very categorically demonstrated that the STAT1 mediated expression of Smad7 negatively regulates the Smad3 activity. Since, CaeA suppress STAT1, the Smad3 signaling is therefore relieved from the negative regulation of Smad7. The dependency of CaeA mediated Tregs induction on Smad3 was revealed by abolishment of Tregs generation under Smad3 suppression. Thus, these experiments very categorically explained that CaeA induces Tregs generation by its synergy with Smad3 signaling.

Overall, our study indicates that CaeA may be employed as a potent future immunosuppressive agent for treating autoimmune disorders and promoting the survival of the organ transplants by inducing the generation of Tregs.

Conclusion and prospects

We conclude that CaeA ameliorates autoimmune disease arthritis by inducing the generation of Tregs. The mode of action revealed that suppression of STAT1 contributes in the improvement of Smad3

uling, thereby enhancing Tregs generation. Thus, our data provide a insight into a novel mechanism of Tregs generation. Finally, this / suggests that the Tregs generated by CaeA in combination of β can have a potential therapeutic application in the treatment of mmune diseases and rejection of organ transplants.