CHAPTER 5

SUMMARY AND CONCLUSION

Histamine, the product of histidine decarboxylation, is an evolutionary conserved signaling molecule. It acts as a powerful stimulant of gastric acid secretion, neurotransmission, bronchocontriction, vasodilation, and immune modulation. Histamine plays a central role in innate and acquired immunity: in allergy and inflammation, closely associated with mast cell functions, in immunomodulation regulating T-cell and B-cell function. Histamine synthesis, signaling, and function is controlled by a variety of immune signals and in turn, modulates antibody and cytokine networks and function. Histamine modulates its function via four receptors (H1R, H2R, H3R and H4R). All these four receptors are members of the 7-transmembrane (heptahelical) spanning family of G protein-coupled receptors, and are expressed on various histamine responsive target tissues and cells and suggest an important critical role of histamine in immune regulation, allergic disorder and autoimmunity. Histamine can selectively recruit the major effector cells into tissue sites and affect their maturation, activation, polarization, and other functions leading to chronic inflammation. Histamine also regulates dendritic cells, T-cells and B-cells, as well as related antibody isotype responses. Histamine modulates antibody production. It directly affects B-cell antibody production as co-stimulatory receptor on B-cells. Histamine H1R- and H2R-deficient mice have an imbalance in Th1/Th2 cell function and a lower susceptibility to develop autoimmunity. Histamine H1R predominantly expressed on Th1 cells may block humoral immune responses by enhancing Th1 type cytokine IFN-γ. In contrast, through histamine H2R, histamine enhances humoral immune responses. Allergen-specific IgE production is differentially regulated in histamine H1R and H2R-deficient mice. Histamine H1R-deleted mice show increased allergen-specific IgE production, whereas histamine H2R-deleted mice show suppressed IgE production. In contrast, more sever autoimmune diseases are observed in mice lacking H3R, the receptor confined to the CNS. H4R on immune cells regulate cell migration and allergic responses in the periphery. Thus, humoral immune role of histamine in immunomodulation by histamine H1- and H2-receptors is documented (however it seems preliminary in nature), while its role by histamine H3- and H4-receptors are still unclear or elementary. However, the studies evaluating the role with receptors intact, but blocked by respective agonists/antagonists, and especially studying the immunomodulatory profile over a span of time are lacking in the existing literature.

Moreover, the studies in rabbit model are elementary, and the existing studies (histamine H1- and H2-receptors) have demonstrated the immunomodulatory role studying only single blood samples taken after immunizing the animal. Therefore, the present study has been planned to fulfill the requirement of histamine role in immune modulation and regulation through histamine H1-, H2-, H3- & H4-receptors against sheep red blood cells (SRBC) (a T-cell dependent antigen) and has been presented in two parts.

In the first part of study we have delineated the histamine role in immune modulation and regulation. To study the humoral immune aspects of histamine in immunomodulation, the present part of study is divided into seven chapters. In the first chapter we have studied *in vivo* immunomodulation and immunomodulatory profile of histamine receptors. Furthermore, on the basis of histamine dose, immunization schedule and time factor, this section has been presented in two sub-chapters: (i) role of histamine on antibody profile generated by primary- and secondary-immunization and (ii) dose-dependent effect of histamine on antibody generation profile. In the first sub-chapter we have demonstrated potential role of histamine on antibody (total immunoglobulins (Igs), immunoglobulin-M and immunoglobulin-G) generation. These results have provided evidence that histamine has a short-term effect (until its presence in the body) on humoral immune system and affects B-cell for antibody class switching and potentially modulates antibody (Igs, IgM & IgG) generation titer. Secondary-immunization study has provided support to primary-immunization results of histamine role in antibody generation. In the second sub-chapter we have studied dose-dependent effect of histamine on antibody generation profile, and evaluated the antibody (Igs, IgM & IgG) generation titer *in vivo* affected by the concentration of histamine.

In the second chapter we have studied *in vivo* immunomodulation and immunomodulatory profile of histamine H1-receptors and demonstrated that the histamine treated-rabbits showed immunopotentiating properties by enhancing the anti-SRBC-antibodies levels as compared to control group (strengthen the previous findings of chapter first). H1R-antagonist-treated group suppressed the anti-SRBC-IgM profile as compared to positive control group, on the other hand H1R-agonist-treated group enhanced the anti-SRBC-IgM levels as compared to all experimental groups and demonstrated that H1-receptors have role of IgM generation. While anti-

SRBC-IgG profiles showed that H1R-antagonist-treated group suppressed the anti-SRBC-IgG levels during complete study as compared to positive control group, while in contrast, H1R-agonist increased anti-SRBC-IgG profile as compared to other experimental group and further illustrated that H1-receptors have an important role in IgG generation. In this study, we noticed that exogenously added histamine *in vivo* (until its presence in the body) enhanced the antibody generation profile against SRBC whereas; on further metabolism its effect disappeared on the antibody generation profile bringing it in the range of control antibody level. We also observed that H1R-antagonist (until their presence) suppressed the antibody profile, which came to normal level (control range) in later phase due to their metabolism. Therefore, on the basis of the present study we reached to a conclusion that the results obtained due to histamine and H1R-antagonist were of short duration which disappeared in latter stage due to clearance of the body from these substance in rabbits. The results of both groups (histamine-treated and antagonist-treated) after metabolism of drugs were identical to each other and also similar to control group. Thus, histamine H1-receptors showed principle role in modulation of immunoglobulins and demonstrate important immunomodulatory effects in IgG and IgM generation.

In the third chapter we have evaluated *in vivo* immunomodulation and immunomodulatory profile of histamine H2 receptors and showed that H2R-antagonist-treated group enhanced IgM profile as compared to positive control-group, while agonists study showed different profile i.e., H2R-agonist-treated group enhanced the anti-SRBC-IgM levels as compared to H2R-antagonist-treated group and positive control. These data showed that H2-receptors have dominant role in IgM generation. While anti-SRBC-IgG profiles revealed that H2R-antagonist-treated group suppresses the anti-SRBC-IgG level during complete study as compared to positive control, and in contrast, H2R-agonist increased IgG profile as compared to all experimental groups and showed that H2-receptors have dominant modulatory role in IgG generation. In this study, we noticed that H2R-antagonist (until their presence) suppressed the antibody profile, which came to normal level (control range) in later phase due to their metabolism. Therefore, on the basis of the present study we reached to a conclusion that the results obtained due to H2R-antagonist were of short duration which disappeared in later stage due to clearance of the body from these substance in

rabbits. Thus, the result of H2R-antagonist-treated group, after metabolism of drug was similar to control group. This study provided evidence that histamine H2-receptors have dominant role in IgM generation.

In the fourth chapter we have studied *in vivo* immunomodulation and immunomodulatory profile of histamine H3-receptors and demonstrated suppressed generation profile of total anti-SRBC-Igs, anti-SRBC-IgM and anti-SRBC-IgG in H3R-agonist-treated group. In contrast, H3R-antagonist-treated group demonstrated increased generation profile of total anti-SRBC-Igs, anti-SRBC-IgM and anti-SRBC-IgG during whole of the study period. These results are contrast to our previous study of histamine H1- and H2-receptors-agonists/antagonists (chapter 2 and 3) and demonstrated that H3-receptors on inhibition by H3R-antagonist showing modulatory activity (enhancing the antibody generation levels). On the other hand H3-receptors on stimulation by H3R-agonist showing modulatory activity (suppressing the antibody generation levels). These data provide evidence that histamine H3-receptors in biological system serve as regulator for other histamine H1-, H2-, or H4-receptors and auto-regulator for itself. Therefore, present study exhibits that H3-receptors regulate biological histamine level and its function in the body, and modulate histamine role in antibody production. In this study, we noticed again that exogenously added histamine *in vivo* (until its presence in the body) enhanced the antibody generation profile against SRBC whereas; on further metabolism its effect disappeared on the antibody generation profile bringing it in the range of control antibody level (strengthen previous histamine results of chapters 1, 2 and 3). Furthermore, H3R-antagonist (until their presence) increased the antibody profile, which came to normal level (control range) in later phase due to their metabolism. The present results provided evidence that histamine through histamine H3-receptors have important role in modulation of total immunoglobulins (Igs, IgM and IgG) generation.

In the fifth chapter we have evaluated *in vivo* immunomodulation and immunomodulatory profile of histamine H4-receptors and showed enhanced generation profile of total anti-SRBC-Igs, anti-SRBC-IgM and anti-SRBC-IgG in H4R-agonist-treated group. In contrast, H4R-antagonist-treated group revealed initially suppressed and then later enhanced the profile of total anti-SRBC-Igs, anti-SRBC-IgM and anti-SRBC-IgG throughout the study. These results demonstrated that

H4-receptors by H4R-antagonist-treatment showing similar profile of antibody (Igs, IgM and IgG) generation as positive control group. On the other hand H4R-agonist-treatment showing modulatory activity (enhancing the antibody generation levels) as compared to other experimental groups. These data provided evidence that histamine through histamine H4-receptor modulates immunological function and stimulates antibody production *in vivo* biological system. Again in this study, exogenously added histamine *in vivo* (until its presence in the body) enhanced the antibody generation profile against SRBC whereas; on further metabolism its effect disappeared (showed same findings of histamine as chapter 1-4). H4R-antagonist (until their presence) showed normal level of antibody profile, which was similar to control range whereas; on further metabolism its effect appeared as modulator and enhanced the antibody generation profile, which came to normal level (control range) at the last experimental day (in later phase). The conclusion of present study provided evidence that histamine H4-receptors have role in modulation of Igs, IgM and IgG generation.

In the sixth chapter we have studied *in vivo* immunomodulation and immunomodulatory profile of histamine receptors (H1-, H2-, H3- & H4-): a comparative agonists study and demonstrated that enhanced generation profile of anti-SRBC-antibody (anti-SRBC-Igs, IgM and IgG) in H1R-agonist-, H2R-agonist- and H4R-agonist-treated rabbits, while it showed suppression in H3R-agonist-treated rabbits as compared to positive control rabbits during the whole study period (same results were analyzed in chapter 2 - 5). Further we noticed that exogenously added histamine *in vivo* (until its presence in the body) enhanced the antibody generation profile against SRBC on 7^{th} day post-I whereas; in later stages its effect disappeared on the antibody generation profile bringing it in the range of control antibody level (same as chapter 1 - 5). The conclusion of the present comparative study has provided evidence that histamine receptors (H1R, H2R and H4R) on stimulation have important role in modulation by enhancing the antibody generation, in which H1R have dominant role in immune modulation as compared to H2R and H4R. On the other hand, H3R on stimulation have dominant immune suppressive role and regulates other histamine receptors (H1, H2 and H4) in immune system.

In seventh chapter we have evaluated *in vivo* immunomodulation and immunomodulatory profile of histamine receptors (H1-, H2-, H3- & H4-): a comparative antagonists study and showed that the histamine treated-rabbits

illustrated immunopotentiating properties by enhancing the anti-SRBC-antibodies levels as compared to positive control group (strengthen our previous histamine findings documented in earlier chapters) and the comparative study of H1-, H2-, H3- & H4-antagonists-treated groups demonstrated enhanced generation profile of anti-SRBC-Igs, IgM and IgG in H3R-antagonist-treated rabbits as compared to positive control rabbits, while H4R-antagonist, H2R-antagonist- and H1R-antagonist-treated rabbits revealed suppressed generation profile of anti-SRBC-Igs, IgM and IgG as compared to positive control rabbits (until the presence in the body). Whereas, on further metabolism of antagonists in the body showed enhanced anti-SRBC-Igs, IgM and IgG generation profile in H4R- & H2R-antagonists-treated rabbits (H4R- showed more marked immune modulation as compared to H2R-) as compared to positive control rabbits. H1R-antagonist-treated rabbits showed suppressed antibody generation profile throughout the study period as compared to other experimental groups. The conclusion of the present study has provided evidence that histamine receptors (H1R, H2R, H3R & H4R) have important role in modulation of antibody generation, in which H1R have dominant immune enhancing role as compared to H2R and H4R (H2R revealed more marked immune enhancing role as compared to H4R). On the other hand, H3R have dominant immune modulatory role in suppressing the antibody generation. Therefore, H3R have regulatory role and regulates other histamine receptors (H1, H2 and H4) in immune system.

In the second part of study we have delineated toxicological effects of histamine receptors (H1, H2, H3 & H4)-agonists/antagonist used in the present immunomodulation study. To study the toxicological effects of HRs-agonists/antagonist, the present part of study is divided into two chapters: (i) Hepatotoxicity by histamine receptors (H1, H2, H3 & H4)-agonists in rabbits: a histopathological & biochemical study, and (ii) Hepatotoxicity by histamine receptors (H1, H2, H3 & H4)-antagonists in rabbits: a histopathological & biochemical study.

In the first toxicological chapter we have demonstrated that AST and ALT levels were significantly increased in all drug-treated groups as compared to control and showed the increased rates of liver degeneration in histamine and HRs-agonists-treated groups, which support hepatotoxic findings in all drug-treated groups compared to control group. Furthermore, increased alkaline phosphatase and bilirubin levels in all drug-treated groups as compared to control group also support liver

degeneration and sustain our histological findings of liver damage. The histopathological study of liver revealed that short-term treatment by histamine and its receptors-agonist produce differential patterns of heptotoxicity in terms of hepatic congestion (histamine and H2R-agonist), centrilobular necrosis (H1R-agonist), binucleated (H4R-agonist) and multinucleated hepatocytes (H2R- and H3R-agonist), and prominent KCs (H4R-agonist) as compared to control group. Thus, the conclusion of these results suggests that histamine receptors on induction via their specific-agonist produce differential pattern of hepatotoxicity warranting further studies.

In the second toxicological chapter we have demonstrated the maintained hepatic lobular architecture, portal triad, hepatic cords and sinusoids in control, histamine and DMSO-treated groups. The notable findings included increased binuclearity in H1R, trinuclearity in H2R, oxyphilic clusters of hepatocytes in H3R, and moderate centrilobular necrosis in H3R and H4R-antagonist-treated groups without obvious lymphocytic infiltration and Kupffer cell prominence. Thus, the conclusion of present study revealed that short-term treatment by histamine receptors-antagonists produce different patterns and intensities of hepatotoxicity in terms of hepatocellular necrosis and hepatocyte polyploidy congruent with biochemical alteration in the blood but had minimal effects on hepatic congestion, inflammatory cells infiltration or Kupffer cell prominence.