Modulation of *in vivo* immunoglobulin production by endogenous histamine and H1R and H2R agonists and antagonists

Trivendra Tripathi¹, Mohammad Shahid², Haris M. Khan², Mahendra Pal Singh Negi³, Mashiattullah Siddiqui¹, Rahat A. Khan⁴

¹Department of Biochemistry, ²Section of Immunology, Department of Microbiology, ⁴Department of Pharmacology; Faculty of Medicine, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh-202002, India

³Biometry and Statistics Division, Central Drug Research Institute, Lucknow-226001, India

Correspondence: Mohammad Shahid, e-mail: shahidsahar@yahoo.co.in; drmohdshahid123@yahoo.com

Abstract:

The present study was designed to delineate the immunomodulatory role of histamine receptors (H1R and H2R) and their antibody generation in a rabbit model. Six groups containing 18 rabbits each received either vehicle (sterile distilled water, 1 ml/kg × b.i.d.), histamine (100 µg/kg × b.i.d.), H1R agonist (HTMT, 10 µg/kg × b.i.d.), H2R agonist (amthamine, 10 µg/kg × b.i.d.), H1R antagonist (pheniramine, 10 mg/kg × b.i.d.) or H2R antagonist (ranitidine, 10 mg/kg × b.i.d.). All animals were subsequently immunized with an intravenous injection of sheep red blood cells (SRBC). Estimations of total serum immunoglobulins (Igs), immunoglobulin M (IgM) and immunoglobulin G (IgG) were performed by ELISA and hemagglutination assay (HA) at days 0 (pre-immunization), 7, 14, 21, 28 and 58 (post-immunization). Both the ELISA and the HA showed similar production of Igs, IgM and IgG but the results were found comparatively more significant by ELISA as opposed to HA. Results showed that histamine could influence a detectable antibody response to SRBC early (i.e., at day 7), which lasted until day 58. Immunomodulatory processes showed suppression of an Ig generation in the H1R-antagonist group with enhancement in the H2R-antagonist group. The H1R-agonist group showed an increased Ig production in comparison to the H2R-agonist group. The IgM production was inhibited in the H1R-antagonist group as compared to the H2R-antagonist group, and it was also suppressed in H1R-agonist group as compared to H2R-agonist group. IgG production was inhibited in the H1R-antagonist group as opposed to the H2R-antagonist group. In contrast, the H1R-agonist group increased IgG production as compared to the H2R-agonist group. All the results were found to be statistically significant ($p < 0.05$ or $p < 0.01$). In conclusion, histamine and its receptor (H1R and H2R) agonists enhance antibody production by triggering the histamine receptors (H1R and H2R), and both the H1R antagonist and the H2R antagonist positively or negatively regulate the antibody production. The findings of this study may have clinical significance.

Key words:

histamine receptors, immunomodulation, T-cell dependent antibody response, humoral immune response, H1 antagonists, H2 antagonists, H1 agonists, H2 agonists

Abbreviations: b.i.d. – two times per day, ELISA – enzyme linked immunosorbent assay, HA – hemagglutination assay, H1R – histamine receptor type 1, H2R – histamine receptor type 2, HRP – horseradish peroxidase, HTMT – histamine trifluoro-

methyl toluidide, *im* – intramuscular, 2-ME – 2-mercaptoethanol, PBS – phosphate buffer saline, Post-I – post-immunization, Pre-I – pre-immunization, *sc* – subcutaneous, SRBC – sheep red blood cells, Th – T helper, – TMB – tetramethylbenzidine

Introduction

Histamine, a biogenic amine, is considered not only as a mediator of chronic inflammation and hypersensitivity but also as a regulator of several essential events in the immune response. Histamine regulates dendritic cells, T-lymphocytes, B-lymphocytes and related antibody isotype responses. Recently, accumulating evidence has highlighted the importance of histamine receptors in immunomodulation [3, 6, 8, 13]. Histamine can modulate the immune response when it is released from mast cells and basophils during inflammatory reactions [3]. Histamine's immunosuppressive and immunomodulatory effects on both humoral immune and cell-mediated immune responses have been well documented [9, 12].

Histamine receptors have previously been shown to enhance delayed hypersensitivity and antibody-mediated immune responses. This has been observed in many pathological processes regulating several essential events in allergy and autoimmune diseases in experimental animals, especially in knock-out mice (either H1- or H2-deficient) [8, 11, 12]. However, studies evaluating the role of intact histamine receptors blocked by their respective agonists or antagonists and the resulting immunomodulatory profile over time are lacking in the existing literature. Moreover, studies in rabbit models are elementary with existing studies demonstrating an immunomodulatory role in single blood samples taken after immunizing the animals.

Our study explored the regulatory mechanisms of histamine receptors that control the immune process through the use of effector cells derived histamine (in control group), exogenous histamine and histamine receptor agonists and antagonists. Histamine receptor type 1 (H1R) and histamine receptor type 2 (H2R) both belong to the G-protein-coupled receptor family [13]; however, they trigger different biochemical intracellular events upon activation [13, 14]. H1Rs and H2Rs are also regulated by specific cytokines present in the immune system. Jutel et al. [6] investigated the H1R-specific antagonist tripeleminamine and found that it inhibited histamine binding in T helper (Th) 1 but not in Th2 cells. This study also demonstrated predominant H1R expression on Th1 cells. Neither H2R antagonist (ranitidine) nor H3R antagonist/H4R-partial agonist (clobenpropit) had any impact on histamine

binding to the Th1 cells. This study demonstrated the expression of H1Rs on Th1 cells and H2Rs on Th2 cells by antibodies generated against the H1R and H2R [6].

Based on the previously mentioned studies, the present study was designed to evaluate the immunomodulatory effects and the immunoglobulin production of H1R and H2R agonists and antagonists in rabbit models over a period of 58 days.

Materials and Methods

Experimental design

To evaluate the systemic antibody response, 108 (54 male and 54 female) New Zealand adult healthy rabbits weighing 1–1.5 kg were randomized equally into six treatment groups (i.e., 18 rabbits (9 male and 9 female) in each group). Group I was treated with vehicle (sterile distilled water) and considered a positive control; group II was histamine treated; group III was treated with an H1R agonist; group IV was treated with an H2R agonist; group V was treated with an H1R antagonist; group VI was treated with an H2R antagonist. Each treatment group was also immunized with sheep red blood cells (SRBC). The animals were housed in a well-maintained animal facility in the central animal house at J. N. Medical College & Hospital, Aligarh Muslim University, Aligarh. This animal house was located in the Bioresources unit under a 12 h light/dark cycle and a temperature of $22 \pm 2^\circ\text{C}$ and the animals were allowed free access to standard laboratory diet including green vegetables and tap water during the studies. All studies were carried out during the light cycle and were approved by the Institutional Animal Ethical Committee at J. N. Medical College & Hospital.

Materials

Materials were obtained from the following manufacturers: Monoclonal anti-rabbit Ig horseradish peroxidase (HRP) conjugated and monoclonal anti-rabbit IgG-HRP conjugated (Sigma, USA); anti-rabbit IgM-HRP conjugated (G Biosciences, Maryland Heights, USA); tetramethylbenzidine (TMB) and TMB diluent (J Mitra and Co, India); polystyrene MaxiSorp micro-

titre flat and round bottom ELISA plates (NUNC, Denmark); glutaraldehyde solution (Central Drug House, India); skim milk (Nestle India Ltd., New Delhi); and 2-mercaptoethanol (2-ME, Merck KGaA, Darmstadt, Germany). All chemicals were of analytical grade.

Drugs

The following pharmaceuticals were used: histamine dihydrochloride (Himedia Laboratories Pvt. Ltd., India); the H1R agonist (histamine trifluoromethyl toluide (HTMT)-dimalate) and the H2R agonist (amthamine dihydrobromide) (Kindly donated by Tocris Bioscience, Tocris Cookson Ltd., United Kingdom); Avil[®] (pheniramine maleate) (Unimark Remedies, India); and Rantac[®] (ranitidine hydrochloride) (J. B. Chemicals and Pharmaceuticals, India). All doses refer to the weight of the salts used.

Dosage regimen

Histamine dihydrochloride (100 µg/kg), the H1R agonist (HTMT-dimalate; 10 µg/kg) and the H2R agonist (amthamine dihydrobromide; 10 µg/kg), were administered twice a day [12 hourly (8 a.m. and 8 p.m. every day)] subcutaneously (*sc*). The H1R antagonist (pheniramine maleate; 10 mg/kg) and the H2R antagonist (ranitidine hydrochloride; 10 mg/kg) were administered twice a day [12 hourly (8 a.m. and 8 p.m. every day)] intramuscularly (*im*). These doses were given three days before immunization and every day after that until seven days after immunization.

Antigen

Sheep blood diluted 1:1 in sterile Alsevier's solution was obtained from the Department of Microbiology, J. N. Medical College & Hospital, Aligarh Muslim University, Aligarh. It was then washed with PBS (10 mM sodium phosphate buffer containing 150 mM NaCl, pH – 7.4) three times and the concentration was adjusted to 5% (1×10^9 cells/ml) in PBS for immunization before use.

Immunization of rabbits

The rabbits in all experimental groups (I–VI) were immunized intravenously *via* their marginal ear vein with 1 ml of 5% (1×10^9 cells/ml) SRBC in PBS.

Sample collection

To determine the systemic antibody response, blood samples were collected from rabbits through their marginal ear veins prior to immunization (day 0) and on days 7, 14, 21, 28 and 58 post-immunization. Blood samples were kept at room temperature for 2 h and then at 4°C overnight. These samples were centrifuged for 10 min at $580 \times g$ and then the serum was isolated and heated at 56°C for 30 min to inactivate complement proteins. Samples were stored in aliquots containing sodium azide as a preservative at –20°C until analyzed.

Serological analyses

Hemagglutination assay (HA)

To determine the antibody's response to SRBC, a direct hemagglutination technique [4] was used with some modifications. Briefly, 100 µl of PBS was dispensed into each well of a round-bottomed 96-well microplate. Serum samples (100 µl) were then added using serial two-fold dilutions (1:2, 1:4, 1:8, 1:16, 1:32) in the wells from columns two to twelve. The first column of wells (PBS only) was considered blank. Next, 100 µl of 2% SRBC in PBS was added to all wells for a final volume of 200 µl. The plates were then shaken for 1 min and incubated at 37°C for 1 h and then stored overnight at 4°C to determine agglutination titres. A positive result was recorded when at least a 50% SRBC agglutination was observed. To measure anti-SRBC immunoglobulin-M (IgM) and immunoglobulin-G (IgG), serum samples were treated with 0.2 M 2-ME for 1 h at 37°C. This treatment inactivates the IgM antibody and the hemagglutination observed after treatment with 2-ME is due mostly to the presence of the IgG antibody. The difference between total antibodies and IgG antibody titres were the titres of IgM antibody.

ELISA using whole SRBC

To determine the SRBC-IgG, SRBC-IgM and SRBC-IgG response, the whole SRBC enzyme linked immunosorbent assay (ELISA) [7] was also carried out on polystyrene plates with some modifications. Briefly, polystyrene MaxiSorp immunoplates were coated with an SRBC suspension (5×10^6 cells/100 µl PBS). The plates were incubated overnight at 4°C. Each

sample was added to the plates in duplicate and half of the plates served as control devoid of antigen coating (each sample put on in duplicate two times, one time on the antigen-coated side and one on the side without antigen). Without disturbing the cell layer, 20 μ l/well of 1.8% glutaraldehyde solution was then gently added to each plate and the plates were incubated at 25°C for 30 min. Each plate was washed four times with 200 μ l of PBS to remove unbound SRBC with complete decanting between each wash and then non-specific binding sites were blocked with 1% skim milk in PBS for 2 h at 37°C. After incubation, the plates were washed four times with 200 μ l of PBS as before. Each rabbit serum sample was then diluted 1:100 in PBS (100 μ l/well), incubated for 1.5 h at 37°C, and then overnight at 4°C. Each plate was then washed with PBS four times as described earlier. The secondary antibodies HRP-conjugated monoclonal anti-rabbit Igs, HRP-conjugated monoclonal anti-rabbit IgM and HRP-conjugated monoclonal anti-rabbit IgG were then added (100 μ l/well) to their respective plates and incubated at 37°C for 1 h. The plates were then washed with PBS four times as before and 100 μ l/well of TMB substrate was added. The plates were then incubated at 25°C for 1 h. The enzymatic reaction was stopped by adding 50 μ l/well of 5% H₂SO₄. Absorbance was determined at 405 nm on an automatic ELISA plate reader (Micro scan MS5608A, ECIL, India). The control wells were treated similarly but were devoid of antigen. Thus, total serum Igs, total IgM and total IgG production were studied *in vivo* in six experimental groups separately

and absorbances were estimated ($A_{\text{test-control}}$) on day 0 (pre-immunization) and on days 7, 14, 21, 28 and 58 (post-immunization).

Statistical analysis

Data were summarized as the mean $\pm$ SD. Groups were compared by using a repeated measures (subjects within groups) two-way analysis of variance (ANOVA) followed by the Newman-Keuls *post-hoc* test. A two-tailed ($\alpha = 2$) probability $p < 0.05$ was considered to be statistically significant. Analyses were performed on SPSS for Windows (version 12.0, Inc., Chicago, IL, USA).

Results

Profile of total anti-SRBC antibody Igs production

The production of total anti-SRBC antibody Igs was estimated by the whole SRBC ELISA method, and direct HA and is summarized in Figure 1 and Table 1, respectively. The ELISA and HA assays both showed similar Igs production; however, the results were found comparatively more significant by ELISA as opposed to HA. No antibody response was detected in any experimental group (control or drug-treated) at day 0 (pre-immunization). An initial increase and then a subsequent decrease in Igs were observed over a time span of 58 days in all groups (Tab. 1 and Fig.

Fig. 1. SRBC-specific immunoglobulins (Igs) production in six different treatment groups over 58 days. To test for significance, each time point value was compared to the control value and then each time point value was also compared to the day 7 value. This was repeated for every treatment group by a two-way analysis of variance (ANOVA) followed by the Newman-Keuls *post-hoc* test. Treatments and days were found to be significant at either $p < 0.05$ or $p < 0.01$ compared to the control group or day 7, respectively. Data are represented as the mean $\pm$ SD

Tab. 1. Antibody responses (the mean $\pm$ SD, n = 18) to different treatments over the study period to SRBC by hemagglutination assays

Groups	Igs					IgM					IgG				
	Post-I					Post-I					Post-I				
	Pre-I	7 th Day	14 th Day	21 st Day	28 th Day	58 th Day	Pre-I	7 th Day	14 th Day	21 st Day	28 th Day	58 th Day	Pre-I	7 th Day	14 th Day
Control	0.00 $\pm$ 0.00	4.67 $\pm$ 0.54	5.00 $\pm$ 0.65 ^b	4.00 $\pm$ 0.92 ^b	3.50 $\pm$ 0.57 ^b	2.33 $\pm$ 0.54 ^b	0.00 $\pm$ 0.00	4.13 $\pm$ 0.91	4.00 $\pm$ 0.65	2.33 $\pm$ 1.25 ^b	1.50 $\pm$ 0.54 ^b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	2.33 $\pm$ 0.52	4.00 $\pm$ 0.65 ^b
Histamine	0.00 $\pm$ 0.00	5.87 $\pm$ 0.65 ^a	4.17 $\pm$ 0.42 ^{bc}	3.83 $\pm$ 0.42 ^b	3.47 $\pm$ 0.54 ^b	1.67 $\pm$ 0.54 ^{bc}	0.00 $\pm$ 0.00	5.29 $\pm$ 0.14 ^a	3.17 $\pm$ 0.42 ^{bc}	2.33 $\pm$ 1.25 ^b	1.67 $\pm$ 0.65 ^b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	4.27 $\pm$ 0.17 ^a	3.17 $\pm$ 0.42 ^{bc}
H1R-agonist	0.00 $\pm$ 0.00	6.20 $\pm$ 0.92 ^a	6.35 $\pm$ 0.65 ^a	5.76 $\pm$ 0.65 ^{ab}	5.22 $\pm$ 0.92 ^{ab}	4.32 $\pm$ 0.65 ^{ab}	0.00 $\pm$ 0.00	5.48 $\pm$ 0.65 ^a	4.55 $\pm$ 0.65 ^b	4.06 $\pm$ 0.62 ^{ab}	2.41 $\pm$ 0.70 ^{bc}	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	4.84 $\pm$ 0.92 ^a	5.12 $\pm$ 0.65 ^{ad}
H2R-agonist	0.00 $\pm$ 0.00	6.58 $\pm$ 0.66 ^a	6.21 $\pm$ 1.30 ^{ab}	5.47 $\pm$ 1.30 ^{ab}	4.59 $\pm$ 0.66 ^{ab}	4.00 $\pm$ 0.92 ^{ab}	0.00 $\pm$ 0.00	6.03 $\pm$ 0.65 ^a	5.59 $\pm$ 0.66 ^{ab}	4.42 $\pm$ 0.71 ^{ab}	2.59 $\pm$ 0.10 ^{ab}	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	4.92 $\pm$ 0.11 ^a	4.70 $\pm$ 0.79 ^d
H1R-antagonist	0.00 $\pm$ 0.00	3.67 $\pm$ 0.54 ^a	4.33 $\pm$ 0.54 ^b	3.67 $\pm$ 1.06	3.00 $\pm$ 1.30 ^b	1.67 $\pm$ 0.57 ^{ab}	0.00 $\pm$ 0.00	3.33 $\pm$ 0.60	3.70 $\pm$ 0.66 ^d	2.10 $\pm$ 1.34 ^b	1.67 $\pm$ 0.84 ^b	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.33 $\pm$ 0.54 ^a	2.67 $\pm$ 1.06 ^{ab}
H2R-antagonist	0.00 $\pm$ 0.00	4.33 $\pm$ 0.54	4.80 $\pm$ 0.42 ^b	4.17 $\pm$ 0.42	3.67 $\pm$ 0.54 ^b	2.17 $\pm$ 0.42 ^b	0.00 $\pm$ 0.00	3.52 $\pm$ 1.68	4.13 $\pm$ 0.64 ^b	3.17 $\pm$ 0.42 ^{cd}	3.03 $\pm$ 0.42 ^{ad}	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	1.67 $\pm$ 0.84 ^c	3.38 $\pm$ 0.48 ^{cb}

^a p < 0.01 in comparison with control group; ^b p < 0.01 in comparison with day 7; ^c p < 0.05 in comparison with control group; ^d p < 0.05 in comparison with day 7

1). The two-way ANOVA revealed that the effect of treatment ($F = 31.74$, $DF = 5,102$; $p < 0.01$) and day ($F = 2416.20$, $DF = 4,408$; $p < 0.01$) on SRBC was statistically significant. The interaction (treatments $\times$ days) effect on SRBC was also found to be significant ($F = 57.89$, $DF = 20,408$; $p < 0.01$).

When comparing the total anti-SRBC Igs produced in each treatment group to their respective control group on each day, the mean Igs differed significantly ($p < 0.05$ or $p < 0.01$) (Tab. 1) except H2R antagonist on day 7; H1R antagonist and H2R antagonist on day 14; Histamine, H1R antagonist and H2R antagonist on day 21 and day 28; and H2R antagonist on day 58. Similarly, when comparing the total anti-SRBC Igs produced each day within all groups, the mean Igs produced from days 14 to 58 in all groups differed significantly ($p < 0.01$) to day 7 except day 14 in H1R agonist and day 21 in H1R antagonist and H2R antagonist (Tab. 1).

Profile of total anti-SRBC antibody IgM production

The production of total anti-SRBC antibody IgM was estimated by the whole SRBC ELISA method, and direct HA and is summarized in Figure 2 and Table 1, respectively. Similar to Igs production, the production of IgM in the ELISA and HA assays were similar; however, the results were found comparatively more

significant by ELISA as opposed to HA. In contrast to the Igs data, no antibody response was detected in all experimental groups (control or drug treated) on day 0 (pre-immunization) and also day 58 (post immunization). In all groups, similar to the Igs production, there was an initial increase and subsequent decrease in IgM production over the time span of 58 days (Tab. 1 and Fig. 2). As compared to day 0, the IgM titers increased at day 7 where they obtained the highest peak and then decrease gradually up to day 58 in all experimental groups except in the H1R antagonist and H2R antagonist groups where the IgM titer increased up to day 14 (Tab. 1 and Fig. 2). The two-way ANOVA revealed that the effect of treatment ($F = 19.28$, $DF = 5,102$; $p < 0.01$) and day ($F = 828.48$, $DF = 3,306$; $p < 0.01$) on SRBC were statistically significant. The interaction effect on SRBC was also found to be significant ($F = 58.39$, $DF = 15,306$; $p < 0.01$).

When comparing the total anti-SRBC IgM production between the treatment groups, the mean IgM production differed significantly ($p < 0.05$ or $p < 0.01$) from their respective control group each day (Tab. 1) except H1R antagonist and H2R antagonist on day 7; H1R agonist, H1R antagonist and H2R antagonist on day 14; and histamine and H1R antagonist on day 21 and day 28. Similarly, when comparing the total anti-SRBC IgM produced each day within all groups, the mean IgM produced from days 14 to 58 in each group

Fig. 2. SRBC-specific immunoglobulin-M (IgM) production in six different treatment groups over 58 days. To test for significance, each time point value was compared to the control value and then each time point value was also compared to the day 7 value. This was repeated for every treatment group by a two-way analysis of variance (ANOVA) followed by the Newman-Keuls *post-hoc* test. Treatments and days were found to be significant at either $p < 0.05$ or $p < 0.01$ compared to the control group or day 7, respectively. Data are represented as the mean $\pm$ SD

Fig. 3. SRBC-specific immunoglobulin-G (IgG) production in six different treatment groups over 58 days. To test for significance, each time point value was compared to the control value and then each time point value was also compared to the day 7 value. This was repeated for every treatment group by a two-way analysis of variance (ANOVA) followed by the Newman-Keuls *post-hoc* test. Treatments and days were found to be significant at either $p < 0.05$ or $p < 0.01$ compared to the control group or day 7, respectively. Data are represented as the mean $\pm$ SD

differed significantly ($p < 0.05$ or $p < 0.01$) to day 7 except day 14 in control group (Tab. 1).

Profile of total anti-SRBC antibody IgG production

The production of total anti-SRBC antibody IgG was estimated by the whole SRBC ELISA method and direct HA and is summarized in Figure 3 and Table 1, respectively. Like the Igs and IgM results, the production of IgG in the ELISA and HA assays were similar; however, the results were found comparatively more significant by ELISA as opposed to HA. The antibody IgG response was also not detected in all experimental groups (control or drug treated) at day 0 (pre-immunization). Similar to the Igs and IgM results, there was an initial increase and subsequent decrease in IgG production in all groups over the time span of 58 days (Tab. 1 and Fig. 3). When compared to day 0, the IgG titer increased at day 7 and obtained its highest peak at day 14. It then decreased gradually in all treatment groups except for the H1R agonist and antagonist groups where the increase occurred up to day 21 (Tab. 1 and Fig. 2). The two-way ANOVA revealed that the effect of treatment ($F = 39.91$, $DF = 5,102$; $p < 0.01$) and day ($F = 288.88$, $DF = 4,408$; $p < 0.01$) on SRBC were statistically significant. The interaction effect on SRBC was also found to be significant ($F = 73.76$, $DF = 20,408$; $p < 0.01$).

When comparing the total anti-SRBC IgG produced in each treatment, the mean IgG production in each observed day differed significantly ($p < 0.05$ or $p < 0.01$) from their respective control group (Tab. 1)

except H2R antagonist on day 14; Histamine, H1R antagonist and H2R antagonist on day 21 and day 58; and H1R antagonist and H2R antagonist on day 28. Similarly, when comparing the total anti-SRBC IgG produced each day within all groups, the mean IgG produced from days 14 to 58 in all groups differed significantly ($p < 0.05$ or $p < 0.01$) to day 7 except day 58 in control group and day 28 in H1R agonist (Tab. 1).

Discussion

In the present study, we investigated the production of total serum Igs, total serum IgM and total serum IgG to SRBC, a T lymphocyte-dependent test antigen [7, 10] modulated by endogenous histamine released from effector cells. Our investigation consisted of control (untreated), histamine, H1R agonist (HTMT), H2R agonist (amthamine), H1R antagonist (pheniramine) and H2R antagonist (ranitidine) treated groups.

To provide evidence relating our investigations to *in vivo* immunoregulatory processes, we used healthy rabbits with and without H1R and H2R agonist or antagonist treatments. Here we demonstrate that rabbits treated with H1R and H2R specific agonists or antagonists were characterized by a marked deviation in their immune response as compared to control rabbits (untreated).

In this study, we demonstrated that histamine released by immunological stimuli from effector cells

(i.e., mast cells and basophils) *in vivo* [13] could influence a detectable antibody response to SRBC. To the best of our knowledge, no other report has demonstrated the anti-SRBC antibody production over a multi-day period except our earlier report during a study period of 28 days [15].

This comparative study on *in vivo* immunomodulatory processes demonstrates a suppressed generation of total anti-SRBC-IgG in the H1R antagonist-treated group and enhancement in the H2R antagonist-treated group during the study period. In contrast, the H1R agonist-treated group demonstrates an increased total anti-SRBC-IgG production as compared to H2R agonist-treated group. These data provide evidence that H1Rs have a dominant role in total IgG production.

Histamine is a potent agonist of all four receptors (H1–H4) [6, 13, 14] that are distributed in different parts of body. These receptors modulate different biochemical, pharmacological and immunological reactions, both *in vivo* and *in vitro*, by activating signal transduction pathways for endogenous histamine release [13]. It is known that histamine directly affects B-cell antibody production as a co-stimulatory receptor on B-cells [1, 2, 5, 12]. It has been demonstrated in mice that histamine enhances the anti-IgM induced proliferation of B-cells, and this is abolished in H1R-deleted mice. In H1R-deleted mice, the antibody production against a T-cell-independent antigen TNP-Ficoll is decreased [3], and this suggests the role of H1R signaling in response triggered from B-cell receptors. Jutel et al. [6] showed a different pattern of antibody responses to T-cell-dependent antigens like ovalbumin. They demonstrated that H1R-deleted mice produced high ovalbumin-specific IgG1 and IgE in comparison to wild-type mice. In the present *in vivo* study, we demonstrate that the histamine-treated rabbits showed immunopotentiating properties by enhancing the anti-SRBC antibody levels (Igs, IgM and IgG) as compared to the control group over the study period.

The anti-SRBC IgM results demonstrate that the H1R antagonist suppresses the IgM production as compared to the H2R antagonist-treated group. The agonists studied also demonstrate the same IgM production as the antagonists. Similar to the antagonists used, the production of IgM increase was significantly lower in the H1R agonist as compared to H2R agonist-treated group. Thus, it can be concluded that H2Rs show a dominant role for IgM generation as compared to the H1Rs. The anti-SRBC IgG results demonstrate that the H1R antagonist-treated group

suppressed the anti-SRBC IgG levels during the study as compared to the H2R antagonist-treated group. In contrast, the H1R agonist increased IgG production as compared to the H2R agonist-treated group and this demonstrates that H1Rs show a dominant role in IgG generation as compared to H2Rs.

In this study, we noticed that exogenously added histamine *in vivo* enhanced antibody generation against SRBC at first; however, further metabolism made its effect disappear and the antibody generation was brought into the range of the control antibody. We also observed that the H1R and H2R antagonists suppressed the antibody productions at first but then these levels were brought into the control range due to being metabolized. Therefore, based on the present study we conclude that the results obtained due to histamine and its antagonists were of short duration due to clearance of these substances from the rabbits. Thus, results from both the histamine and antagonist-treated groups were identical to each other and similar to the control group after metabolism of the drugs.

In this study, the analysis of the results by two serological techniques (ELISA and HA) is noteworthy. When comparing the results between the HA and the ELISA, similar conclusions were drawn and hence we suggest that laboratories that do not have facilities for ELISA may use HA to perform base line studies on antibody production.

To conclude, our results provide evidence that histamine H1Rs have a principle role in the modulation of total Igs and demonstrate potent effects in IgG generation. H2Rs show a dominant role in IgM generation and B-cell differentiation and proliferation by positively and negatively regulating antibody production. To the best of our knowledge, this is the first report describing the potential immunoregulatory role of H1 and H2 receptor agonists and antagonists, and also the first report studying the immunomodulatory profile in experimental rabbits over a continued study period of 58 days with pre- and post-immunization.

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