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Effects of Histamine Receptor Antagonists Metiamide and Cimetidine on Antibody Formation *in Vitro* by Murine Cells (41335)

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Abstract. The effects of the H_2 receptor antagonists cimetidine and metiamide on antibody responsiveness by murine splenocytes immunized *in vitro* with sheep erythrocytes were examined. These agents had a dose-related effect on antibody formation. Incubation of normal mouse spleen cells with 10 μg metiamide or cimetidine resulted in a marked stimulation of the antibody response, but increasing the dose to 1000 μg or more depressed the response. The higher doses also suppressed viability of the cells, but to a lesser extent than suppression of antibody formation. There was no effect on the cytokinetics of appearance of antibody-forming cells, since the peak day of the response was similar regardless of presence or absence of the drugs. Optimum enhancement occurred when the drugs were added to cultures on Day 0 or plus one day, indicating that immunomodulation was associated with early events of the immune response.

Histamine (H_2) receptors on the surface of lymphocytes appear to be involved in various aspects of immune activation, especially in immediate and delayed hypersensitivity reaction. Henny and Lichtenstein *et al.* reported in 1971 that histamine as well as β catecholamines and prostaglandins inhibit the cytotoxic effects of lymphocytes sensitized to fiberblasts or mast cells (1). Little is known, however, concerning the effects of H_2 antagonists on antibody-producing lymphocytes. In the present study the histamine receptor antagonists metiamide and cimetidine were studied in regard to their effects on the antibody response of murine splenocytes immunized *in vitro* with sheep erythrocytes. Parameters examined in this study included the effects of these agents on the cytokinetics of the immune response, the magnitude of the response, and the viability of the cells treated. In addition, the effects of time of addition of the H_2 receptor antagonist relative to the time of *in vitro* immunization were examined.

Experimental Methods. *Animals.* BALB/c male mice obtained from Jackson Memorial Laboratories, Bar Harbor, Maine, were used for these studies. They were 6–8 weeks of age and maintained in groups of 6–10 in individual mouse cages. The animals were fed Purina mouse chow and water *ad libitum*.

Antigen and immunization. Sheep erythrocytes (SRBC) were obtained from Baltimore Biological Laboratories, Cockeysville, Maryland. Cell cultures (see below) were immunized with 0.1% suspensions of washed erythrocytes (approximately 2×10^6 RBCs) at the time of culture initiation.

Spleen cell cultures. The spleen was obtained at autopsy from individual mice and pooled cell suspensions obtained by teasing with needles and forceps. Cells were washed several times in tissue culture medium (RPMI 1640 containing 10% fetal calf serum) and cultures prepared in Marbrook vessels (Bioresearch Corp., Vineland, N.J.) so that each vessel contained 5×10^6 nucleated viable splenocytes in 1.0 ml medium on a dialysis membrane in the inner chamber (3). The outer reservoir chamber contained 11–12 ml medium. In each experiment at least six cultures were immunized with SRBC per group. Some of the cultures were immunized similarly and treated with cimetidine or metiamide (see below).

H_2 Antagonists. Cimetidine and metiamide, obtained from Smith, Kline and French Laboratories, Philadelphia, Pennsylvania,

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suspended in 1.0 ml tissue culture medium in graded concentration ranging from 100 to 2000 μg per culture, were added in 0.1-ml volumes to triplicate sets of vessels. All chambers were incubated at 37°C in an atmosphere of 73% N_2, 10% CO_2, and 7% O_2 without shaking according to the method first described by Marbrook (3, 4).

Antibody assay. At varying times after culture initiation and *in vitro* immunization, spleen cells were harvested from the chambers, washed several times with medium, and the numbers of antibody plaque-forming cells (PFC) per million viable spleen cells determined by the Jerne plaque assay in agar gel (5). For this purpose, 0.1 ml of a washed suspension of cultured cells was added to 2 ml melted Difco agar containing 0.1 ml of a 0.1% suspension of washed RBCs. The mixture of cells and agar was carefully poured onto the surface of a previously prepared 60-mm-diameter petri plate containing a base layer of sterile solidified agar. After the upper layer solidified, the plates were incubated for 1 hr at 37°C and treated with approximately 1–2 ml of a 1:10 dilution of sterile guinea pig serum as a source of complement. The plates were incubated further for 1 hr and the numbers of zones of hemolysis, indicating PFCs, were enumerated. The number of PFCs per million nucleated spleen cells plated or per culture was calculated and the average number of three to six duplicate cultures per experiment determined

Experimental Results. Addition of either metiamide or cimetidine to spleen cell cultures resulted in an alteration of the number of antibody-forming cells depending upon the dose of compound used. As is evident in Tables I and II, addition of 10 μg of either compound to 5×10^6 normal mouse spleen cells resulted in a 70–90% increase in the number of PFCs as compared to control cultures. One microgram metiamide also resulted in an increased PFC response, but this dose of cimetidine had only a marginal effect. Increasing the concentration of these agents to 50 or 100 μg per culture resulted in a similar or even greater increase in PFC numbers. On the other hand, 500 μg of either compound resulted in a moderate to marked diminution of the antibody response. One thousand micrograms resulted in approximately a 60–80% decrease in the PFC response, while 2000 μg resulted in an even greater inhibition.

The smaller doses of cimetidine or metiamide (100–500 μg) had only a slight to moderate effect on the number of viable cells recovered per culture. The 10-μg dose resulted often in slight but inconsistent elevation in the number of viable splenocytes. In contrast, the 1000-μg dose resulted in a 40% decrease in spleen cell number, whereas the 2000-μg dose resulted in a 50–60% decrease. The decreases in number of viable spleen cells did not appear to account for the decrease in PFC formation.

TABLE I. EFFECT OF METIAMIDE ON HEMOLYTIC ANTIBODY FORMATION *IN VITRO*

Metiamide concentration (μg/culture)[a]	No. of viable cells per culture ($\times 10^6$)	Hemolytic PFC response[b]			
		Per 10^6 spleen cells	Percentage of control	Per culture	Percentage of control
None (control)	1.40	1050 ± 165	—	1420 ± 210	—
1.0	1.32	1840 ± 230	175	2360 ± 170	166
10.0	1.46	2160 ± 260	206	2950 ± 275	208
50.0	1.36	1750 ± 196	167	2450 ± 168	173
100.0	1.21	1430 ± 250	136	1940 ± 210	137
500.0	1.10	952 ± 160	119	1439 ± 320	101
1000.0	0.75	510 ± 97	49	370 ± 65	26
2000.0	0.59	240 ± 62	23	195 ± 29	14

[a] Indicated concentrations of metiamide added to 5×10^6 normal BALB/c spleen cells in Marbrook vessels on day of culture initiation and immunization with 2×10^6 RBC.

[b] Average PFC response ± SE, for triplicate cultures in five different experiments 5 days after *in vitro* immunization.

TABLE II. EFFECT OF CIMETIDINE ON HEMOLYTIC ANTIBODY FORMATION *IN VITRO*

Cimetidine concentration (μg/culture)[a]	No. of viable cells per culture (×10⁶)	Hemolytic PFC response[b]			
		Per 10⁶ spleen cells	Percentage of control	Per culture	Percentage of control
None (control)	1.32	1120 ± 125	—	1485 ± 230	—
1.0	1.26	1440 ± 143	130	1790 ± 193	121
10.0	1.52	2560 ± 230	229	3700 ± 290	249
50.0	1.40	2630 ± 195	235	3580 ± 250	240
100.0	1.10	1830 ± 125	205	2010 ± 95	318
500.0	1.01	950 ± 86	85	960 ± 70	65
1000.0	0.82	530 ± 60	47	450 ± 30	31
2000.0	0.65	128 ± 52	11	85 ± 20	6

[a] Indicated concentration of cimetidine added to 5 × 10⁶ normal BALB/c spleen cell in Marbrook vessels on day of culture initiation and immunization with 2 × 10⁶ SRBC.

[b] Average PFC response ± SE, for triplicate cultures in five different experiments 5 days after *in vitro* immunization.

For example, calculating the number of PFCs per million viable spleen cells as compared to PFC response per culture still showed a marked alteration in response.

The cytokinetics of the immune response was not significantly affected by any dose of metiamide or cimetidine (Table III). Cell cultures showed the greatest PFC response on Days 4 or 5 after culture initiation. Cells stimulated with 10 μg of either drug showed the expected peak day of response, with increased PFC formation on each day of culture assay. Cultures treated with 1000 μg of these compounds showed a decreased PFC response throughout each of the 6 days of culture assay, but the peak response still occurred on the fourth and fifth day.

TABLE III. CYTOKINETICS OF ANTIBODY RESPONSE *IN VITRO* TO SRBC BY SPLEEN CELLS TREATED WITH METIAMIDE OR CIMETIDINE

Concentration of drug (μg/culture)[a]		Antibody PFC response on day[b]				
		+2	+3	+4	+5	+6
None (controls)		186	795	1280	1640	1030
Metiamide	10	185	970	1650	2440	2100
	100	170	850	1860	2210	1740
	1000	60	210	430	410	210
Cimetidine	10	216	1150	1930	2430	1840
	100	201	876	1750	1950	1130
	1000	95	130	276	350	290

[a] Indicated concentration of drug added to culture of 5 × 10⁶ normal mouse spleen cells immunized *in vitro* with 2 × 10⁶ SRBC.

[b] Average PFC response for three or four cultures on day indicated after *in vitro* immunization.

The time of addition of metiamide or cimetidine to the cell cultures had some effect on the response. As is evident in Table IV, enhanced or depressed responsiveness occurred mainly when the compound was added at or near the time of culture initiation. Addition of the drug 2 to 4 days after culture initiation had little or no depressive effect on the expected immune response.

Discussion and Conclusion. The results of this study indicated that metiamide and cimetidine, known H_2 receptor antagonists, markedly influence the primary humoral antibody response of normal mouse spleen cells immunized *in vitro* with SRBC. The effects noted were dose dependent, as well as dependent upon the time the drug was added to the spleen cell cultures *in vitro*. Marked enhancement of antibody formation occurred when 10 μg cimetidine was added to five million spleen cells on Days 0 or 1. This enhancement was characterized by a marked increase in the magnitude of antibody response on the peak day of antibody formation, as well as throughout the 6-day period of the immune response assayed. A 100-fold greater dose of the drugs (i.e., 1000 μg) markedly depressed the antibody response when added to cultures on Day 0.

The effects noted were most marked in terms of the magnitude of the response and not the cytokinetics. For example, there was no shift of the peak day of response in cultures suppressed with 1000 μg or more of these drugs. Thus, it appears that these

TABLE IV. EFFECT OF TIME OF ADDITION OF METIAMIDE OR CIMETIDINE ON SPLEEN CELL CULTURES ON *IN VITRO* ANTIBODY RESPONSIVENESS TO SRBC

Day drug added to cultures[a]	Antibody PFC response[b]			
	Metiamide		Cimetidine	
	10 μg	1000 μg	10 μg	1000 μg
Day 0	2830 ± 310	420 ± 65	2640 ± 230	320 ± 28
+1	2010 ± 240	970 ± 130	3100 ± 195	876 ± 140
+2	1560 ± 150	1130 ± 97	1840 ± 160	1430 ± 210
+3	1310 ± 97	1400 ± 145	1460 ± 128	1210 ± 185
+4	1410 ± 140	1370 ± 160	1230 ± 200	1330 ± 280

[a] 5×10^6 normal spleen cells immunized on Day 0 with 2×10^6 SRBC and treated on indicated day with either 10 or 1000 μg dose of metiamide or cimetidine.

[b] Average PFC response ±SE, for four to six cultures per group on Day 5 after *in vitro* immunization with SRBC; response of normal, untreated control cultures at same time was 1496 ± 230 PFCs.

agents, in excessive doses, depress the antibody response but do not alter the kinetics of the response. It should be noted, however, that the high dose of drug not only suppressed the antibody-forming ability of the spleen cells, but also resulted in an approximate 50–60% decrease in the number of viable cells recovered from the cultures on the fifth day of *in vitro* cultivation. Nevertheless the decrease in antibody-forming ability was greater than that which could be attributed only to the decrease in the number of viable nucleated spleen cells.

The depressive activity of the 1000-μg dose was most evident when the drug was added to cultures on the day of immunization. Little significant suppression occurred when the drugs were added 2 days or more after immunization. Enhancement occurred also when the drugs were added either on Days 0 or 1. However, this effect was generally lost when the drugs were added to cultures on Day 2 or later after *in vitro* immunization.

Studies are in progress to determine whether the immunomodulating effects of cimetidine or metiamide are mediated by reaction with adherent macrophages, T lymphocytes, or B cells. Earlier studies have revealed that H_2 receptors are present on regulatory T lymphocytes (1, 2). Thus, a most plausible target for the action of these drugs are such T cells. However, cocultures and cell separation studies, currently in progress, are necessary to understand the cellular site of action. Therefore, although it is not yet clear which cell class, if any, is preferentially affected, it appears clear from the present results that the H_2 antagonists cimetidine and metiamide affect not only hypersensitivity reactions such as histamine release and cellular cytotoxicity *in vitro,* but also modulate the primary *in vitro* immune response to sheep RBCs *in vitro*.

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