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HISTAMINE 2-RECEPTOR-MEDIATED IMMUNOMODULATION IN THE MOUSE. II. IMMUNOMODULATION BY THE H_2 ANTAGONIST METIAMIDE

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Abstract—Modulation of delayed hypersensitivity and antibody formation to sheep red cells by metiamide were studied in the mouse system. Depending on the time and dose of antigen and metiamide administration suppression or enhancement of the delayed hypersensitivity response was observed. The effects in this system did not differ from those reported for the H_2 agonist tolazoline, which were most probably mediated by suppressor cells. As far as the humoral response was concerned metiamide tended to stimulate the IgM response. Optimal stimulation was reached if 50 mg metiamide/kg was administered 3 days before immunization with 2×10^8 SRBC. Under all conditions tested the IgG response was unaffected. These results suggest antagonistic effects of metiamide for tolazoline and adduce further evidence for the presence of H_2 receptors on B cells. The IgM production per plasmacell was enhanced suggesting different H_2 receptors to be involved in differentiative and proliferative B cell responses. The possible consequences of H_2 antagonist application in human therapy are discussed.

To explain the different effects of histamine two kinds of receptors were postulated by Ash & Schild in 1966, designated H_1 and H_2. Classical antihistaminic drugs would be H_1 receptor antagonists. It was not until 1972, however, that the existence of H_2 receptors was definitively proven in the synthesis of the first H_2 antagonist burimamide (Black, Duncan, Durant, Ganellin & Parsons). Afterwards the analogues metiamide and cimetidine were developed (Galmiche, Colin, Al-Saati & Geoffroy, 1977). As cimetidine has the greatest therapeutic index (Durant, Emmett & Ganellin, 1977) this drug has been reserved for human use (Gill, 1978), mostly in the treatment of peptic ulcers.

In the study of the effects of histamine analogues on the immune system differential sensitivities of animal species to H_1 and H_2 effects of histamine have to be concerned. The mouse is rather insensitive to H_1 effects (Bergman, Milner & Munoz, 1977), whereas guinea-pigs and humans are comparably sensitive (Askenase, 1977a). Therefore the mouse seems to be the animal of choice for the study of H_2 effects.

As far as the immune system is concerned, subpopulations of murine, guinea-pig and human T lymphocytes were shown to bear H_2 receptors (Rocklin, 1976; Roszkowski, Plaut & Lichtenstein, 1977; Plaut & Berman, 1978). This was demonstrated in rosette formation of lymphocytes with histamine-linked sheep erythrocytes (Kedar & Bonavida, 1974) and in adhesion of cytotoxic (Shearer, Simpson, Weinstein & Melmon, 1977) and suppressor T cells (Shearer, Weinstein & Melmon, 1974) to histamine-linked Sepharose particles. *In vitro* experiments revealed that maturation (Singh & Owen, 1976) and functioning of murine T cells is under H_2 control (Plaut, Lichtenstein, Gillespie & Henney, 1973; Plaut, Lichtenstein & Henney, 1973). The importance of H_2 receptors for an *in vivo* immune response was demonstrated in the inhibition of a delayed hypersensitivity (DH) reaction in guinea pigs by histamine (Rocklin, 1976). Suppressor T cells seems to release a soluble factor which is responsible for this suppression (Rocklin, 1977; Rocklin, Greineder, Littman & Melmon, 1978). It is likely that also in the human system T cell functions are sensitive to H_2 effects of histamine (Ballet & Merler, 1976; Verhaegen, De Cock & De Cree, 1977; De Cock, De Cree & Verhaegen, 1977; Wang & Zweiman, 1978). There exists some controversion on the presence of histamine receptors on B cells (Wigzell, 1973; Melmon, Bourne, Weinstein, Shearer, Kram & Bauminger, 1974; Fallah, Maillard & Voisin, 1975; Ballet & Merler, 1976; Roszkowski *et al.*, 1977). Some effects on antibody formation can also be explained by stimulation of T suppressor cells. If present, H_2 receptors on B cells would quantitatively and functionally be less important.

In a recent paper we described the immunological effects of a H_2 receptor agonist (tolazoline; Yellin,

Sperow & Buck, 1975) in the mouse (Van Dijk, Rapis, Jacobse-Geels & Willers, 1979). Marked effects dependent on the time and dose of tolazoline administration were observed. Tolazoline has, however, also α-blocking effects, so that a combined study with a H_2 antagonist seems indicated. The present study, which is directed to possible immunomodulating effects of the H_2 antagonist metiamide, precedes this combined study. This study might moreover be of interest for the human situation as H_2 antagonists are administered to renal allograft patients to treat side-effects of glucocorticoids on the stomach. Some authors found indications for enhanced cellular inflammatory reactions as DH and allograft rejection in guinea pigs and man (Askenase, 1977a; Avella, Madsen, Binder & Askenase, 1978; Goodwin, 1978; Primack, 1978), whereas many others did not (McGregor, Ogg, Smith, Cochran, Gray & Gillespie, 1977; De Pauw, Lamers, Wagener & Festen, 1977; Jones, Askenase & Greaves, 1978; Doherty & McGeown, 1978; Rudge, Jones, Bewick, Weston & Parsons, 1978; Charpentier & Fries, 1978).

EXPERIMENTAL PROCEDURES

Animals

Swiss inbred mice were bred and maintained in the Laboratory of Microbiology, Utrecht, The Netherlands. Unless otherwise stated female mice were used at an age of 10–12 weeks. Mice were immunized by intraperitoneal (i.p.) injection of extensively washed sheep red blood cells (SRBC).

Delayed hypersensitivity

Immune mice received a subcutaneous (s.c.) eliciting injection of 1.25×10^8 SRBC suspended in 50 μl saline in the left hind footpad. The 24 h footpad swelling, measured semi-electronically (Van Dijk, Versteeg & Hennink, 1976), was used as parameter for DH.

Direct and indirect plaque forming cells.

The numbers of direct (19S) plaque forming cells (PFC) in the spleens of immune mice were determined according to Jerne & Nordin (1963) and expressed per 10^6 spleen cells. Indirect (IgG) plaque forming cells were developed by the adaption of Sterzl & Riha (1965). The rabbit anti-IgG serum required was prepared according to Zaalberg, Van der Meul & Van Twisk (1968) and used in a dilution of 1:240.

Haemolysin production

The amount of 19S (IgM) anti-SRBC antibodies formed *in vitro* by spleen cells in liquid medium was quantified as described previously (Van Dijk & Bloksma, 1977) and expressed in haemolysin units (HU50). One HU50 is the amount of antibodies required for 50% lysis of 5×10^8 SRBC in a total volume of 1.5 ml VSB^{++} (Mayer, 1961) in the presence of 20 μl of fresh guinea-pig serum, by incubation during 1 h at 37°C.

The haemolysin production per PFC was calculated by dividing the haemolysin production per 10^6 spleen cells of individual animals by the corresponding number of direct PFC per 10^6 spleen cells.

Statistical analysis

Results have been expressed as the arithmetic mean of n independent observations±standard error of the mean (s.e.m.) Unless otherwise stated the two-sided t-test of Student was performed to analyse the statistical significance of the results. Values of $P>0.05$ were considered to be not significant (N.S.)

Materials

Antigen. SRBC stored in Alsever's old solution were obtained from the National Institute of Public Health (RIV, Bilthoven, The Netherlands).

Metiamide. Metiamide was kindly provided by Dr. L. P. Mensinga (Smith Kline & French B.V., Rijswijk, The Netherlands). Each week a fresh stock-solution was prepared by dissolving metiamide in 1N HCl and adjusting the pH to 6.0 with 0.1 N NaOH. Mice were i.p. injected with a dilution of the stock-solution in saline.

RESULTS

Direct toxicity of metiamide

To investigate the sensitivity of the Swiss strain to metiamide, groups of male and female mice received i.v. injections with increasing doses of the drug in 0.5 ml saline. Intraperitoneal toxicity was only tested in male animals. The animals were daily inspected over a period of 8 weeks (Table 1). The i.v. LD_{50} both in males and in females amounted between 80 and 100 mg/kg, but females tended to be somewhat more sensitive. Intraperitoneal gifts up to 350 mg/kg were not lethal. In survivors no long-term toxicity was observed.

Effects on delayed hypersensitivity

Mice were i.p. immunized with 10^6 SRBC on Day 0 and treated with 20, 50 or 80 mg metiamide/kg body weight on Day -3, -1, 0, $+1$, or $+5$. Controls received saline instead of metiamide. On Day 5 the animals were challenged for DH and 24 h later the reaction was measured. In all cases the dose of 80 mg/kg was the most effective (data not given). Using this dose the effects of metiamide in relation to

the day of its administration and the dose of SRBC used for immunization were studied (Fig. 1). When administered 3 days before immunization, metiamide did not influence the response to numbers of SRBC below 10^9. The response to 10^9 SRBC was significantly enhanced. If metiamide was given on Day -1, the responses to 10^5 and to 10^6 SRBC were non-significantly decreased, whereas the reaction to 10^8 SRBC was higher than in the control animals. After metiamide treatment on Day 0 and $+1$ the response was in general impaired with a minimum for the immunizing dose of 10^6 SRBC, whereas the reaction to the same number of SRBC after metiamide administration on Day $+5$ was significantly increased.

Table 1. Direct toxicity of metiamide

	Route of metiamide administration					
	Intravenously				Intraperitoneally	
Dose of metiamide (mg/kg body weight)	♂ Survival* (%)	*n*	♀ Survival (%)	*n*	♂ Survival (%)	*n*
20	100	3				
50	100	6				
80	100	9	100	3		
100	39†	18	0†	6	100	6
120	11†	9	0†	3	100	6
200	0†	6			100	6
250					100	6
300					100	6
350					100	6

* Survival over an 8 weeks period was followed.
† Death occurred within a few minutes after injection.

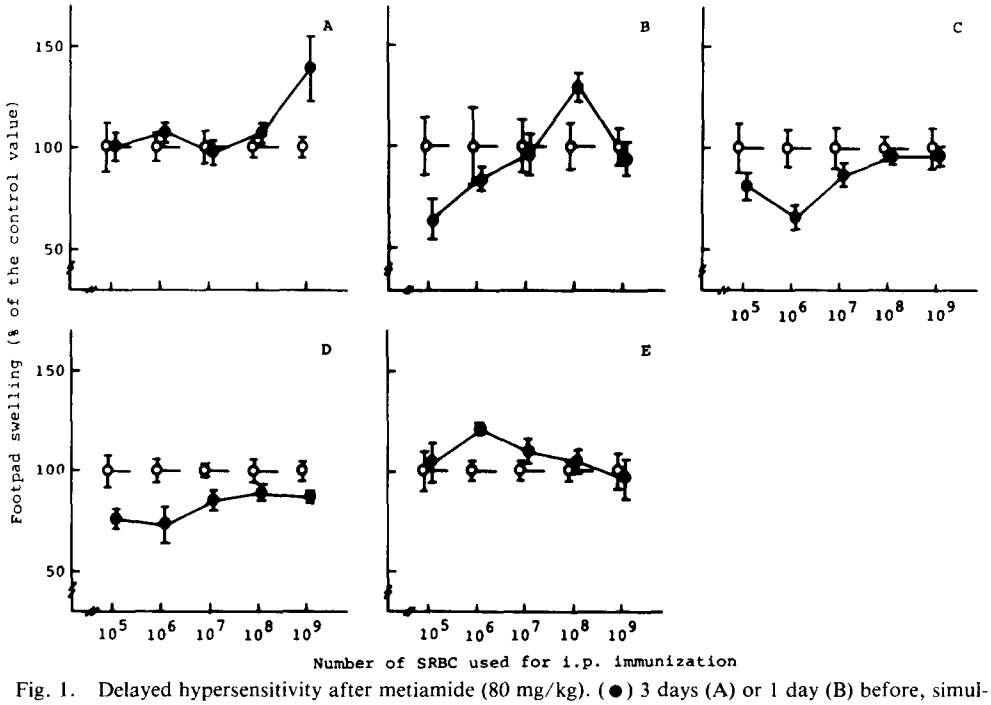

Fig. 1. Delayed hypersensitivity after metiamide (80 mg/kg). (●) 3 days (A) or 1 day (B) before, simultaneously with (C) or 1 (D) or 5 days (E) after i.p. immunization with graded numbers of SRBC. Controls (○) received saline instead of metiamide. On Day 5 the mice ($n = 7$) were challenged and 24 h later footpad swelling was measured. Vertical bars indicate the s.e.m. The enhancement for 10^9 SRBC in A, for 10^8 SRBC in B, and for 10^6 SRBC in E is significant ($P<0.05$, $P<0.05$ and $P<0.001$ respectively). The decrease for 10^6 SRBC in C, and for 10^5, 10^6 and 10^7 SRBC in D is significant ($P<0.001$ and thrice $P<0.05$ respectively). The decrease for 10^5 SRBC in B is not significant.

Effects on the plaque-forming cell response

Male mice were treated with 20, 50 or 80 mg of metiamide/kg or saline on Day −1, 0, or +4 and immunized i.p. on Day 0 with 2×10^8 SRBC which is the optimal number for a direct PFC response. Day 5 the direct splenic PFC response was measured. In general an enhancement of the response was observed (Fig. 2) with an exception for the dose of 80 mg metiamide/kg administered on Day 4. The dose of 50 mg/kg appeared the most discriminative and was used in further experiments.

In the next experiment metiamide (50 mg/kg) was administered 1 or 3 days before, simultaneously with, or 1 or 4 days after immunization of male mice with 2×10^8 SRBC. The direct (IgM) and IgG splenic PFC responses were measured 5 days after immunization. Figure 3 shows that administration of metiamide on Day 3 before immunization resulted in a significant enhancement of the IgM response, whereas none of the treatments influenced the IgG response. Because of these results administration of metiamide (50 mg/kg) on Day −3 was chosen for the study of the influence on the antibody response to graded numbers of SRBC. Figure 4 indicates that in that case only the IgM response to 10^8 SRBC was significantly enhanced. The IgM response to other antigen concentrations and the IgG response to all numbers of SRBC tested remained unaltered.

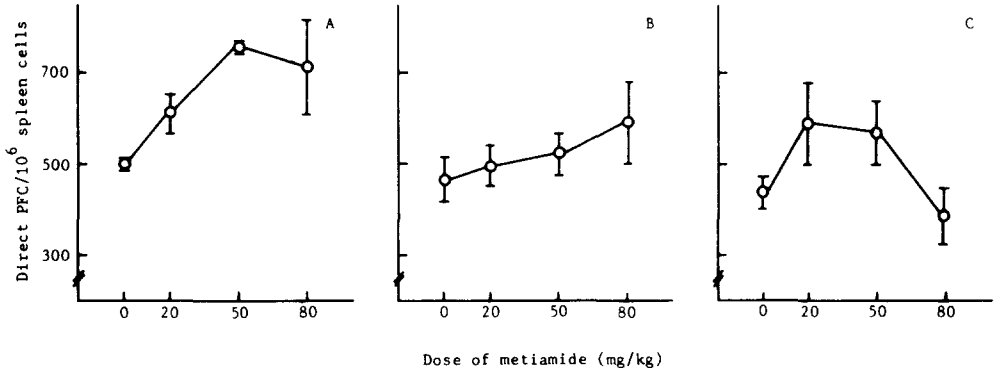

Fig. 2. Effect on antibody formation of different doses of metiamide administered i.p. 1 day before (A), simultaneously with (B), or 4 days after (C) i.p. immunization of mice ($n=5$) with 2×10^8 SRBC. Vertical bars indicate the s.e.m. The increases for 20 and 50 mg metiamide/kg administered Day −1 are significant ($P<0.025$ and 0.0001 respectively).

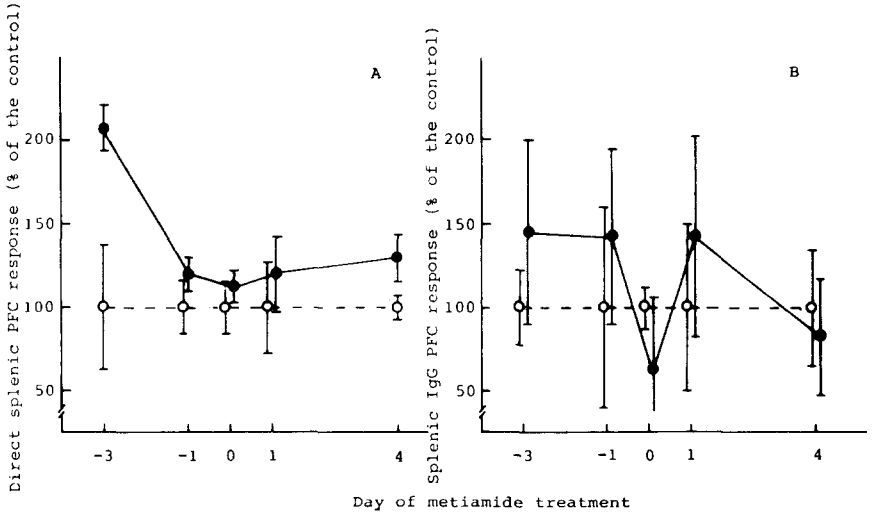

Fig. 3. Effect on the direct (A) and IgG (B) splenic PFC response of metiamide (50 mg/kg), (●) treatment on different days before or after i.p. immunization with 2×10^8 SRBC. Controls (○) received saline instead of metiamide. Vertical bars indicate the s.e.m. Only the increase of the IgM response after metiamide administration on Day −3 is significant ($P<0.025$).

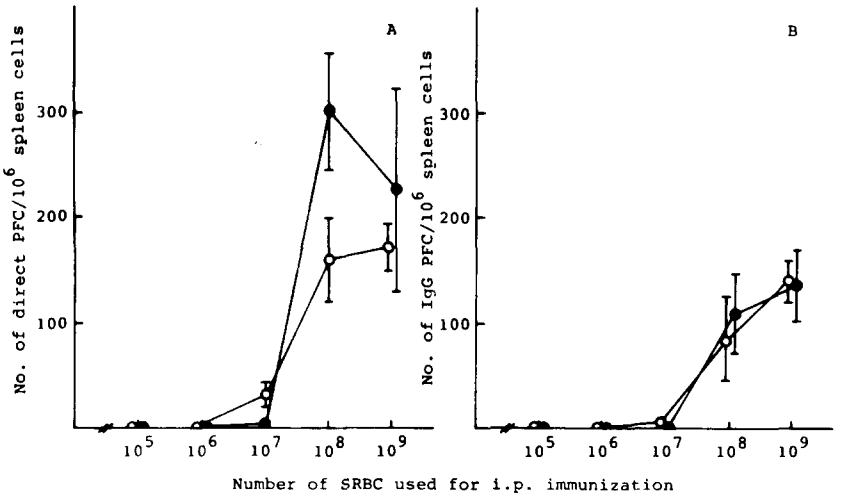

Fig. 4. Effect on the direct (A) and IgG (B) splenic PFC response of metiamide (50 mg/kg) (●) administration 3 days before i.p. immunization with decimally increasing numbers of SRBC. Control mice (○) received saline instead of metiamide. Vertical bars indicate the s.e.m. ($n = 5$). Only the difference observed for the IgM response to 10^8 SRBC is significant ($P < 0.05$).

Table 2. Influence of metiamide on antibody production*

	Treatment		
	Metiamide	Saline	*P*-value
Number of IgM PFC/10^7 spleen cells	3391±507† (59)‡	2137±254	<0.05 (single-sided)
Haemolysin production/10^7 spleen cells§	3.18±0.48 (172)	1.17±0.17	<0.01
Haemolysin production/IgM PFC§	$(9.65 \pm 1.32) \times 10^{-4}$ (76)	$(5.48 \pm 0.56) \times 10^{-4}$	<0.025

* Mice were injected with metiamide (50 mg/kg) or saline (0.5 ml) 3 days before i.p. immunization with 10^8 SRBC. The spleens were removed on Day 5 after immunization and tested for the number of IgM PFC and the amount of 19S antibodies (haemolysin production) secreted.
† Mean±s.e.m. ($n = 5$).
‡ Percentage enhancement of the response.
§ Expressed in haemolysin units (HU50's).

Effects on antibody production

Administration of 50 mg metiamide/kg on Day 3 before immunization with 10^8 SRBC was also used to study a possible effect on the splenic antibody production and the antibody production per plasmacell. Day 5 after immunization the IgM production per 10^6 spleen cells and per single PFC was determined with a haemolysis in liquid procedure (Van Dijk & Bloksma, 1977) in combination with the Jerne plaque assay. Table 2 shows that besides enhancing the number of PFC metiamide stimulated the splenic haemolysin production even more, which is also demonstrated in an increased amount of antibody produced per PFC.

DISCUSSION

In this study the possible immunomodulating properties of the H_2 antagonist metiamide were investigated in the mouse system. Introductory experiments suggested an acute i.v. LD_{50} of between 80–100 mg/kg both in males and in females and the absence of longterm effects after one metiamide injection.

The LD_{50} in our Swiss mouse strain was lower than that of 141 mg/kg as reported for mice of a Charles-River strain (Brimblecombe, Duncan & Walker, 1973). Respiratory failure was mentioned as most probable cause of acute death. Intraperitoneal administration, which appeared far less toxic, was chosen for the study of the effects on the immune response.

The effects of metiamide on DH were very similar to those of tolazoline (Fig. 1; Van Dijk *et al.*, 1979). This suggests that the effects of tolazoline on the cellular response were most probably H_2 charactered, although α-adrenergic effects cannot be excluded at this moment. In view of literature data targets of metiamide and tolazoline action were most likely suppressor cells. It might be that they gain an altered antigen-sensitivity (Weitzman, Shen & Cantor, 1976; Rocklin, 1976; Askenase, 1977a; Avella *et al.*, 1978; Van Dijk *et al.*, 1979). The similarity of the effects of tolazoline and metiamide suggest that both drugs behave as (ant)agonist for the same receptor in this *in vivo* test: biotransformation of metiamide might result in metabolites with H_2 agonist activity or tolazoline might compete with a more potent physiological agonist for H_2 receptor sites on lymphocytes. The suppression or enhancement of the cellular response dependent on the time of metiamide administration and on the immunizing dose might explain some discrepancies in literature concerning H_2 antagonist effects on DH, acquired tolerance to dinitrochlorbenzene, and renal allograft rejection (Askenase, 1977b; Daman & Rosenberg, 1977; McGregor *et al.*,1977; De Pauw *et al.*, 1977; Avella *et al.*, 1978; Goodwin, 1978; Jones *et al.*, 1978; Primack, 1978; Doherty & McGeown, 1978; Rudge, 1978; Charpentier & Fries, 1978).

In the humoral response the effects of metiamide deviated from those of tolazoline, suggesting different target cells of H_2 directed action in the humoral and cellular response. In general, the IgM response was stimulated, whereas the IgG response remained unaffected (Figs. 2–4), whereas tolazoline depressed the IgM and stimulated the IgG response (Van Dijk *et al.*, 1979). The combination of these observation supports the earlier proposed idea of a H_2 control of the shift from IgM→IgG synthesis. The location of the H_2 receptor would rather be the membrane of a B cell than that of some regulatory cell. The enhancement of the IgM production per plasma cell observed for both metiamide (Table 2) and tolazoline (Van Dijk *et al.*, 1979) indicate that this phenomenon is mediated by distinct receptors possibly presented on some regulatory cell.

In conclusion our results indicate that metiamide as representative of a number of drugs with H_2 antagonist properties influences the immune response. Dependent on the time and dose of both antigen and metiamide administration a suppression or an enhancement of DH was observed. In general, the humoral response was stimulated as far as the IgM response was concerned, whereas the IgG response was unaffected. These side-effects of a H_2 antagonist on immune reactions might be important in the application of similar drugs in the treatment of peptic ulcers accompanying glucocorticoid therapy in renal transplantation. Further study will be directed to possible immunomodulating interreactions of H_2 agonists and antagonists in the mouse.

REFERENCES

ASH, A. S. F. & SCHILD, H. O. (1966). Receptors mediating some actions of histamine. *Br. J. Pharmac. Chemother.*, **27**, 427–439.

ASKENASE, P. W. (1977a). Role of basophils, mast cells, and vasoamines in hypersensitivity reactions with a delayed time course. *Prog. Allergy*, **23**, 199–329.

ASKENASE, P. W. (1977b). Histamine 2-antagonist inhibition of delayed hypersensitivity (DH). *Clin. Res.*, **25**, 481–492.

AVELLA, J., MADSEN, J. E., BINDER, H. J. & ASKENASE, P. W. (1978). Effect of histamine H_2-receptor antagonists on delayed hypersensitivity. *Lancet*, **i**, 624–626.

BALLET, J. J. & MERLER, E. (1976). The separation and reactivity *in vitro* of a subpopulation of human lymphocytes which bind histamine. Correlation of histamine reactivity with cellular maturation. *Cell. Immun.* **24**, 250–269.

BERGMAN, R. K., MILNER, K. C. & MUNOZ, J. J. (1977). New test for endotoxin potency based upon histamine sensitization in mice. *Infec. Immun.*, **18**, 352–355.

BLACK, J. W., DUNCAN, W. A. M., DURANT, C. J., GANELLIN, C. R. & PARSONS, E. M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature, Lond.*, **236**, 385–390.

BRIMBLECOMBE, R. W., DUNCAN, W. A. M. & WALKER, T. F. (1973). Toxicology of metiamide. In *International Symposium on Histamine H_2-receptor Antagonists.* (eds. WOOD, C. J. & SIMKINS, M. A.), pp. 53–69. Smith Kline & French Laboratories Ltd. Welwyn Garden City, England.

CHARPENTIER, B. & FRIES, D. (1978). Cimetidine and renal-allograft rejection. *Lancet*, **i**, 1265.

DE COCK, W., DE CREE, J. & VERHAEGEN, H. (1977). Restoration by levamisole of histamine-inhibited E rosette formation of T lymphocytes of patients with allergies. *Int. Archs Allergy appl. Immun.*, **54**, 176–182.

DAMAN, L. A. & ROSENBERG, E. W. (1977). Acquired tolerance to dinitrochlorobenzene reversed by cimetidine. *Lancet,* **ii,** 1087.

VAN DIJK, H. & BLOKSMA, N. (1977). Quantification of *in vitro* antibody secretion by immune spleen cells. *J. Immun. Meth.,* **14,** 325–331.

VAN DIJK, H., RAPIS, M., JACOBSE-GEELS, H. E. L. & WILLERS, J. M. N. (1979). Histamine 2-receptor mediated immunomodulation in the mouse. I. Immunomodulation by the H_2 agonist tolazoline. *Clin. exp. Immun.,* **35,** 470–477.

VAN DIJK, H., VERSTEEG, H. & HENNINK, H. J. (1976). A semi-electronic device for measuring delayed hypersensitivity in the mouse by footpad swelling. *J. Immun. Meth.,* **12,** 261–265.

DOHERTY, C. C. & MCGEOWN, M. G. (1978). Cimetidine and renal-allograft rejection. *Lancet,* **i,** 1048.

DURANT, G. J., EMMETT, J. C. & GANELLIN, C. R. (1977). The chemical origin and properties of histamine H_2-receptor antagonists. In *Cimetidine V,* Proceedings of the second international symposium on histamine H_2-receptor antagonists, (eds. BURLAND, W. L. & SIMKINS, M. A.), pp. 1–12. Experta Medica, Amsterdam.

FALLAH, H. A., MAILLARD, J. L. & VOISIN, G. A. (1975). Regulatory mast cells. I. Suppressive action of their products on an *in vitro* primary immune reaction. *Ann. Immun. (Inst. Pasteur)* **126C,** 669–682.

GALMICHE, J. P., COLIN, R., AL-SAATI, M. N. & GEFFROY, Y. (1977). Effect of cimetidine on pancreatic exocrine secretion. *Lancet,* **i,** 647.

GILL, G. V. (1978). Cimetidine overdose. *Lancet,* **i,** 99.

GOODWIN, J. S. (1978). Cimetidine and delayed hypersensitivity. *Lancet,* **i,** 934.

JERNE, N. K. & NORDIN, A. A. (1963). Plaque formation in agar by single antibody producing cells. *Science,* **140,** 405.

JONES, F. G. C., ASKENASE, P. W. & GREAVES, M. W. (1978). Cimetidine and the delayed-hypersensitivity response. *Lancet,* **i,** 880.

KEDAR, E. & BONAVIDA, B. (1974). Histamine receptor-bearing leukocytes. (HRL). I. Detection of histamine receptor bearing cells by rosette formation with histamine-coated erythrocytes. *J. Immun.* **113,** 1544–1552.

MAYER, M. M. (1961). *Experimental Immunochemistry,* (eds. KABAT, E. A. & MAYER, M. M.) 2nd edn. p. 149. Thomas Publishers, Springfield, Illinois, U.S.A.

MCGREGOR, C. G. A., OGG, L. J., SMITH, I. S., COCHRAN, A. J., GRAY, G. R. & GILLESPIE, G. (1977). Immunological and other laboratory studies of patients receiving short-term cimetidine therapy. *Lancet,* **i,** 122–123.

MELMON, K. L., BOURNE, H. R., WEINSTEIN, Y., SHEARER, G. M., KRAM, J. & BAUMINGER, S. (1974). Hemolytic plaque formation by leukocytes *in vitro*. Control by vasoactive hormones. *J. clin. Invest.,* **53,** 13–21.

DE PAUW, B. E., LAMERS, C. B. H. W., WAGENER, D. J. T. & FESTEN, H. P. M. (1977). Immunological studies after long-term H_2-receptor antagonist therapy. *Lancet,* **ii,** 616.

PLAUT, M. & BERMAN, I. J. (1978). Histamine receptors on human and mouse lymphocytes. *J. Allergy clin. Immun.,* **61,** 132–133.

PLAUT, M., LICHTENSTEIN, L. M., GILLESPIE, E. & HENNEY, C. S. (1973). Studies on the mechanism of lymphocyte-mediated cytolysis. IV. Specificity of the histamine receptor on effector T cells. *J. Immun.,* **111,** 389–394.

PLAUT, M., LICHTENSTEIN, L. M. & HENNEY, C. S. (1973). Increase in histamine receptors on thymus derived effector lymphocytes during the primary immune response to alloantigens. *Nature, Lond.,* **244,** 284–286.

PRIMACK, W. A. (1978). Cimetidine and renal-allograft rejection. *Lancet,* **i,** 824–825.

ROCKLIN, R. E. (1976). Modulation of cellular immune responses *in vivo* and *in vitro* by histamine receptor bearing lymphocytes. *J. clin. Invest.,* **57,** 1051–1058.

ROCKLIN, R. E. (1977). Histamine-induced suppressor factor (HSF): Effect on migration inhibitory factor (MIF) production and proliferation. *J. Immun.,* **118,** 1734–1738.

ROCKLIN, R. E., GREINEDER, D., LITTMAN, B. H. & MELMON, K. L. (1978). Modulation of cellular immune function *in vitro* by histamine receptor-bearing lymphocytes: Mechanism of action. *Cell. Immun.,* **37,** 162–173.

ROSZKOWSKI, W., PLAUT, M. & LICHTENSTEIN, L. M. (1977). Selective display of histamine receptors on lymphocytes. *Science,* **195,** 683–685.

RUDGE, C. J., JONES, R. H., BEWICK, M., WESTON, M. J. & PARSONS, V. (1978). Cimetidine and renal-allograft rejection. *Lancet,* **i,** 1154.

SHEARER, G. M., SIMPSON, E., WEINSTEIN, Y. & MELMON, K. L. (1977). Fractionation of lymphocytes involved in the generation of cell-mediated cytotoxicity over insolubilized conjugated histamine columns. *J. Immun.,* **118,** 756–761.

SHEARER, G. M., WEINSTEIN, Y. & MELMON, K. L. (1974). Enhancement of immune response potential of mouse lymphoid cells fractionated over insolubilized conjugated histamine columns. *J. Immun.,* **113,** 597–607.

SINGH, U. & OWEN, J. J. T. (1976). Studies on the maturation of thymus stem cells. The effects of catecholamines, histamine and peptide hormones on the expression of T cell alloantigens. *Eur. J. Immun.,* **6,** 59–62.

STERZL, I. & RIHA, I. (1965). A localized haemolysis in gel method for the detection of cells producing 7S antibody. *Nature, Lond.,* **208,** 858–859.

VERHAEGEN, H., DE COCK, W. & DE CREE, J. (1977). Histamine-receptor bearing peripheral T lymphocytes in patients with allergies. *J. Allergy clin. Immun.,* **59,** 266–268.

WANG, S. R. & ZWEIMAN, B. (1978). Histamine suppression of human lymphocyte responses to mitogens. *Cell. Immun.,* **36,** 28–36.

WEITZMAN, S., SHEN, F. W. & CANTOR, H. (1976). Maintenance of hypo-responsiveness to antigen by a distinct subclass of T lymphocytes. *J. Immun.*, **117**, 2209–2212.

WIGZELL, H. (1973). The functional significance of surface structures on lymphocytes. *Ann. Immun. (Inst. Pasteur)*, **124C**, 7–15.

YELLIN, T. O., SPEROW, J. W. & BUCK, S. H. (1975). Antagonism of tolazoline by histamine H_2-receptor blockers. *Nature, Lond.*, **253**, 561–563.

ZAALBERG, O. B., VAN DER MEUL, V. A. & VAN TWISK, M. J. (1968). Antibody production by isolated spleen cells. A study of the cluster and plaque techniques. *J. Immun.*, **100**, 451–458.