*al.*, 1977), this result demonstrates its action on T helper cells.

In kinetic experiments using cell numbers as the parameter, CS-A (0.1 μg/ml) was added to mouse spleen cell cultures either at the beginning of or 48 h after onset of stimulation by Con A. The drug affected lymphoid cell proliferation only at an early stage of mitogenic triggering and did not interfere with blast cells since no inhibition was observed after addition at 48 h. Inhibition of cell transformation was not due to a cytolytic action of CS-A as no significant differences in cell number nor in percentage of dye exclusion were observed between drug-treated, stimulated and non-treated, unstimulated control cultures.

The reversibility of the inhibitory effect of CS-A was studied using the same *in vitro* system. To a mouse spleen cell culture 0.1 μg/ml of the compound was added for 1 hour. The culture was then washed to remove the drug and Con A added for the next 72 h. The proliferation as measured by cell number was inhibited by 65% of control value compared to 87% in cultures containing the drug during the full incubation period. In another experiment CS-A was washed out after 1 h and the cells were incubated 24 h without drug before Con A was added for the last 72 h. Now the inhibition was only 22% of control value, thereby indicating that the effect of the compound was partly reversible.

Using biochemical assays the content of RNA, DNA and protein from drug-treated and Con A stimulated cultures were shown to be equal to those measured in unstimulated controls. It was further demonstrated that the incorporation of the tritium-labelled precursors (uridine, thymidine and leucine) was strongly inhibited.

These results led us to conclude that CS-A totally inhibits Con A stimulation of resting T cells. It seems to interfere at the very early stage of the cell cycle and does not affect the lymphocyte once it has been triggered.

**References**


BOREL, J.F. & WIESINGER, D. (1977). Effect of cyclosporin A on murine lymphoid cells. *In: Regulatory Mechanisms in Lymphocyte Activation.* (Ed. by D.O. Lucas), 716–718 (Academic Press, New York).

BOREL, J.F., FEURER, C., MAGNÉE, C. & STÄHELIN, H. (1977). Effects of the new anti-lymphocytic peptide cyclosporin A in animals. *Immunology*, **32**, 1017–1025.

MISHELL, R.I. & DUTTON, R.W. (1966). Immunisation of normal mouse spleen cell suspensions *in vitro*. *Science*, **153**, 1004–1006.


## The effect of several immunomodulating agents on a model of humoral and cell mediated immunity in the mouse


J. BRUIN, J. COTTNEY, D.K. GEMMEL & A.J. LEWIS

*Scientific Development Group, Organon Laboratories Ltd, Newhouse, Lanarkshire, ML1 5SH*


Pharmacological modulation of the humoral (antibody mediated) and cell mediated immune response is complicated by numerous factors that include species, timing and dose of drug administration, antigen, etc. (Heppner & Calabresi, 1976). In attempts to affect selectively the different limbs of the immune system we decided to examine the effects of single doses of a variety of drugs in a combined humoral and cell mediated model of immunity in the mouse. These studies were prompted by the observations that drugs such as cyclophosphamide could enhance the cellular response when administered prior to antigen sensitization (Turk & Poulter, 1972; Turk, Parker & Poulter, 1972; Kerkhaert, van den Berg & Willers, 1974; Lagrange, Mackaness & Miller, 1974).

Male mice (CFLP strain, 30–45 g) were used in groups of 8 for these experiments. Drugs or drug vehicle (5% mulgofen in distilled water) were administered i.p. on the day prior to antigen sensitization. The mice were sensitized s.c. with 0.1 ml of a 0.125% emulsion of methylated bovine serum albumin (MBSA) in Freund's complete adjuvant to elicit a cell mediated immune response and i.p with a suspension of $10^8$ sheep red blood cells to produce a humoral immune response. Seven days after sensitization one hind paw of each animal received 0.05 ml of 0.05% MBSA in saline and the contralateral paw received saline alone. Hind paw thickness was measured 24 h after challenge and the mean change in paw thickness between MBSA and saline injected paws was calculated. Blood samples were obtained from the retro-orbital plexus immediately after the hind paw measurements and the serum from individual mice was used to measure haemagglutinating antibody titres. Vehicle and drug treated groups were compared using the Student's *t*-test and Mann Whitney U-test for the cell mediated immune response and humoral immune response, respectively.

Of the range of drugs examined only the nitrogen mustard type alkylating agents such as cyclophosphamide (200 mg/kg), chlorambucil (30 mg/kg) and melphalan (5 mg/kg) enhanced the cell mediated immune response. A number of other immunomodulating agents such as cycloleucine (300 mg/kg), azathioprine (250 mg/kg), levamisole (30 mg/kg), methotrexate (100 mg/kg), oxisuran (300 mg/mg) and procarbazine (300 mg/kg), and anti-inflammatory drugs such as indomethacin (10 mg/kg) and prednisolone (30 mg/kg) were inactive. Only

cyclophosphamide suppressed the antibody production whereas oxisuran and prednisolone slightly enhanced the humoral response.

In conclusion, using two different antigens in the mouse we have produced a model that is capable of detecting drugs that modulate either limb of the immune system. This model may be of use in the discovery of new immunomodulator agents.

**References**


HEPPNER, G.H. & CALABRESI, P. (1976). Selective suppression of humoral immunity by antineoplastic drugs. *Ann. Rev. Pharmac. Toxicol.*, **16**, 367–379.

KERKHAERT, J.A.M., VAN DEN BERG, G.J. & WILLERS, J.M.N. (1974). Influence of cyclophosphamide on the delayed hypersensitivity of the mouse. *Ann. Immunol.*, **125C**, 415–426.

LAGRANGE, P.H., MACKANESS, G.B. & MILLER, T.E. (1974). Potentiation of T-cell mediated immunity by selective suppression of antibody formation with cyclophosphamide. *J. exp. Med.*, **139**, 1529–1539.

TURK, J.L., PARKER, D. & POULTER, L.W. (1972). Functional aspects of the selective depletion of lymphoid cells of cyclophosphamide. *Immunology*, **23**, 493–501.

TURK, J.L. & POULTER, L.W. (1972). Selective depletion of lymphoid tissue by cyclophosphamide. *Clin. exp. Immunol.*, **10**, 285–296.


## Histamine $H_1$ antagonists and histamine release from human lung *in vitro*


M.K. CHURCH & CAROLYN F. GRADIDGE

*Clinical Pharmacology Group, Faculty of Medicine, University of Southampton, Centre Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY*


Lichtenstein & Gillespie (1975) reported that histamine $H_1$ antagonists inhibited antigenic histamine release from human leucocytes at low concentrations whereas at higher concentrations they caused histamine release in the absence of antigen. We have examined the activity of representatives of the five major classes of antihistamines (Douglas, 1970) in passively sensitized human lung *in vitro*.

Surgical specimens of human lung were chopped finely, divided into replicates of approximately 200 mg and sensitized for 18 h at room temperature and 1 h at 37°C in 2 ml of Tyrode's solution containing 0.2 ml of serum from an allergic donor. In experiments on the inhibition of antigen induced histamine release, antihistamines ($10^{-10}$–$10^{-3}$g/ml) were added 30 s before antigen (1/1000 dilution of anti-IgE – Miles Yeda) and the tissue incubated for 15 min. To assess histamine released by antihistamines alone, antigen was omitted. Histamine release was expressed as a percentage of the total histamine content of each lung sample.

Mepyramine, an ethylenediamine, inhibited antigen induced histamine release only at the highest concentrations used, $10^{-4}$ and $10^{-3}$ g/ml. It did not release histamine. Similarly, the alkylamine derivative chlorpheniramine, was a weak inhibitor of antigen induced histamine release and only caused release of small amounts of histamine at $10^{-4}$ and $10^{-3}$ g/ml. Diphenhydramine and cyclizine, ethanolamine and piperazine derivatives respectively, were approximately equiactive. Both inhibited antigen induced histamine release by approximately 50% at $10^{-6}$ g/ml and caused histamine release at higher concentrations. The most active antihistamine tested was the phenothiazine derivative, promethazine, which inhibited antigen induced histamine release by 70% at $1 \times 10^{-6}$ g/ml. Above this concentration promethazine caused histamine release both in the presence and absence of antigen.

Because of the pharmacological relationships between phenothiazine antihistamines and central nervous depressant drugs, other compounds used primarily for their central effects were tested. The phenothiazine major tranquillizers, chlorpromazine and trimeprazine, and the tricyclic antidepressant, amitriptyline, were marginally more active than promethazine both in inhibiting antigen induced histamine release and releasing histamine. The monoamine oxidase inhibitor, phenelzine, was approximately equiactive with promethazine. The results obtained with these compounds correlate quite closely with similar results obtained using human leucocytes (Lichtenstein & Gillespie, 1975). It is concluded that the ability of these drugs to inhibit antigen induced histamine release is not due to their interaction with classical histamine $H_1$ receptors. An investigation of their mechanism of action is proceeding.

**References**


DOUGLAS, W.W. (1970). Histamine and antihistamines. In *The Pharmacological Basis of Therapeutics*, 4th edn. Ed. Goodman, L.S. & Gilman, A. Macmillan, N.Y. P. 636.

LICHTENSTEIN, L.M. & GILLESPIE, E. (1975). The effects of $H_1$ and $H_2$ antihistamines on allergic histamine release and its inhibition by histamine. *J. Pharmac. exp. Ther.*, **192**, 441–450.