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CONCANAVALIN A, A MODEL 'ANTIGEN' FOR THE *IN VITRO* DETECTION OF CELL-BOUND REAGINIC ANTIBODY IN THE RAT

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SUMMARY

Incubation of sensitized peritoneal or pleural cells, or isolated mast cells, taken from rats previously infected with the nematode, *Nippostrongylus brasiliensis*, but not of cells taken from parasite-free controls, with concanavalin A *in vitro* induces the release of histamine. The release mechanism triggered by Con A is virtually completed within 60 sec, depends on temperature, but not on the presence of complement, and is inhibited by sympathomimetic amines. It therefore shows striking similarities to the anaphylactic reaction induced by the specific allergen.

The mechanism by which histamine release is triggered by Con A is discussed, considering the observations that trypsinized Con A is no longer able to induce histamine release, that the presence of high concentrations of trypsinized Con A inhibits the release of histamine by subsequently added Con A or allergen, and that certain carbohydrates inhibit the release of histamine induced by both Con A and allergen. Although some of the data suggest a similarity in the structure of the antigenic determinant in the Fab fragment of IgE and the receptor site for Con A, it seems more likely that Con A is reacting mainly by cross-linking of Fc regions of the immunoglobulin molecule.

INTRODUCTION

The understanding of the manner in which human immunoglobulin E (IgE) or IgE-type antibodies of other species interact with target cells, for example tissue mast cells (Mota, 1963), and of the mechanism of interaction between cell-bound reaginic antibody and the allergen has made exciting advances in the last few years (reviewed by Stanworth, 1970, 1971; Ishizaka & Ishizaka, 1970). It has recently been demonstrated that the rat infected with the nematode, *Nippostrongylus brasiliensis*, is a good experimental model for the study of the reaginic antibody response (Ogilvie, 1964; Keller, 1970a, b). The present paper reports that concanavalin A (Con A), a phytagglutinin or lectin isolated from jack bean

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meal (Sumner & Howell, 1936), when added *in vitro* to cells taken from rats which had been infected with *N. brasiliensis*, triggers a release mechanism which is similar to that elicited by the specific worm allergen. Cells taken from untreated, parasite-free rats do not give this reaction with Con A.

MATERIAL AND METHODS

Rats

Colony-bred Osborne-Mendel or CNRS rats with an initial weight between 180 and 220 g were used.

Parasite

Methods for maintenance and recovery of the nematode, *Nippostrongylus brasiliensis*, for counting eggs in rat faeces, for estimation of allergen and for immunization of animals were as described previously (Keller, 1970a).

Allergen was obtained by collecting the supernatant fluid of freshly harvested, washed *N. brasiliensis* adult worms incubated for 60 min at 37°C in physiological saline.

Cells from the peritoneal or pleural cavities of sensitized rats or untreated controls were obtained by irrigating the cavities with phosphate-buffered isotonic solution (pH 7·60) supplemented with 0·1 mg human serum albumin (Blutspendedienst SRK, Bern) instead of serum (Keller & Beeger, 1963) and by sucking off the fluid after gentle massage for 2 min. Sensitized cells were taken either 30 days after a primary or 5–10 days after a fourth infection with *N. brasiliensis* larvae (Keller, 1970a). In most experiments, the total yield of peritoneal or pleural cells from several animals was pooled, washed and aliquots of cells containing 60,000–100,000 mast cells were incubated at 37°C for various time intervals with different concentrations of worm allergen or of Con A (Sigma). In some experiments, the peritoneal mast cells were separated by centrifugation over 50% bovine serum albumin (Behringwerke, Marburg/Lahn), processed as previously described (Keller, 1970b; Keller & Beeger, 1963), and incubated with worm allergen or Con A.

Histamine was assayed biologically on the guinea-pig ileum (Keller, 1970b), the percentage released in comparison to controls calculated (Keller *et al.*, 1968) and corrected for spontaneous release of 1–3%. In the experiments with sympathomimetic amines, isoprenaline (Isuprel Winthrop) or adrenaline (Fluka AG, Buchs SG) were added to sensitized cells at the same time as worm allergen or Con A, using the special precautions taken to avoid artefacts given by Assem & Schild (1969). Carbohydrates (Fluka AG, Buchs SG) were added to the cell suspension briefly before worm allergen or Con A.

Trypsinized Con A was obtained by tryptic digestion (Trypsin, Fluka AG, Buchs SG) of Con A as described by Burger & Noonan (1970), and the reaction was stopped by the addition of 1·0% soybean trypsin inhibitor (Sigma).

RESULTS

Release of histamine by Con A from sensitized rat cells in vitro

When peritoneal or pleural cells or isolated peritoneal mast cells taken from nematode-infested rats were incubated *in vitro* for 10 min at 37°C with the nematode allergen or Con A, each agent released a similar, significant percentage of total histamine (Table 1). On the

TABLE 1. The percentage of total histamine released from rat pleural cells by different concentrations of worm allergen, untreated or trypsinized concanavalin A (incubation 10 min at 37°C)

	Worm allergen (worm equivalents/ml)			Untreated Con A (μg/ml)					Trypsinized Con A (μg/ml)		
Concentration of agents	10	50	100	1	5	10	50	100	50	100	500
Sensitized cells	22·0* (±10·0)	31·2* (±9·2)	28·2* (±10·1)	3·7 (±2·8)	17·0* (±8·6)	26·5* (±4·6)	25·9* (±9·1)	17·2* (±7·2)	0·8 (±1·3)	1·2 (±1·3)	2·0 (±2·6)
Normal cells	0 (±1·1)	0·8 (±1·0)	1·1 (±2·1)	0 (±0·8)	1·3 (±1·9)	1·4 (±2·1)	1·7 (±1·9)	1·8 (±2·7)	1·5 (±2·6)	0·8 (±1·3)	0·9 (±1·4)

* Significantly different from controls ($P<0·001$).

contrary, no release of histamine was detectable following incubation of the same agents with cells obtained from untreated, parasite-free controls. The results in Table 1 also show that 10 μg/ml Con A produced the greatest response whereas lower or higher concentrations were less effective in inducing histamine release from sensitized cells. When cells obtained from parasite-sensitized donors were incubated at 37°C for various time intervals with 10 μg/ml Con A, the percentage of histamine released after 2 min of incubation was almost the same as after 10 min of incubation (Table 2). No substantial release of histamine was detected after incubation at 4°, 10° or 48°C (Table 2). Furthermore, the percentage of

TABLE 2. Time and temperature dependency of Con A-induced histamine release from sensitized rat pleural cells (10 μg/ml Con A)

Conditions of incubation	Percentage of total histamine release (mean of 10 experiments)
2 min 37°C	18 (±4·2)
5 min 37°C	22 (±4·8)
10 min 37°C	24 (±3·2)
10 min 4°C	0 (±0·8)
30 min 4°C	1 (±0·6)
10 min 10°C	2 (±1·0)
10 min 48°C	4 (±1·2)

histamine released by Con A or the allergen were the same irrespective of whether the serum in the incubation fluid had been previously heat-inactivated (60 min 56°C) or not. A number of other compounds such as phytohaemagglutinin (10–500 μg/ml; Difco) and agents known to react with human blood group substances such as extracts from *Dolichos biflorus*, *Ulex europaeus*, *Bauhinia purpurea* or *Vicia graminea* (which were a gift from Dr M. Metaxas, Zürich), were without effect in the present system.

When isoprenaline (10^{-6} M) or adrenaline (10^{-5} M) were added to sensitized rat pleural cells at the same time as the allergen or Con A, histamine release induced by these agents was inhibited (Table 3).

TABLE 3. Inhibition by sympathomimetic amines of allergen or Con A-induced histamine release from sensitized rat pleural cells

	Molar concentration of sympathomimetic amines in the incubate		
	None	Isoprenaline 10^{-6}	Adrenaline 10^{-5}
Allergen (50 worm equivalents/ml)	28 (±5·1)	5 (±1·2)*	6 (±1·8)*
Con A (10 μg/ml)	27 (±2·7)	4 (±0·8)*	8 (±0·6)*

Percentage of histamine released after 10 min at 37°C; each value represents the mean of 15 experiments.

* Significantly lower than controls ($P<0·001$).

TABLE 4. The effect of carbohydrates on the percentage of total histamine released from sensitized rat peritoneal cells by concanavalin A (10 μg/ml) or worm allergen (10 worm equivalents/ml) *in vitro*. Time of incubation 10 min at 37°C

Carbohydrate present in incubate		Mannose		Methyl α-D-mannopyranoside		Methyl α-D-glucopyranoside		Glucose		Glucosamine		Galactose	
	None	0·1 M	0·01 M	0·1 M	0·01 M	0·1 M	0·01 M	0·1 M	0·01 M	0·1 M	0·01 M	0·1 M	0·01 M
Concanavalin A	49·5 (±7·9)	0·6* (±0·8)	35·2* (±12·6)	0·2* (±0·5)	10·0* (±3·5)	13·0* (±5·7)	35·4* (±4·4)	10·1* (±4·9)	4·2* (±4·9)	0·2* (±0·6)	37·6* (±4·8)	39·0* (±5·3)	59·8 (±2·5)
Worm allergen	44·8 (±6·1)	13·7* (±4·0)	32·7* (±5·5)	22·4* (±4·1)	53·7 (±8·1)	24·1* (±5·1)	43·7 (±3·9)	17·6* (±3·9)	42·6 (±6·8)	7·7* (±3·0)	53·7 (±5·5)	27·9* (±4·2)	46·3 (±9·4)

Each value represents the mean of 30 experiments. * Significantly lower than controls ($P<0·001$).

The effect of carbohydrates on histamine release by worm allergen or by Con A

To see whether saccharide binding sites of Con A were responsible for its effects in the present *in vitro*-system, various carbohydrates were tested. The presence of such compounds in concentrations which produced no spontaneous release of histamine, markedly suppressed the capacity of Con A to trigger the release mechanism in sensitized cells (Table 4); and additional experiments revealed that the allergen-induced release mechanism was also inhibited in the presence of carbohydrates (Table 4).

Experiments with trypsinized Con A

When trypsinized Con A in concentrations up to 500 μg/ml was added to sensitized peritoneal or pleural cells, only a very low percentage of total histamine was released (Table 1). When sensitized cells had been first preincubated for 10 min at 37°C with 500 μg/ml of trypsinized Con A, the percentage of total histamine released by subsequent addition of either worm allergen or untreated Con A was always markedly lower than that released from cells in the absence of trypsinized Con A (Table 5). On the other hand,

TABLE 5. The percentage of total histamine released by worm allergen or untreated Con A from sensitized rat pleural cells preincubated with trypsinized Con A (500 μg/ml 10 min at 37°C)

	Worm allergen (worm equivalents/ml)		Untreated Con A (μg/ml)	
Concentration of agents	10	50	10	100
Untreated sensitized cells	49	48	49	40
Sensitized cells pre-incubated with trypsinized Con A	21	25	26	24

Each value represents the mean of 20 experiments.

pretreatment of sensitized cells with the same concentrations of trypsin or of soybean trypsin inhibitor alone did not affect their subsequent response to either worm allergen or untreated Con A.

DISCUSSION

The data showing that Con A and the specific allergen are similarly able to induce the release of histamine from appropriately sensitized peritoneal or pleural cells or isolated sensitized rat mast cells, but not from cells taken from parasite-free controls, strongly suggest that the release mechanism induced by both agents is triggered by their interaction with cell-bound IgE-type antibody. Con A-induced release of histamine was found to be a rapid event not dependent on complement, and was fully suppressed when incubation was performed at 4°, 10° or 48°C, and thus shows similar characteristics as antigen-induced release of histamine (Mongar & Schild, 1957; Perera & Mongar, 1963). These data indicated that Con A might induce a sequence of cellular events similar to that elicited by the specific allergen in the anaphylactic reaction.

It seems probable that in immediate hypersensitivity, IgE-type immunoglobulins combine

with appropriate tissues through the Fc portion of the molecules, and binding of the allergen to the Fab portions produces bridging of cell-bound antibody molecules (Stanworth, 1970, 1971; Ishizaka and Ishizaka, 1970; Ishizaka *et al.*, 1971b). This bridging may induce enzymatic sequences triggering the cellular release mechanism. It has recently been demonstrated that drugs which increase the cellular level of cyclic 3′, 5′-adenosine monophosphate are able to prevent allergen-induced release of histamine (and of other biologically active agents) from human lung (Assem & Schild, 1969), leucocytes (Lichtenstein & Margolis, 1968), basophils (Ishizaka *et al.*, 1971a), monkey lung (Ishizaka *et al.*, 1971b) or rat mast cells (Keller, unpublished). Present experiments have shown that the release of histamine from sensitized cells by both allergen or Con A is inhibited to the same degree by sympathomimetic amines (Table 3) and thus suggest that allergen and Con A trigger similar cellular enzymatic sequences.

There is some evidence which indicates that the specific allergen may achieve the bridging effect by binding to two neighbouring cell-bound IgE-type antibody Fab fragments (Ishizaka & Ishizaka, 1968; Stanworth, 1971), and which thus presumes that the antigen molecule is at least bivalent. If it is accepted that trypsinization of Con A results in monovalent pieces (Burger & Noonan, 1970), the present observations showing that trypsinized Con A has lost its ability to elicit the release mechanism (Table 1) make it probable that the effect of the intact Con A molecule is also achieved by cross-linking of adjacent cell-bound antibody molecules.

There is increasing evidence showing that Con A combines with polysaccharides and glycoproteins having terminal α-D-mannopyranoside, α-D-glucopyranoside, and related structures (Sumner & O'Kane, 1948; Goldstein *et al.*, 1965; Poretz & Goldstein, 1970). The experiments demonstrating that the capacity of Con A to induce the release mechanism *in vitro* was markedly suppressed when such carbohydrates were present in concentrations which produced no spontaneous release of histamine (Table 2), suggests that saccharide binding sites of Con A were responsible for its effects. Interestingly, histamine release induced by the specific allergen was similarly inhibited when high concentrations of the carbohydrates were present in the incubate.

These observations can easily be understood if it is accepted that both IgE-type antibody and the worm allergen molecules exhibit free sugar groupings. However, they do not answer the question whether or not Con A and the allergen bind to similar receptor sites on the immunoglobulin molecule. When sensitized cells were preincubated with high concentrations of trypsinized Con A, the release mechanism induced by subsequent addition of both untreated Con A and the worm allergen was markedly suppressed. These data make it conceivable that the antigenic determinant in the Fab region of IgE-type rat anti-worm antibody is similar or identical with the receptor site of Con A and that its effects are due to binding to this site. Various facts, for example the large difference between the molecular weights of Con A (Sumner *et al.*, 1938) and the worm allergen (Jones & Ogilvie, 1967), and also the differences observed in the degree of inhibition of allergen- and Con A-induced release of histamine by some carbohydrates make it more probable, however, that Con A reacts predominantly with Fc regions of the immunoglobulin. Under these circumstances, cross-linking of the cell-bound IgE molecules brought about by Con A *in vitro* would take place in their Fc-regions, as with antibody directed specifically against determinants within their Fc-regions (Stanworth, 1970) or with protein A from *Staphylococcus aureus* (Stanworth, 1971).

It seems highly probable that the binding of Con A to IgE-type antibody is immunologically non-specific. This is supported by the observation that in one of the experiments, cells obtained from rats not infested with *N. brasiliensis* released of their histamine on incubation with Con A but not with *N. brasiliensis* allergen and these rats were found to be infected with *Syphacia muris*, a parasite not related to *N. brasiliensis*. Other experiments have shown that Con A and the carbohydrates were not able to reproduce the *in vitro*-effects under various *in vivo*-conditions. This is probably due to interaction of these agents with other components of the body (Leon, 1967; Goldstein *et al.*, 1969).

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