

BRIEF COMMUNICATIONS

On the measurement of blocking antibody

Blocking of antigenic histamine release compared
to maximal antigen binding by antibodies in serum

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A distinction is made between maximum antigen-binding capacity of serum and the degree to which antibodies in serum responsible for such binding may actually block interaction between antigen and sensitized cells. Measurement of histamine release from leukocytes by antigen previously incubated with serum was compared with release by antigen added simultaneously with serum. Antigenic release of histamine was much less blocked when cells, antigen, and serum antibody were brought together simultaneously. Under such circumstances, measurement of the in vitro blocking effect of antibodies on the functional action of antigen in histamine release yielded results which may be found more relevant to clinical manifestations than maximal antigen-binding capacity of serum.

In 1935 Cooke and associates¹ introduced the use of the term "blocking" in connection with antibodies found in serum of allergic individuals, differing from skin-sensitizing antibodies, but able to specifically combine with antigens from allergen extracts when mixed with the serum in vitro. The term "blocking" was originally chosen to convey the idea that blocking antibody "prevented" the interaction between allergen and skin-sensitizing antibody on cells and so afforded protection from symptoms. This connotation has greatly influenced the views of subsequent investigators and practitioners.

In the ensuing 25 years, numerous in vitro procedures have been devised for measurement of the so-called blocking antibody, but the role it may play in modification of symptoms has not been clarified. All the procedures advocate incubation of serum and antigen together for an hour or more before applying some method for measuring the amount of antigen which has combined with

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PROCEDURE I

30 min. ANC	0 min. ANC
0.1 ml. Antigen 0.1 ml. Serum Incubate 30 min. Add 1 ml. leukocyte suspension, mix	1 ml. Leukocyte suspension 0.1 ml. Serum Mix, add to 0.1 ml. antigen Mix
Incubate 15 min. Centrifuge without delay Extract aliquot of supernate for histamine analysis	

or been neutralized by antibody. Presumably this was done to determine the maximal content of "blocking" antibody or antigen-binding capacity.

In the body, antigen encounters sensitized cells without prolonged prior exposure to antibody in serum. Perhaps the uncertainty regarding the correlation between total titer of "blocking" antibody and clinical manifestations stems from failure to clearly separate two aspects: (1) the measurement of the maximum capacity of the serum to bind antigen, and (2) the determination of the degree to which such binding capacity may actually exert any blocking effect on the functional interaction between antigen and sensitized cells. Expressed in current terminology: How much does specific IgG circulating in the blood actually block the interaction of antigen with cell-fixed IgE and, consequently, cellular release of histamine?

As originally proposed,³ the procedure using in vitro histamine release from leukocytes in the measurement of antigen-neutralizing capacity of serum called for incubation of antigen and serum together for an hour before adding sensitized leukocytes. An outstanding advantage of the in vitro histamine release system is that conditions for the in vitro study of the interaction between antigen, antibody in serum, and sensitized cells can be arranged to simulate in vivo circumstances more closely than other methods for measurement of the antigen-binding antibody, loosely referred to as blocking antibody.

Prompted by the preceding consideration, we undertook determination of the blocking of histamine release from sensitized leukocytes by antibodies in serum, when it was added simultaneously with antigen, in comparison with the release observed when serum and antigen were incubated together for 30 minutes before mixing with sensitized leukocytes. In other words, the antigen-neutralizing capacity (ANC) of serum without prior incubation (0 min. ANC) was compared to antigen-neutralizing capacity of serum incubated with the antigen 30 minutes (30 min. ANC).

PROCEDURE

Antigen-neutralizing capacity of serum was determined as already described,^{4, 5} except for the modifications of the protocol shown in Procedure I.

In both cases 4 serial concentrations of antigen were tested with a normal serum and the allergic subject's serum. Maximal binding of antigen occurs within 30 minutes incubation at 37° C.³ A 15 min. period was allowed for histamine release as this proved adequate for abundant release (usually maximal release) and because any blocking effect of antibody in

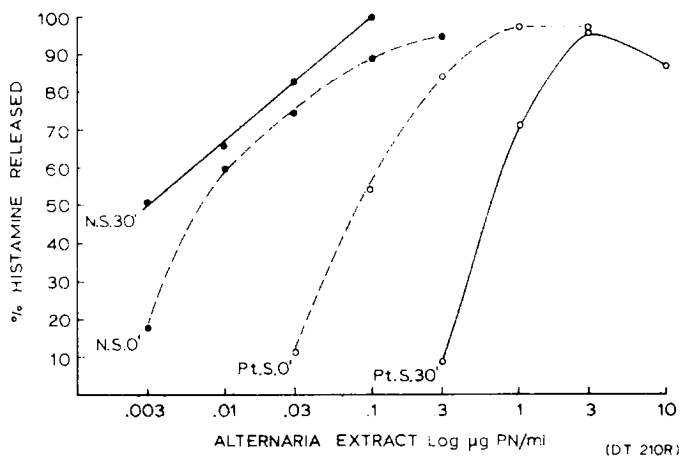


FIG. 1. Concentration-response curves of antigenic histamine release from leukocytes of an allergic subject in 0 min. and 30 min. ANC procedures described in text. **N.S.** = normal serum; **Pt.S.** = patient's serum.

serum on the early phase of release of mediator is likely to be most pertinent to clinical symptoms of the immediate type of hypersensitivity.

RESULTS

Fig. 1 shows the type of concentration-response curves obtained. It is evident that a much greater concentration of antigen is required to give the same release of histamine where antigen and serum are incubated together prior to addition of sensitized leukocytes (30 min. ANC) than is the case without prior incubation (0 min. ANC). (Also interesting, but not pertinent to the present thesis, are the slight differences between histamine release by antigen with and without prior incubation with normal serum repeatedly observed in the experiments.)

Table I presents the values of ANC determined at 0 min. and 30 min. for sera from 17 subjects, representing a wide range of antigen-neutralizing capacity in sera as measured in the 30 min. ANC procedure. It is clear that, especially in subjects with high 30 min. ANC, the value of the 0 min. ANC is considerably less than that for the 30 min. ANC. This means that histamine release from the sensitized leukocytes by antigen was considerably less "blocked" in the 0 min. ANC procedure when cells, antigen, and antibody encountered one another simultaneously (as occurs in the body) than in the 30 min. ANC procedure, where antigen could be bound by antibody during incubation with the serum before the leukocytes were added.

Apparently when the antibody content of sera is quite high, as sometimes seen following injection therapy, some blocking of histamine release does occur under 0 min. conditions, but to a much less impressive degree than the 30 min. ANC value might lead one to expect.

The protocol shown under Procedures is most convenient to perform, but the antigen is incubated at 8 per cent concentration of serum in the 0 min. ANC and 50 per cent serum in the 30 min. ANC. This was shown not to affect the

TABLE I

Subject	ANC*		Ratio 30 min./0 min.	Injection therapy	Allergen†
	30 min.	0 min.			
364T	1,300	26	50.0	+	A
364C	400	83	4.8	+	A
210R	223	11	20.3	+	A
52N	75	1	75.0	+	D
72L	43	23	2.0	+	A
148O	27.5	3.7	7.5	0	A
208K	18.0	1	18.0	0	A
251H	12.0	1	12.0	0	A
178J	7.8	6.8	1.2	+	D
329O	6.9	1.7	4.1	+	D
230J	6.3	1.0	6.3	0	A
300F	6.3	2.3	2.7	+	A
307B	4.0	2.0	2.0	0	D
223H	4.0	1.0	1.0	0	A
205G	3.3	2.1	1.6	0	D
165J	2.8	1.0	2.8	0	D
36N	2.3	1.3	1.8	+	A

*ANC: The ratio of concentrations of antigen required to release same percentage of histamine after prior incubation with subjects' serum and a normal serum, e.g.,

$$\frac{0.42 \text{ } \mu\text{gPN/ml.}}{0.085 \text{ } \mu\text{gPN/ml.}} = \text{ANC of } 5.5$$

†A = Alternaria; D = house dust.

PROCEDURE II

50 % Serum	8 % Serum
0.1 ml. Antigen	0.1 ml. Antigen
0.1 ml. Serum	0.1 ml. Serum
Incubate 30 min.	1.0 ml. Buffer
Add 1 ml. leukocyte suspension	Incubate 30 min.
0.1 ml. buffer	Add leukocytes in 0.1 ml. (contains sufficient number to attain final concentration equal to that in 50 per cent serum procedure)

results significantly by carrying out the incubations with the serum concentrations at 8 per cent and 50 per cent as shown in Procedure II.

Comparisons of 30 min. ANC gave the results shown in Table II. Which procedure gives most precise results could be determined by more extensive comparisons.

CONCLUDING COMMENTS

The true functional blocking action of serum antibodies on antigenic release of mediators in immediate hypersensitivity disorders would seem to be more nearly estimated by the 0 min. ANC procedure. Even closer relevance to events in the body could probably be obtained by development of in vitro procedures, whereby antigen could be added simultaneously with serum or other body fluids directly to cells or tissues; for example, antigen could be added to leukocytes resuspended in 100 per cent serum from an allergic subject compared to resuspension in compatible serum from a nonallergic subject. The term "blocking" should be abandoned in connection with quantities of antibodies in serum

TABLE II

Subject	ANC	
	50 % Serum	8 % Serum
395K	288	280
382H	94	85
396H	30	35

determined by measurement of maximal capacity for binding antigen and should be restricted to measurements of functional capacity of antibody to inhibit cellular responses to antigen under physiologic conditions. This may be designated the functional inhibitory capacity (FIC').

Some threshold titer of antibody in serum may be required to block mediator release sufficiently to affect production of symptoms, and marked variation among individuals may be anticipated.

Further efforts to appraise the role of antibodies in serum in pathogenesis of symptoms or evaluation of injection therapy may advantageously incorporate a measurement such as 0 min. ANC by the in vitro histamine release procedure.

REFERENCES

- 1 Cooke, R. A., Barnard, J. H., Hobald, S., and Stull, A.: Serological evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J. Exp. Med.* **62**: 733, 1935.
- 2 Loveless, M. H.: Immunologic studies of pollinosis. VI. Shortening the treatment of hay fever, *J. ALLERGY* **15**: 311, 1944. (Contains historical review of early work on "blocking" antibody.)
- 3 Lichtenstein, L. M., and Osler, A. G.: An in vitro study of the reaction between ragweed pollen antigen, allergic human serum and ragweed-sensitive human leukocytes, *J. Immunol.* **96**: 169, 1966.
- 4 May, C. D., Lyman, M., Alberto, R., and Cheng, J.: Procedures for immunochemical study of histamine release from leukocytes with small volume of blood, *J. ALLERGY* **46**: 12, 1970.
- 5 May, C. D., Lyman, M. S., Alberto, R., and Cheng, J.: Immunochemical evaluation of antigenicity of house dust extract, *J. ALLERGY* **46**: 73, 1970.
- 6 May, C. D., Lyman, M. S., Alberto, R., and Aduna, N.: Comparison of immediate "blocking" of antigenic release of histamine by serum and maximal antigen neutralizing capacity, *J. ALLERGY* **47**: 111, 1971. (Abst.)