

INHIBITION OF PRAUSNITZ-KÜSTNER
REACTION BY PROTEOLYTIC-CLEAVAGE
FRAGMENTS OF A HUMAN MYELOMA
PROTEIN OF IMMUNOGLOBULIN CLASS E

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Summary Inhibition of Prausnitz-Küstner testing, using proteolytic digestion fragments of an atypical myeloma protein of immunoglobulin class E, has provided evidence that human skin-sensitising antibodies (reagins) bind to isologous tissues through sites within the Fc regions of the molecules.

Introduction

WE have described elsewhere (Stanworth, Humphrey, Bennich, and Johansson, 1967) the specific inhibition of the Prausnitz-Küstner (P.-K.) reaction in a healthy person, by local injection of an atypical myeloma protein, initially designated "IgND" and now referred to as IgE or γ E (World Health Organisation 1968). We concluded that the myeloma protein blocked the reagin-binding skin sites in the healthy recipient.

Similar inhibition tests have now been done with various fragments of the same myeloma protein, obtained by proteolytic cleavage, in an attempt to throw some light on the mode of fixation of reagins to isologous tissues.

Technique

The well-defined horse-dander reagin-allergen system was used; the P.-K. testing procedure adopted was similar to that described previously (Stanworth, Humphrey, Bennich, and Johansson, 1967); and, as before, inhibition testing was carried out in two different ways. In test series A duplicate sites on the healthy recipient's back were passively sensitised by intradermal injection (0.1 ml.) of a mixture (1/1) of allergic serum from donor "P. R." and either IgE fragment or, as a control, intact immunoglobulin. In test series B duplicate sites on the

TABLE I—RESULTS OF TESTING FOR COMPETITIVE INHIBITION OF P.-K. REACTION

Test substance	Molecular weight	Results of duplicate P.-K. tests					
		10 min. weal areas (sq. mm.)			20 min. weal areas (sq. mm.)		
		(1)	(2)	Mean	(1)	(2)	Mean
Saline (control)	..	94	85	90	168	135	152
Myeloma IgE:	196,000	2	4	3	4	6	5
Fc fragment	98,000	5	3	4	4	4	4
Fc-like fragment (1) ..	..	38	55	47	90	96	93
Fc-like fragment (2) ..	..	70	47	59	126	88	107
Intermediate 7S fragment	(150,000)	14	5	10	70	91	81
F(ab') ₂ fragment	104,000	61	92	77	126	101	114
Fab fragment	(~50,000)	71	40*	56	132	67*	100
λ -like fragment	..	78	71	75	188	109	149
Normal IgG	150,000	88	56	72	147	95	121

* <0.1 ml. injected.
Mean area of wheals produced by direct prick testing with allergen: 5 ($\pm$ 1) sq. mm. at 10 minutes, 9 ($\pm$ 4) sq. mm. at 20 minutes.

recipient's forearms were injected with test substance 24 hours before passive sensitisation with allergic serum alone. In both series, the recipient was challenged by pricking horse-dandruff protein solution into the sensitised sites 24 hours after the transfer of the serum (or serum/inhibitor mixture). The proteolytic cleavage products tested—viz., " λ -like", Fab, F(ab')₂, "Fc", "Fc-like" (Bennich and Johansson 1967)—were isolated from papain digests of myeloma IgE, by gel filtration on 'Sephadex G150' and chromatography on diethylaminoethyl cellulose (Bennich and Johansson 1968). They were tested for competitive inhibitory activity (in test series A) at a protein concentration of 50 μ g. per ml.; whilst twice this concentration was used in test series B (involving pre-injection into the recipient's forearms). These concentrations are well within the dose range of whole myeloma protein which showed complete inhibition of P.-K. activity in the previous study.

Results

The results obtained, by determining the areas of tracings of weals appearing 10 and 20 minutes after challenge, are given in tables I and II.

In test series A, for competitive inhibition, parent myeloma IgE (from patient "ND") and normal IgG were tested, in addition to the various fragments of the myeloma protein listed in table I. Only the "Fc" fragment was as effective as the intact IgE myeloma protein in inhibiting the P.-K. reaction completely. Some of the other kinds of fragment suggested relatively weak inhibitory activity. Of these, the "7S Fc" fragment (produced by less than 3 hours papain digestion of IgE) seemed to be most effective. The P.-K. reactions developing at sites injected with mixtures containing this material took longer, and were ultimately smaller than the positive reactions occurring at the other sites (table I). The light chain(λ)-like material proved completely non-inhibitory.

The results of tests (B) (table II), in which the test substances were injected before the sensitising serum, confirmed that the "Fc" fragment of the myeloma IgE was completely inhibitory at the concentration tested (100 μ g. per ml.). None of the other fragments tested showed significant inhibition of P.-K. activity: nor did two IgG myeloma proteins of G1 and G2 subclass which had produced mild swelling and redness lasting several hours at the injection site, but which had subsided completely before challenge with allergen.

Discussion

The results confirm our earlier observations that the

TABLE II—RESULTS OF P.-K. TESTING AT SITES INJECTED 24 HOURS PREVIOUSLY WITH Ig OR Ig FRAGMENT

Test substance	Volume of solution (100 μ g./ml.) injected (ml.)	Results of subsequent P.-K. testing with undiluted allergic serum (24 hr. interval between transfer and challenge)					
		10 min. weal areas (sq. mm.)			20 min. weal areas (sq. mm.)		
		Left arm	Right arm	Mean	Left arm	Right arm	Mean
Saline (control)	0.10	43	47	45	49	65	57
Myeloma IgE:	0.10	5	11	8	4	9	7
Fc fragment	0.05	2	4	3	2	4	3
Fc-like fragment	0.05	43	24	34	59	34	47
F(ab') ₂ fragment	0.10	51	63	57	66	91	79
Fab fragment	0.05	50	41	46	46	60	53
Myeloma IgG:							
1	0.10	32	2	18	58	35	47
2	0.10	9	22	16	51	78	65
Horse-dander allergen (direct prick-test control) ..		6	1	4	6	6	6

Test conditions as for table I except that sites on forearms used.

IgE myeloma protein (previously termed IgND) can inhibit P.-K. activity, in contrast to normal IgG and certain myeloma proteins of this class. Moreover, this inhibitory activity is retained by an "Fc" fragment (molecular weight 98,000) of the myeloma IgE

None of the other IgE fragments, tested at the same concentration, showed convincing inhibition of P.-K. activity. The suggestion of a weak inhibitory activity, in the direct competition tests (A), by the "Fc-like" and "7S Fc" preparations might indicate that these preparations had retained some skin-binding sites within their structures. However, the possibility of contamination with traces of "Fc" fragment cannot be excluded, particularly since the "7S Fc" preparation was isolated after less than 3 hours' digestion (in the presence of cysteine). The " λ -like" material, which failed to react with antiserum directed against the "Fc" fragment of IgE, was completely devoid of P.-K. inhibitory activity.

These findings indicate that human skin-sensitising antibodies (reagins) bind to isologous tissues through their Fc regions, in a manner analogous to the binding of precipitating rabbit antibodies (of the IgG class) to heterologous (guineapig) skin (Ovary and Karush 1961). This would leave the Fab regions free for subsequent combination with allergen, by the mode of interaction proposed elsewhere (Stanworth 1968). It is probably relevant that the "Fc" fragment isolated from the myeloma IgE possesses distinctive chemical properties, and seems to be about twice the size of the Fc fragment from IgG.

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AN ASSOCIATION BETWEEN SERUM-MAGNESIUM AND TREMOR AND CONVULSIONS IN INFANTS AND CHILDREN

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Summary Thirteen cases of convulsions or tremors in children with hypomagnesiæmia but no associated hypocalcæmia are reported. On recovery, either spontaneously or after administration of magnesium, serum-magnesium returned to normal levels. The cause of the depressed serum-magnesium in these cases is not known. The possible mechanisms whereby a low magnesium concentration can cause hyperexcitability in nervous tissue are also discussed.

Introduction

HYPOMAGNESÆMIA has often been associated with hyperirritability of the nervous system in adults (Flink et al. 1957, Vallee et al. 1960, Welt and Gitelman 1965). Similar cases in children are rare: a likely explanation for this is the lack of reliable methods for the estimation of magnesium in small quantities of serum. We describe here thirteen cases of tremor and fits in infants and children associated with hypomagnesiæmia but not hypocalcæmia. The total and ultrafiltrable magnesium and calcium concentrations were measured in each case since we felt that the ultrafiltrable fraction might provide a more reliable indication of the ionic (biologically active) concentrations of these elements in the serum than would an estimation of total concentration. Direct measurement of ionised magnesium and calcium in small quantities of serum is technically difficult.

Patients and Methods

During the period December, 1967, to March, 1968, forty cases of convulsions, tremors, or muscular twitchings were selected at random from the pædiatric wards, and serum-levels of magnesium and calcium were determined. Fifty-eight healthy non-fasting children aged 1 day to 10 years were selected as controls; these children were all clinically well.

2-4 ml. of blood was withdrawn from the femoral vein and allowed to clot under paraffin; the serum was separated by centrifugation. The ultrafiltrate of the serum was obtained by a modification of the method of Munday and Mahy (1964). The serum after equilibration with 5% carbon dioxide and 95% oxygen was introduced into a cellulose bag which was then suspended in a 25 ml. centrifuge tube. The tube and contents were centrifuged at 800 g in an atmosphere of 5% carbon dioxide and 95% oxygen at 36-38°C. After 2-4 hours, 0.15-0.25 ml. of crystal-clear ultrafiltrate collected at the bottom of the centrifuge tube.

Magnesium was estimated fluorometrically by the 8-hydroxy-quinoline method while calcium was titrated with disodium edetate using calcein indicator under ultraviolet light; these estimations were carried out on the ultramicro-scale (O'Brien and Ibbott 1962). The reliability of the ultrafiltration procedure and the estimation methods was tested by sampling pooled serum six times on different occasions. The coefficient of variation of the results for the pooled serum did not exceed 5%.

Blood was collected when symptoms were present or as soon as possible after a convulsion. Serum magnesium and calcium measurements were repeated on recovery. Routine estimations of serum potassium, sodium, chloride, and bicarbonate were also performed when symptoms were present.

Results

Out of the forty cases studied, thirteen showed a definite lowering of the serum-magnesium but no hypocalcæmia when symptoms were present. The remaining twenty-seven patients were excluded either because their serum-magnesium levels were normal or because there was an associated hypocalcæmia. Clinically, it was not possible to differentiate between these cases and the thirteen who had hypomagnesiæmia alone. In the thirteen cases, other electrolyte levels were normal when symptoms were present except for patient 10 who had a slight degree of hyponatræmia (125 meq. per litre).

Clinically, none of the thirteen cases showed any evidence of malnutrition, dehydration, or overhydration. The clinical data together with the individual values of serum magnesium and calcium during hypomagnesiæmia and on recovery are listed in tables I and II. The following is a summary of the case histories of the patients.

Cases 1-7 (table I)

Hypomagnesiæmia developed while the patients were in the